THEMATIC REVIEW

RISING STARS

Sex differences in toxicant-associated fatty liver disease

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This paper is part of a collection of articles highlighting the breadth and depth of research being undertaken across the field of basic endocrinology by early- and mid-career researchers. The collection is published across the *Journal of Endocrinology* and the *Journal of Molecular Endocrinology*.

Abstract

Based on biological sex, the consequential health outcomes from exposures to environmental chemicals or toxicants can differ in disease pathophysiology, progression, and severity. Due to basal differences in cellular and molecular processes resulting from sexual dimorphism of organs including the liver and additional factors influencing 'gene–environment' interactions, males and females can exhibit different responses to toxicant exposures. Associations between environmental/occupational chemical exposures and fatty liver disease (FLD) have been well-acknowledged in human epidemiologic studies and their causal relationships demonstrated in experimental models. However, studies related to sex differences in liver toxicology are still limited to draw any inferences on sex-dependent chemical toxicity. The purpose of this review is to highlight the present state of knowledge on the existence of sex differences in toxicant-associated FLD (TAFLD), discuss potential underlying mechanisms driving these differences, implications of said differences on disease susceptibility, and emerging concepts. Chemicals of interest include various categories of pollutants that have been investigated in TAFLD, namely persistent organic pollutants, volatile organic compounds, and metals. Insight into research areas requiring

Key words

- sex differences
- toxicant
- liver
- TAFLD
- TASH

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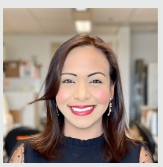

Banrida Wahlang is an Assistant Professor and an NIH Early-Stage Investigator at the University of Louisville, School of Medicine. Her research background includes understanding the impacts of environmental exposures to persistent and volatile organic pollutants on liver and cardio-metabolic diseases. Dr Wahlang was formerly a University of Kentucky and University of Louisville Superfund Research Center trainee and an NRSA postdoctoral fellow. Dr Wahlang's current work focuses on sex differences in the environmental health sciences and underlying sex-dependent mechanisms that drive metabolic disease outcomes with chemical exposures. She received a NIEHS K01 Award to help fund this research. Dr Wahlang's research interests and goals also include women's environmental health, sex and gender studies in environmental toxicology, socio-cultural determinants of health and community science, and sex-specific intervention strategies for combatting pollution effects on human health.

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further development is also discussed, with the objective of narrowing the knowledge gap on sex differences in environmental liver diseases. Major conclusions from this review exercise are that biological sex influences TAFLD risks, in part due to (i) toxicant disruption of growth hormone and estrogen receptor signaling, (ii) basal sex differences in energy mobilization and storage, and (iii) differences in chemical metabolism and subsequent body burden. Finally, further sex-dependent toxicological assessments are warranted for the development of sex-specific intervention strategies.

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Introduction

Scope of the review

Fatty liver disease (FLD), the most common form of chronic liver disease, impacts about 25.2% of the global population (Younossi *et al.* 2016). Traditionally, genetic predisposition, lifestyle factors, and comorbidities such as obesity and diabetes were blamed for FLD development. However, emerging studies encompassing human epidemiologic data to experimental model systems have also demonstrated the detrimental effects of environmental chemicals or toxicants on the liver leading to FLD. Notably, more recent data have also illustrated the importance of considering biological sex when assessing environmental contributions to FLD. Therefore, this review examines the topic of sex differences in liver toxicology by first introducing general concepts such as sex and gender in scientific research, sexual dimorphism of the primary organ of discussion – the liver, and an overview of FLD. Second, the review briefly discusses mechanisms of toxicant-associated FLD (TAFLD) that have been described, independent of biological sex considerations. Finally, the review highlights the present state of knowledge on sex differences in TAFLD and expands the discussion on potential underlying mechanisms driving these differences for categories of pollutants implicated in TAFLD, namely, persistent organic pollutants (POPs) and other endocrine-disrupting chemicals (EDCs), volatile organic compounds (VOCs), and heavy metals.

Sex and gender

The existence of sex differences in scientific research studies has been observed over several decades and dates back to scientific publications from the 1950s where differences in coronary artery disease outcomes were reported in male and female patients (Keys *et al.* 1956, Masini *et al.* 1961). Nonetheless, the overall research topic pertaining to sex differences in health and disease is still deemed as 'poorly understudied' with a lack of thorough findings and insufficient knowledge preventing one from drawing definitive conclusions on how disease etiology, pathogenesis, and drug treatment can differ by sex. Perhaps, the most conspicuous reason for this could be the faulted general assumption that men and women may not respond differently to drug treatments, and the exclusion of women as vulnerable subjects during drug clinical trials in the 1970s (Institute of Medicine 2001, Liu & Mager 2016). Or perhaps, another culpable reason could be the reluctance of scientific researchers to incorporate female experimental models in their research designs so as to avoid 'hormonal' and other 'unpredictable' effects that female models may introduce into the experiments (Institute of Medicine 2001), hence altering already-established mainstream study protocols. In fact, in 1985, the Public Health Service Task Force on Women's Health concluded that studies on women's health have been compromised (Public Health Service Task Force on Women's Health Issues 1985); this was closely followed by NIH guidelines recommending funded researchers to incorporate female models in their experimental designs and acknowledge sex differences, if applicable (Liu & Mager 2016). These regulatory efforts have generated more extensive discussions on the importance of considering both sex and gender differences in health and disease while also contributing to the recent surge in publications evaluating and reporting sex differences. However, these improvements and related awareness also harbingered notable challenges in this research area including the interchangeable use of sex and gender in epidemiologic and animal scientific studies and species differences when interpreting and translating sex-specific findings.

Although 'sex' and 'gender' are not mutually exclusive and both factors can align to influence human health and disease development, the two terms, however, are antonymous with distinct originations and entail different meanings. Sex refers to the basic biological component that arises as a consequence of possessing different sex chromosomes at birth, thereby influencing genetic traits, physiological and phenotypic characteristics,

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and hormone make-up as reflected by the gonads, sex hormones, and external and internal reproductive organs (Clayton & Tannenbaum 2016). In stark contrast, gender is a construct formed and driven by social, historical, cultural, and behavioral factors, and choices, thereby influencing an individual's self-identity and societal traits (Clayton & Tannenbaum 2016). Gender encompasses gender identity and dysphoria, as well as gender norms and relations, thus reflecting the complex and intricate aspects of this term (Schiebinger & Stefanick 2016). In this review, the term 'sex' will be used as the predominant focus will be on research and clinical studies that identify 'sex' as a biological variable.

The liver as a sexually dimorphic organ

Given that biological sex can impact organ development, function, and accompanying cellular and molecular processes, its consideration is imperative in elucidating disease etiology in addition to disease initiation, progression, and treatment; drug and xenobiotic responses; as well as chemical toxicity. Sexual dimorphism of organs including the liver has been well-recognized and appreciated with mechanistic studies attributing the nature of growth hormone secretion, which is pulsatile in males and continuous in females, as a partial reason driving sexually dimorphic hepatic gene expression profiles (Holloway *et al.* 2008, Waxman & Holloway 2009). Furthermore, sex steroid hormones play a crucial role in defining the sexually dimorphic liver during both early life and puberty onset. In males, testosterones exert a 'programming' effect on the liver during the prenatal period, while both androgens and estrogens exert 'activational' effects on the liver during puberty onset, resulting in two distinct biological systems (Mauvais-Jarvis 2015). In addition, hepatic estrogen receptor alpha (ERα) expression also influences sex-specific liver functions including gluconeogenesis and lipid metabolism during different life stages (Le Magueresse-Battistoni 2021). The liver is an indispensable organ in living organisms, based on its critical location in the abdominal cavity along with hepatic portal vein blood supply, and its requisite functions include detoxification of drugs and other xenobiotics and maintenance of general energy homeostasis through endobiotic metabolism including synthesis and breakdown of lipids, carbohydrates, and proteins (Trefts *et al.* 2017, Beier & Arteel 2021). The liver is also pivotal in regulating blood chemistry through the production and release of proteins like albumin, clotting factors, and hepatokines (Rhyu & Yu 2021); nutrient uptake and drug excretion through bile acid (BA) synthesis and secretion (Lefort & Cani 2021); along with iron and copper storage (Roberts & Sarkar 2008, Vogt *et al.* 2021). In addition, another vital role of the liver includes the clearance of bacteria from the blood stream as part of its filtering process and regulation of inflammatory responses through the production of immune factors (Gao *et al.* 2008). Due to its unique anatomic characteristics which render it the initial organ that ingested drug compounds and nutrients encounter and due to its intrinsic functions in xenobiotic biotransformation including phase I and II metabolisms, the liver consequently becomes a primary target organ for foreign chemicals including environmental pollutants or toxicants.

Fatty liver disease

Fatty liver disease (FLD) is often characterized as a spectrum of disorders initially manifested by steatosis which is the accumulation of fat droplets in the liver (Wahlang *et al.* 2018). Steatosis may be accompanied by inflammation and Kupffer cell activation leading to steatohepatitis; this can further progress to the generation of pro-fibrotic factors and stellate cell activation resulting in fibrosis and subsequent tissue scarring or cirrhosis (Wahlang *et al.* 2018). Incompetent interventions and treatment failure can drive cirrhosis to terminal FLD stages such as hepatocellular carcinoma and fulminant liver failure. However, liver regeneration and repair mechanisms coupled with intervention strategies often halt or slow down disease progression (Beier & Arteel 2021). Based on the etiology or probable cause, different nomenclatures for FLD have been adopted and broadly classified as alcoholic-associated liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD) (Wahlang *et al.* 2018). As the terminology suggests, ALD is brought upon by excessive alcohol consumption with a global prevalence of approximately 0.5% for alcoholic cirrhosis (Asrani *et al.* 2021), a more severe pathology of the disease. While ALD was thought to affect males more than females, more recent knowledge has suggested otherwise (Wagnerberger *et al.* 2013, Kezer *et al.* 2021). In contrast, NAFLD development has been associated with a myriad of factors ranging from genetic predisposition, lifestyle choices such as hyper-caloric intake, certain medications, comorbidities including but not limited to diabetes, and exposures to occupational chemicals and environmental toxicants (Younossi *et al.* 2018, Powell *et al.* 2021). Because of the wide array of potential causes and multifactorial risks, NAFLD has a higher prevalence than

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ALD (Younossi *et al.* 2016), and its current observable impact is higher in males and post-menopausal females (Lonardo *et al.* 2019, DiStefano 2020, Shaheen *et al.* 2021). Despite the etiology, ALD and NAFLD typically have similar pathogenesis and disease progression. About a decade ago, terminologies were also defined for NAFLD and non-alcoholic steatohepatitis (NASH) brought upon by toxicant exposures, namely toxicant-associated fatty liver disease (TAFLD) and toxicant-associated steatohepatitis (TASH), respectively (Wahlang *et al.* 2013*a*). However, unlike ALD and NAFLD, there are still limited data (Deierlein *et al.* 2017) to draw any inferences on the sex-specific prevalence and impact of TAFLD. Yet, biological sex can modulate environment–gene interactions and define how toxicants impact and modify physiological processes. Moreover, biological processes throughout the lifespan are different in males and females and confounded by factors including pregnancy, lactation, menstruation, and menopause, further emphasizing the significance of addressing sex differences in toxicology.

Therefore, the purpose of this review is to highlight the present state of knowledge on the existence of sex differences in TAFLD and associated mechanisms, in addition to the implications of said differences on disease risk and susceptibility. The review also provides insight into uncharted areas of research with the purpose of narrowing the knowledge gap on how environmental pollution differentially impacts liver health in males and females.

Currently known mechanisms in TAFLD and TASH

TAFLD and TASH were originally described in industrial workers that exhibited steatohepatitis and cirrhosis caused by occupational exposures to organic chemicals like 1,3-butadiene, styrene, and vinyl chloride (Cave *et al.* 2010, 2011). From a historical perspective, toxicant insults to the liver and subsequent TAFLD development were documented as early as the 1970s in human populations that were accidentally exposed to POPs, specifically, the 'Yucheng' and 'Yusho' poisoning incidents that occurred in Taiwan and Japan, respectively (Masuda 1985), and in rubber-manufacturing workers exposed to vinyl chloride (Creech & Johnson 1974). Environmental contributions to FLD have now been investigated for divergent classes of toxicants extending from POPs like polychlorinated biphenyls (PCBs), dioxins, organochlorine pesticides (OCPs) and perfluoroalkyl substances (PFAS) to VOCs, heavy metals, and polycyclic aromatic hydrocarbons (Wahlang *et al.* 2019*c*, 2013*a*). While TAFLD and TASH were originally connected to liver injury and toxicity observed in chemical workers which oftentimes occurred at relatively higher doses, it is also applied to chemical exposures that occur in everyday life, often viewed as 'environmental' rather than 'occupational' and occurring at lower doses. As such, environmental exposures today arise from ambient outdoor and indoor air, household and personal products, ingestion of chemical-laden food and contaminated water, and exposures from daily activities.

Distinguishable mechanisms that induce or promote liver toxicity have been substantially studied in toxicological and experimental models, particularly for POPs and VOCs, with the predominant mechanism of action often dictated by the chemical type (Wahlang *et al.* 2019*c*). Some of the common mechanistic concepts implicated in toxicant-mediated FLD include alterations in normal physiologic metabolism, disruptions of endocrine and signaling processes, formation of reactive intermediates, epigenetic modulations, and activation of inflammation and cell death pathways (Wahlang *et al.* 2019*c*). Indeed, several sub-classes of POPs have been demonstrated to act as EDCs by interacting with thyroid and sex hormone receptors including the estrogen (ER) and androgen (AR) receptors (Cano *et al.* 2021), inhibiting insulin production and subsequent glucose uptake (Lee *et al.* 2018), and altering secretory levels of hormones like leptin and glucagon-like peptide-1 (Shannon *et al.* 2019, Wahlang *et al.* 2013*b*). Apart from activation of the hormone receptors, PCBs have also been postulated to act as EDCs partially through the destruction of pancreatic beta cells and interference with normal beta cell function, thus hampering insulin synthesis and secretion (Mimoto *et al.* 2017, Shi *et al.* 2019). Certain heavy metals like arsenic, mercury, and cadmium also promote beta cell dysfunction through the induction of oxidative stress and generation of reactive oxygen species (ROS) (Mimoto *et al.* 2017). Because of the critical role of the endocrine system in maintenance of overall energy homeostasis in the body, EDCs also have the capacity to stimulate metabolic perturbations which can result in obesity, diabetes, and FLD development. Hence, EDCs can also act as metabolism-disrupting chemicals (MDCs) and the vast majority of POPs induce metabolic toxicity as EDCs/MDCs (Heindel *et al.* 2017, Wahlang *et al.* 2019*c*). Exclusive of endocrine system interactions, MDCs can also facilitate FLD development by interacting with hepatic xenobiotic and endobiotic receptors. Studies have demonstrated that PCBs promote TASH, in part, through

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activation of the aryl hydrocarbon receptor (AHR), constitutive androstane receptor (CAR), and pregnane-xenobiotic receptor (Wahlang *et al.* 2019*a*). While their major role is xenobiotic detoxification, these receptors also play distinct roles in hepatic energy metabolism and inflammation through receptor–receptor crosstalk (Cave *et al.* 2016). Interactions with endobiotic receptors have also been illustrated for multiple PFAS that activate peroxisome-proliferator activated receptors (PPARs) and thereby interfere with lipid metabolism (Szilagyi *et al.* 2020), and PCBs' upregulation of liver-X-receptor and farnesoid-X-receptor target genes to influence steatosis and BA homeostasis (Wahlang *et al.* 2019*a*). Toxicants like PCBs and vinyl chloride can also modulate FLD development by altering normal hepatic signaling processes through epidermal growth factor receptor (EGFR) inhibition, mammalian target of rapamycin activation, and transcriptional reprogramming; thus, these toxicants are also considered as signaling disrupting chemicals (Wahlang *et al.* 2019*c*).

Furthermore, multiple VOCs including acrolein and vinyl chloride also incite liver injury through the formation of reactive metabolites or intermediates that can bind to macromolecules and form adducts (Rusyn *et al.* 2021). These reactive intermediates can also induce carbonyl stress on cell organelles and ensue mitochondrial dysfunction, oxidative stress, ROS generation, lipid peroxidation, and endoplasmic reticulum (ER) stress (Lang & Beier 2018, Rusyn *et al.* 2021). In addition, POPs, VOCs, and heavy metals can also drive TASH development and progression through the induction of pro-inflammatory states in the liver including inflammasome activation and stimulation of cell death pathways (Lang & Beier 2018, Wahlang *et al.* 2019*a*, Renu *et al.* 2021). Because these primary mechanisms of toxicity do not sufficiently explain the total observable effects of toxicants in compromising liver health, other mechanisms of toxicity that have been investigated and identified including DNA epigenetics (Jin *et al.* 2020, Cano *et al.* 2021), modulation of microRNA levels (Cave *et al.* 2022, Petri *et al.* 2022), RNA epitranscriptomics (Klinge *et al.* 2021), as well as disruption of the gut–liver axis through gut microbiome alterations (Wahlang *et al.* 2021).

Sex differences in TAFLD associated with POP exposures

Also known as 'forever chemicals', POPs are poly-halogenated organic compounds that persist in the environment due to their resistance to degradation and continue to be a looming environmental health concern. Comprising of numerous man-made chemicals produced intentionally or inadvertently for industrial processes, agriculture, construction, and commercial products, POPs are found in polluted surroundings and landfills. Numerous classes of POPs have been banned over decades, beginning with the US Congress ban on PCBs in the 1970s and the historic Stockholm Convention ban on PCBs, dioxins, and selected OCPs in 2001 (Wahlang 2018). Nonetheless, their ability to migrate across the globe through the 'grasshopper effect' (Kobusinska *et al.* 2020) and to 'bioaccumulate' in living organisms and biomagnifying along trophic levels of the food chain (Beyer & Biziuk 2009) continue to make these 'forever toxicants' highly relevant in environmental health research. The majority of epidemiologic studies previously investigating the effects of POPs on liver toxicity were focused on industrial and pesticide workers who were chiefly males, while other studies investigating exposures in residential populations did not stratify their statistical findings by sex. However, a modest proportion of studies reported divergences in toxicant body burdens and exposure effects between males and females which, to some extent, provided the foundational basis to pursue this area of research. Significantly, due to their ubiquitous nature, current exposures to POPs occur in the entire population, impacting all sexes throughout their lifespan. Hence, elucidating sex-dependent effects will enable better risk assessment and identification of susceptible populations. Based on the principal chemical class, currently known knowledge on sex differences in TAFLD related to POP exposures are highlighted in Table 1.

Polychlorinated biphenyls and dioxins

Polychlorinated biphenyls (PCBs) are halogenated aromatic hydrocarbons that were primarily used as dielectric fluids in capacitors and transformers. PCBs were commercially manufactured as mixtures rather than individual congeners. Monsanto Corporation was the primary PCB manufacturer in North America and marketed PCB mixtures under the trade name 'Aroclor' (Wahlang *et al.* 2014). PCBs are classified as 'coplanar' or 'dioxin-like' due to their structural resemblance and capacity to activate the AHR similar to dioxin; and 'non-coplanar', 'non-dioxin-like' or 'phenobarbital-like' based on their ability to activate CAR similar to phenobarbital (Safe 1993, Wahlang *et al.* 2014). Unlike PCBs, dioxins were mostly by-products of various industrial processes. The correlation between PCBs and dioxins with TAFLD/TASH

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Table 1 Selected persistent organic pollutants (POPs) and other endocrine/metabolism disrupting chemicals (EDCs/MDCs) associated with sex-dependent fatty liver disease and related TASH endpoints that were primarily reported.

Chemical/ chemical group	Experimental exposure models	Sex-specific toxicity		Epidemiology studies/ toxicological evidence	Sex-specific associations	
		Females	Males		Females	Males
Dioxin-like PCBs	Steatosis and energy metabolism: Sub-acute exposure to PCB 126 in SD rats (Eti *et al.* 2022)	Altered lipid metabolism, cholesterol levels, and increased hepatic PPARα activation	Impaired glucose homeostasis			
	Glucose homeostasis: Sub-chronic exposure to PCB 77 in wildtype and AHR knockout mice with diet-indued obesity and subsequent weight loss (Jackson *et al.* 2019)	AHR-deficient, exposed females showed impaired glucose tolerance	Wildtype males were more sensitive to PCB 77-mediated glucose intolerance during weight loss			
Non-dioxin-like PCBs				Obesity & BMI: Prenatal exposures to PCBs and DDE and analysis at 5 and 7 years of age (Tang-Peronard *et al.* 2014)	PCBs associated with increased waist circumference and increased BMI from 5–7 years of age	No associations observed for BMI or waist circumference
Organic pollutant mixtures	TASH, metabolic & endocrine disruption: Acute PCB 126 + Aroclor 1260 exposure in C57BL/6 mice (Wahlang *et al.* 2019*b*)	Increased steatosis, hepatic inflammation, and impaired glucose uptake	No changes observed for steatosis, inflammation, or glucose metabolism	Diabetes & Hypertension: 24-year follow-up on the Yucheng cohort (Wang *et al.* 2008) Hypertension: PCBs, PCDDs, and PCDFs in US NHANES participants (Ha *et al.* 2009)	Positive association between PCBs and PCDFs with diabetes; PCDDs and PCDFs associated with newly diagnosed hypertension	No association with diabetes; positive association between PCBs and hypertension
	Liver tumors: Different sub-chronic and chronic exposures to Aroclors in SD rats (Mayes *et al.* 1998, Whysner & Wang 2001, Brown *et al.* 2007)	More severe liver toxicity and higher incidence of hepatocellular neoplasms with different Aroclors and with increased doses	Significant increase in hepatocellular neoplasms only with high dose Aroclor 1260	Cancer Mortality: Meta-analysis of Yucheng and Yusho cohorts; mortality analysis of electrical capacitor workers (Ruder *et al.* 2014, Li *et al.* 2015)	Elevated liver cancer mortality in Yucheng/ Yusho cohorts and unhealthy worker effect in electrical workers	Elevated mortality for other cancers in Yucheng/Yusho cohorts
	Metabolic & endocrine disruption: Life-long dietary exposure to TCDD, PCB 153, BPA, and DEHP in C57BL/6 mice (Naville *et al.* 2013)	Glucose intolerance associated with reduced estrogenic signaling	Alterations in cholesterol metabolism	Elevated liver enzymes: Associations between POPs and ALT in morbidly obese patients (Rantakokko *et al.* 2015)	Positive association between PCB-118, β-HCH, and trans-nonachlor with ALT post-bariatric surgery	No POPs–ALT interactions
Dioxins	Liver tumors: Sub-chronic TCDD exposure in SD rats with diethyl nitrosamine tumor initiation (Wyde *et al.* 2001)	Increased 8-oxo-deoxyguanosine adduct formation in livers of intact but not ovariectomized rats	No change in 8-oxo-deoxyguanosine adduct levels with TCDD exposure			

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Table 1 Continued.

Chemical/ chemical group	Experimental exposure models	Sex-specific toxicity		Epidemiology studies/ toxicological evidence	Sex-specific associations	
		Females	Males		Females	Males
	Hepatic gene expression: TCDD exposure for 28 days in male and 92 days in female C57BL/6 mice (Nault *et al.* 2017)	Repression of sexually dimorphic hepatic gene expression	Loss of sexually dimorphic hepatic gene expression and impaired GH-JAK2-STAT5 signaling			
OCPs	Liver tumors: Sub-acute HCB exposures in SD rats with or without subsequent diethyl nitrosamine administration (Smith *et al.* 1985, Krishnan *et al.* 1991, Plante *et al.* 2002, Plante *et al.* 2007)	Increased hepatic tumor development; increased susceptibility to porphyria development	Hepatic tumor development only in a few males; less susceptibility to porphyria development	Waist circumference: Prenatal exposures to PCBs and DDE and analysis at 5 and 7 years of age (Tang-Peronard *et al.* 2014)	DDE associated with increased BMI from 5–7 years of age and increased waist circumference in females with overweight mothers	No associations observed for BMI or waist circumference
				Elevated liver enzymes: OCP exposures in a Brazilian population (Freire *et al.* 2015)	No pronounced OCP–ALT/AST interactions	Positive associations for β-HCH, DDE, and HCB with ALT and AST
Other pesticides	Steatosis & obesity: Dietary exposure to a pesticide mix in wildtype and CAR knockout C57BL/6 mice (Lukowicz *et al.* 2018)	Hyperglycemia but no changes in bodyweight or steatosis. Exposed, CAR knockout females showed significant mortality	Increased bodyweight, steatosis, and glucose intolerance vs CAR knockouts			
	Fetal liver proteomics: Vinclozolin exposure in pregnant CD-1 mice (Rister *et al.* 2021)	Activation of xenobiotic metabolism pathways in fetal liver	Activation of oxidative phosphorylation pathways in fetal liver			
PFAS	Steatosis, apoptosis, lipid metabolism, dyslipidemia: PFOS or PFOA exposures in SD rats, C57BL/6 mice, or zebrafish (Kim *et al.* 2011, Rebholz *et al.* 2016, Bagley *et al.* 2017, Schlezinger *et al.* 2020, Khazaee *et al.* 2020, Roth *et al.* 2021)	PFOS-exposed rats showed lower serum cholesterol levels and no steatosis. In contrast, PFOA-exposed mice showed aggravated diet-induced cholesterolemia	PFOS-exposed rats had increased steatosis but lower serum cholesterol and triglyceride levels. PFOS-exposed zebrafish showed enhanced changes in lipid metabolism genes	Elevated liver disease biomarkers: Cross-sectional analyses of PFAS levels and liver disease biomarkers in adult (C8) or adolescent populations (Lin *et al.* 2010, Darrow *et al.* 2016, Attanasio 2019, Bassler *et al.* 2019)	Positive associations between certain PFAS and IL-8, IFNγ, adiponectin; positive association for PFOA with ALT, AST, and GGT, and for PFNA with ALT and AST in adolescents	Positive associations between certain PFAS with C3a and leptin; negative association between PFOA and ALT in adolescents

(Continued)

Table 1 Continued.

Chemical/ chemical group	Experimental exposure models	Sex-specific toxicity		Epidemiology studies/ toxicological evidence	Sex-specific associations	
		Females	Males		Females	Males
	Liver tumors: Chronic exposures to PFOS salts in SD rats (Butenhoff *et al.* 2012*a*,*b*, Seacat *et al.* 2003)	Hepatocellular carcinoma observed in K^+ PFOS high-dose group only; higher serum PFOS concentrations	Increased hepatocellular adenoma with K^+ PFOS exposure			
	Prenatal exposures: Exposure to GenX or PFOA in pregnant CD-1 mice (Cope *et al.* 2021)	Adult offspring displayed more of a liver damage phenotype including hepatocyte necrosis	Adult offspring displayed adverse metabolic outcomes including excess weight gain and adiposity			
PBDEs	Liver tumors: Perinatal and/or adulthood exposures to brominated biphenyls in F344/N rats (National Toxicology Program 1993)	Combined perinatal and adult exposure enhanced liver neoplasm development	Perinatal exposure associated with increased incidence of hepatocellular adenoma	Elevated liver enzymes: Prenatal exposure to EDCs in birth cohort studies from six European countries (Midya *et al.* 2022)	Exposure to PBDEs and PCBs associated with increased CK-18 levels	Exposure to PBDEs and PCBs associated with increased CK-18 levels
	Liver proteomics: sub-acute exposures to a BFR mix in zebrafish (Kling *et al.* 2008)	Downregulation of proteins involved in iron homeostasis and activated oxidative stress responses	Activated oxidative stress responses			
Other EDCs/ MDCs	Liver tumors: Tributyltin exposure in C57BL/6 mice (Katz *et al.* 2020)	Increased adiposity but no enhancement in liver tumor or steatosis	Liver tumor development preceded by disrupted GH-STAT5 signaling			
	Hepatic gene expression & energy metabolism: BPA exposure in pregnant CD-1 mice (Ilagan *et al.* 2017); tolyfluanid in diet in C57BL/6 mice (Ruiz *et al.* 2019)	Increased hepatic ERα, ERβ expression with BPA; reduced body weight and increased insulin sensitivity with tolyfluanid exposure	Decreased hepatic ERα, ERβ expression with BPA; impaired glucose tolerance with tolyfluanid exposure			
	Liver weights: Exposure to DEHPs in wildtype non-agouti and yellow agouti mice (Neier *et al.* 2019)	Increased liver and body weights with exposure	Increased body weight with exposure	The metabolic syndrome and DEHP exposures (Shih *et al.* 2022)	Postmenopausal females with higher ∑DEHP concentrations had more than nine-fold higher odds for the metabolic syndrome	Higher mono-ethyl phthalate metabolite concentrations were associated two- to three-fold increased odds for the metabolic syndrome

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development and progression is considerably established in experimental model systems; likewise, associations between PCB exposures and liver injury have been illustrated in human population studies (Wahlang *et al.* 2019*d*). Sex differences with PCB exposures have been reported for selected TASH endpoints. In a 24-year follow-up study of the 'Yucheng' human cohort in Taiwan, when compared to their respective reference groups, diabetes was twice as prevalent in PCB-exposed females but not in males (Wang *et al.* 2008). Females also exhibited greater obesity than males in a children cohort study investigating prenatal PCB exposures (Tang-Peronard *et al.* 2014). Additionally, an epidemiologic study utilizing the National Health and Nutrition Examination Survey (NHANES 1999–2002) database demonstrated that PCBs were positively associated with hypertension in males and inversely correlated in females (Ha *et al.* 2009). Notably, a human cohort study assessing all-cause and cancer mortality rates among electrical capacitor manufacturing workers who were occupationally exposed to PCBs reported that males manifested more of a 'healthy worker effect' compared to females, while increased total cancer mortality was observed in female long-term workers (Ruder *et al.* 2014). Additionally, a meta-analysis of the 'Yucheng' and 'Yusho' human cohorts reported that females tended to have higher liver cancer mortality than males (Li *et al.* 2015). In concordance, sex-dependent effects were also reported for PCB-induced liver tumors in rodents exposed to different Aroclors or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), with female rats and mice displaying the higher number of liver tumors and mechanistically proposed to be estrogen-dependent (Mayes *et al.* 1998, Whysner & Wang 2001, Wyde *et al.* 2001, Brown *et al.* 2007). With respect to TASH, exposure to a mixture of 'dioxin-like' PCB126 and the commercially produced 'non-dioxin-like' PCB mixture, Aroclor1260, resulted in steatosis, glucose intolerance, and inflammation in C57BL/6 female mice compared to the male counterparts (Wahlang *et al.* 2019*b*). Strikingly, another mixture study investigating the effects of high-fat diet feeding and concurrent low-dose exposures to a pollutant cocktail of TCDD, PCB153, diethylhexyl phthalate (DEHP), and bisphenol A (BPA) from preconception to adulthood demonstrated that diet-induced obese adult female mice exposed to this pollutant mixture displayed signs of glucose intolerance, while male mice showed alterations in hepatic gene expression related to cholesterol metabolism (Naville *et al.* 2013). The study also indicated increased hepatic estrogen inactivation with exposure as a probable reason for the compromised hepatic insulin sensitivity observed in female mice. Enhanced hepatic ERα was also observed in ovariectomized mice with life-long exposure to this particular pollutant mixture, emphasizing the capacity of these chemicals to induce estrogenic effects in the liver (Julien *et al.* 2018).

Other sex-dependent PCB effects on liver endpoints include reported positive correlations between circulating levels of the dioxin-like PCB118 and the liver enzyme, alanine transaminase (ALT) which is frequently elevated in liver injury, only in females in a human study focusing on post-bariatric surgery evaluation (Rantakokko *et al.* 2015). In addition, an experimental study investigating dioxin-like PCB126 effects on hepatic energy metabolism *via* AHR demonstrated that while female rats were more vulnerable to PCB126-mediated disruption in fatty acid metabolism and lipid homeostasis, male rats were more susceptible to PCB126-mediated changes in glucose homeostasis (Eti *et al.* 2022). The susceptibility of males to impaired glucose homeostasis was also observed with dioxin-like PCB77 exposure in obese mice undergoing weight reduction (Jackson *et al.* 2019). Another potential aspect to consider with regards to sex differences is exposure doses to POPs and resultant body burdens. Sex-dependent evaluation of PCB serum levels in the Anniston Community Health Survey (ACHS) cohort, a residential population that was highly exposed to various POPs, revealed that in both the initial (ACHS-I) and follow-up (ACHS-II) studies, females manifested higher circulating PCB and OCP levels (Wahlang *et al.* 2019*d*). Interestingly, liver disease prevalence identified by elevated hepatocyte death markers, namely cytokeratin CK 18 M30 and M65 was reportedly higher in males in ACHS-I (Clair *et al.* 2018). Further studies specifically identifying sex-dependent associations between PCBs and liver disease biomarkers are needed for exposed populations including the ACHS cohort.

Organochlorine pesticides

Knowledge of oOCP exposures in the context of FLD is still evolving. Selected OCPs, relevant to concomitant environmental movements and initiatives, including the classic dichloro-diphenyl-trichloroethane and its metabolite dichloro-diphenyl-dichloroethylene (DDE), hexachlorobenzene (HCB), and chlordane, have been assessed for their contributions to human health complications, particularly as EDCs (Diamanti-Kandarakis *et al.* 2009, Silva *et al.* 2021). While OCPs constituted a majority of the Stockholm Convention's

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'dirty dozen' list, they are continually detected in human blood samples (Centers for Disease Control and Prevention 2018). There is evidence for associations between OCPs and TASH including elevated liver enzymes (Wahlang *et al.* 2020*a*, Sang *et al.* 2022), steatosis (Al-Eryani *et al.* 2015), and altered lipid metabolism (Ji *et al.* 2016, Liu *et al.* 2017) with limited insight on sex differences. Available evidence suggests that, similar to PCBs, females may be more susceptible to liver toxicity with OCP exposures. Indeed, prenatal DDE exposure in humans resulted in increased waist circumference only in females (Tang-Peronard *et al.* 2014), while beta-hexachlorocyclohexane (β-HCH) and the chlordane metabolite, trans-nonachlor, were associated with ALT elevation only in females after post-bariatric surgery, similar to PCB118 (Rantakokko *et al.* 2015). However, positive associations for β-HCH, DDE, and HCB with ALT and aspartate transaminase (AST) were slightly pronounced in males vs females in a residential Brazilian human population (Freire *et al.* 2015). Female rodents were more sensitive to HCB-induced hepatocarcinogenic toxicity that was independent of hepatic HCB concentrations (Smith *et al.* 1985), with one plausible mechanism being sex-specific alterations in intracellular communication reflected by depleted expression of connexins (Plante *et al.* 2002, Plante *et al.* 2007). Another study on chronic dietary exposure to currently used pesticides unrelated to typical OCPs demonstrated that while male mice were more susceptible to pesticide-modulated obesogenic and steatotic effects, CAR knockout female mice manifested higher pesticide toxicity, implicating the importance of CAR expression in female mice in adapting to toxicants that are CAR activators. (Lukowicz *et al.* 2018). Hepatic proteomic analysis of fetal livers from mice exposed to vinclozolin, a model anti-androgenic EDC and pesticide, demonstrated distinct protein signatures with males showing activated pathways related to oxidative damage while protective pathways were activated in females (Rister *et al.* 2021). The evidence on OCPs thus far reinforces the prominence of 'sex–gene–environment' interactions in defining toxicity parameters and outcomes between sexes.

Perfluoroalkyl substances

With respect to PFAS, one of the introductory studies associating this class of contaminants to FLD was reported in sub-populations of the culturally significant C8 Health Project which continues to be one of the largest recruitments in epidemiological studies to date and consisted of participants that were exposed to different PFAS chemicals, primarily perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). The study emphasized the sexually dimorphic PFAS effects on adipocytokines pertinent to TAFLD with positive associations between certain PFAS and pro-inflammatory cytokines (IL-8, IFNγ, adiponectin) observed only in females and other biomarkers (C3a, leptin) only in males (Bassler *et al.* 2019). Sex-dependent PFAS associations with liver enzymes appeared to be age-related, with positive associations reported between PFAS and ALT in adolescent females but not males (Attanasio 2019) and the absence of sex-specific associations in adult populations (Darrow *et al.* 2016). Sex differences in hepatic toxicity indices also appear to be chemical-, species-, and strain-dependent with male Sprague–Dawley rats showing higher sensitivity to PFOS-mediated hepatic necrosis, steatosis and elevated ALT (Seacat *et al.* 2003, Kim *et al.* 2011, Butenhoff *et al.* 2012*a*, Butenhoff *et al.* 2012*b*, Bagley *et al.* 2017) and female C57BL/6 mice being more responsive to PFOA-mediated amelioration of diet-induced hypercholesterolemia (Rebholz *et al.* 2016, Schlezinger *et al.* 2020). Female mice exposed to PFAS mixtures also demonstrated greater susceptibility to liver inflammation and injury than males (Roth *et al.* 2021). Similarly, prenatal exposure to the PFOA replacement chemical, GenX (hexafluoropropylene oxide-dimer acid), resulted in liver damage (necrosis) in female mice but metabolic derangement in males (Cope *et al.* 2021). Some explanations for these sex-specific hepatic PFAS effects include sex-dependent PFAS metabolism, elimination and tissue distribution (Khazaee *et al.* 2020, Huang *et al.* 2021, Costello *et al.* 2022), PFAS interactions with ERα (Bassler *et al.* 2019), and sexually dimorphic expression of key PFAS liver targets (Schlezinger *et al.* 2020, Roth *et al.* 2021).

Other persistent organic pollutants

Evaluations on sex-dependent TAFLD endpoints for additional POPs like brominated flame retardants are still pending with minimal studies looking at hepatic endpoints (National Toxicology Program 1993, Kling *et al.* 2008). Although prenatal exposure to an EDC mixture of POPs that also contain polybrominated diphenyl ethers (PBDEs) showed no sex-specific associations with CK18 in a children cohort study (Midya *et al.* 2022), the current lack of evidence for these chemicals, however, requires further investigations. Other organic pollutants including BPA and phthalates are often classified as POPs. Although these chemicals have short half-lives and are technically not persistent, they are considered

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so due to their ubiquitous and constant presence in the environment (Warner & Flaws 2018). With BPA being a classic EDC, sex differences with *in-utero* BPA exposures were observed in mice including increased hepatic ERα and ERβ expression in ovariectomized female offspring after estradiol treatment, but contrasting effects in male offspring, and consequential impact on glucose and lipid metabolism at adulthood was also observed in both sexes (Ilagan *et al.* 2017). Exposures to DEHP have also been associated with TAFLD endpoints in human studies, with one cross-sectional study demonstrating higher urinary DEHP metabolite concentrations strongly correlating with the metabolic syndrome, NAFLD-related comorbidity, in postmenopausal females (Shih *et al.* 2022). Perinatal exposures to DEHP and two other phthalates, namely diisononyl phthalate and dibutyl phthalate, in food led to increased liver weights only in female mice, possibly due to phthalate-mediated PPARα activation (Neier *et al.* 2019). Sex-specific liver toxicity from exposures to other established EDCs and MDCs such as tributyltin, an organo-metallic biocide, and tolyfluanid, a fungicide, have also been reported. Tributyltin exposure in C57BL/6 mice from preconception to lactation stages led to liver tumor development only in male offspring but not females, with GH signaling proposed as a mechanism driving these differences (Katz *et al.* 2020). Likewise, male offspring showed impaired hepatic energy metabolism including attenuated glucose tolerance from maternal exposures to tolyfluanid, a glucocorticoid receptor agonist, in C57BL/6 mice (Ruiz *et al.* 2019).

Sex-specific mechanistic evidence for POPs' contribution to TAFLD

Generally, NAFLD progression and its severe outcomes including hepatocellular carcinoma development are reportedly more prevalent in male patients, in part due to the pro-fibrotic and carcinogenic-promoting effects of androgens and the protective role of estrogens (Grossmann *et al.* 2019). The global prevalence of diabetes is also higher in males, especially in middle-aged populations, with females showing higher insulin sensitivity (Tramunt *et al.* 2020). However, with regards to TAFLD and toxicant-associated metabolic disorders, the epidemiologic data and experimental studies suggested deviation from these norms. It can be speculated from the evidence thus far, that while females may be more susceptible to PCB and OCP-associated hepatic inflammation and metabolic toxicity, particularly with short-term exposures, males demonstrated other facets of toxicity such as oxidative stress, insulin resistance, and obesogenic effects. One plausible mechanism for these observed sex differences is the ability of POPs, which behave both as EDCs and MDCs, to re-define hepatic identity and function with exposure, partially through the GH and ER signaling pathways. GH signaling through the Janus kinase 2 (JAK2)-signal transducer and activator of transcription (STAT5) pathway is critical in the maintenance of normal liver physiology (Cui *et al.* 2007) while also playing a decisive role in dictating hepatic sex-specific gene expression (Choi & Waxman 2000). Altered GH-JAK2-STAT5 signaling has been associated with metabolic disorders including steatosis and implicated in hepatocellular carcinoma development (Cui *et al.* 2007, Le Magueresse-Battistoni 2021, Haque *et al.* 2022). POPs such as TCDD have been shown to disrupt the GH-JAK2-STAT5 signaling and inhibit sex-biased STAT5B-dependent repressors such as CUX2 leading to masculinization of female livers while male livers appeared more feminized (Nault *et al.* 2017). Metabolic feminization of the male liver was also observed with BPA exposure in adult zebrafish (Sun *et al.* 2021). In addition, PCBs and TCDD have been demonstrated to downregulate transcription factors that co-regulate sexually dimorphic gene expression along with STATB5 including the male-biased activator – hepatocyte nuclear factor 4 alpha (HNF4α) in mouse liver (Wahlang *et al.* 2019*b*, Cholico *et al.* 2022), while female-biased activators such as HNF3α (FOXA1) was increased by a non-dioxin-like PCB mixture in an *in-vitro* model (Fortunati *et al.* 2017).

Sex-specific mechanisms driving the increased susceptibility for PCB-mediated liver injury in females exposed to a mixture of dioxin-like and non-dioxin-like PCBs included enhanced hepatic and systemic inflammatory responses and strikingly, the pro-androgenic and anti-estrogenic effects of this particular PCB mixture in females (Wahlang *et al.* 2019*b*). These proposed mechanisms also highlighted the role of gonadal or sex hormones in modulating hepatic lipid metabolism, insulin resistance, and inflammation. Indeed, estrogen regulates energy homeostasis by potentiating insulin secretion, attenuating hepatic steatosis, and regulating adipocyte differentiation (Mimoto *et al.* 2017, Palmisano *et al.* 2017, Meda *et al.* 2020) while also combatting oxidative damage and ROS production in an FLD setting (Besse-Patin *et al.* 2017, Galmes-Pascual *et al.* 2020). Moreover, estrogen has been implicated to interact with PPARs to mediate immune regulation (Park & Choi 2017), and ER activity can either enhance or dampen pro-inflammatory cytokine production (Kovats 2015, Moulton 2018).

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Further, ERα, which is also the major estrogen receptor in the liver exerts sex-specific regulation on hepatic energy metabolism during different life stages. Hepatic ERα expression is higher in male mice during the neonatal stage and is important for masculinizing liver function in adulthood; however, in adulthood, female mice display higher ERα levels (Della Torre *et al.* 2018). In adult female mice, ERα plays a central role in the maintenance of lipid homeostasis and energy storage, in part, to prepare for reproductive requirements (Mauvais-Jarvis 2015). PCBs appear to interfere with physiological ERα signaling and lipid homeostasis, by upregulating fatty acid translocase (Cd36), an AHR target, and suppressing ERα expression, thereby enhancing lipid uptake to the liver and increasing susceptibility to steatosis in females (Wahlang *et al.* 2019*b*).

In contrast to female mice that favor enhance lipogenesis as an evolutionary strategy to prepare for reproductive needs, male mice appear to utilize carbohydrates as a fuel source for dispensing energy and muscle activity (Mauvais-Jarvis 2015). Thus, in adult male mice, hepatic ERα regulates gluconeogenesis, in addition to lipid homeostasis, and its function appears to be co-dependent on AR expression (Qiu *et al.* 2017). Interestingly, enhanced estrogenic effects in males also led to beneficial effects in white adipose tissue such as decreased inflammation, a phenomenon which was absent in females (Jiang *et al.* 2014). ERs also play a role in cholesterol and BA metabolism and activated ERα can impact both BA synthesis and BA pool composition, in part, by downregulating Cyp7a1, the cholesterol metabolizing enzyme (Phelps *et al.* 2019). Exposure to PFOA, an ERα and PPARα agonist, in conjunction with a high-caloric diet suppressed Cyp7a1 expression which was more pronounced in humanized PPARα female mice than their male counterparts (Schlezinger *et al.* 2020). Overall, these basal differences in energy expenditure, coupled with distinct ERα functions in males and females, partially aid in explaining the differences in metabolic toxicity observed with exposure to POPs.

Sexual dimorphism in hepatic receptor expression including xenobiotic and endobiotic receptors and their target genes has also been illustrated in *in-vitro* and *in-vivo* models (Jalouli *et al.* 2003, Waxman & Holloway 2009, Lau-Corona *et al.* 2022) and could be another sex-specific pathway driving hepato-metabolic toxicity. The reported sex-specific mechanistic studies also underscore the role of hepatic receptor–receptor interactions or the interactome as a sex-specific mechanism. For example, the AHR and ER have a complex but mutual relationship including interactions between their signaling pathways, transcriptional interference, inhibitory crosstalk, and AHR-induced proteasomal degradation of ER (Tarnow *et al.* 2019); this AHR/ER crosstalk may partially explain the anti-estrogenic effects observed for selected PCB congeners. CAR–ER interaction is another critical mechanism that requires further examination when assessing sex-specific liver toxicity and TAFLD outcomes. CAR can also inhibit ER-mediated signaling through coactivator sequestration and influence lipid metabolism (Min *et al.* 2002). Therefore, it can be postulated that a cocktail of POPs that can activate both the AHR and CAR can dampen ER expression and activation. Mechanistically, CAR activation by both PCBs and OCPs has been correlated with EGFR inhibition and downstream signaling disruption (Hardesty *et al.* 2018). EGFR is critical in hepatocyte regeneration and repair mechanisms, and unsurprisingly, sex differences in hepatic EGFR expression and its gene network have been observed in mice (Wang *et al.* 2016, Wahlang *et al.* 2019*b*). However, little is still known about how this CAR–EGFR pathway could be a sex-specific mechanism leading to TAFLD. Perhaps future studies investigating the beneficial effects of EGFR supplementation with toxicant exposures could shed more light on this topic.

In addition to altered sex hormones, another component that may drive these sex-dependent PCB effects could be the nature of POPs bioaccumulation patterns and pharmacokinetics. One study looking at tissue distribution with Aroclor 1254 exposure noted that male and female mice displayed different congener accumulations in the liver (Li *et al.* 2020*b*). This could be due to basal differences in the hepatic expression of cytochrome P450s implicated in PCB metabolism including Cyp2b10, Cyp1a2, Cyp3a11, and sex-dependent hepatic fat content (Wahlang *et al.* 2019*b*, Li *et al.* 2020*b*). For chemicals such as PFAS that rely on transporters (e.g. BA transporters such as Na+/taurocholate co-transporting polypeptide, organic anion transporting polypeptides) for reuptake into the liver (Fragki *et al.* 2021), sex-specific transporter expression, and sex hormone regulation of transporter expression may also influence PFAS concentration in the liver in females and subsequent hepato-toxicity. Moreover, sex differences in PFAS metabolism and elimination rate have been noted with rats eliminating PFOA and perfluorononanoic acid (PFNA) at significantly higher rates than males (Fenton *et al.* 2021), although these differences may be more subtle in humans. The implications of sex-specific chemical pharmacokinetics on hepatic and metabolic toxicity pertinent to human exposures warrant further investigations.

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Sex differences in TAFLD associated with VOC exposures

Increasingly recognized as major drivers of air pollution, this broad category of organic compounds constitutes a plethora of industrial chemicals such as trichloroethylene, vinyl chloride, and styrene known to be widely used in the rubber manufacturing industry; industrial solvents including toluene, benzene, and xylene; chemicals emitted from petroleum and exhaust fumes; components of tobacco and cigarette smoke including acrolein and crotonaldehyde, and multiple household and cosmetic products, thus reflecting multiple sources of exposures. Studies focused on sex differences in TAFLD related to VOC exposures are highlighted in Table 2. Low-dose VC inhalation, in conjunction with high-fat diet feeding, caused sex-dependent hepatic injury with male mice demonstrating higher susceptibility to vinyl chloride-mediated inflammation, apoptosis, and metabolic disruption (Wahlang *et al.* 2020*b*). Postulated factors driving these differences included sex-dependent vinyl chloride metabolism into reactive metabolites, protective estrogenic activity in females, and differential inhalational capacity (Wahlang *et al.* 2020*b*). In contrast, based on epidemiologic findings, females exhibited a greater risk for hepatotoxicity with benzene exposure, in part due to higher rates of benzene metabolization (Poli *et al.* 2022). Interestingly, prenatal, and adult benzene exposure resulted in insulin resistance and metabolic disruption only in male mice suggesting species differences in sex-dependent benzene effects (Debarba *et al.* 2020, Koshko *et al.* 2021). Consistently, a human study investigating blood VOC levels and liver disease markers in the general Canadian population demonstrated significant associations for benzene and total xylenes with circulating liver enzyme, namely gamma-glutamyl transferase (GGT), only in females (Cakmak *et al.* 2020). Likewise, another human study investigating the role of biological sex on associations between urinary VOC metabolites and circulating liver disease biomarkers reported significant associations between VOCs including crotonaldehyde, acrylamide, and acrylonitrile with alkaline phosphatase (ALP) only in females with associations being more pronounced in female smokers (Wahlang *et al.* 2023). Exposure to trichloroethylene in immunocompromised mice resulted in significant hepatic inflammation in female mice (Blossom *et al.* 2020) while female rats manifested greater immunological alterations than males when exposed to an environmentally relevant mixture of VOCs and heavy metals (Azeez *et al.* 2015). Furthermore, exposure to ambient particulate matter ($PM_{2.5}$) impacted TAFLD endpoints in a sex-dependent manner with female mice displaying greater vulnerability to insulin resistance and steatosis, partially through enhanced lipid transport, inhibition of glucocorticoid levels, and disruption of the hypothalamus–pituitary–adrenal axis (Li *et al.* 2020*c*). Overall, sex differences in VOC-mediated TAFLD appears to be dictated by the chemical type, modifiable risk factors including hypercaloric diets and immune-status, as well as differences in VOC metabolism and subsequent urinary metabolite levels which are oftentimes measured as surrogates for VOC body burdens.

Sex-specific mechanistic evidence for VOCs' contribution to TAFLD

The sex-specific studies on VOCs reiterate how sex-specific metabolism of the parent compound to reactive metabolites could be a mechanistic pathway driving sex-dependent hepatotoxicity for VOCs. The amount of reactive metabolites produced can dictate the extent of carbonyl stress induction on cell organelles which subsequently impacts mitochondrial damage, oxidative stress, energy dysregulation, and increase in circulating liver enzymes as an indication of liver injury. More recent studies have also demonstrated the ability of VOCs to interfere with normal endocrine hormone physiology and VOC interaction with sex hormone receptors (Kassotis *et al.* 2018), which may contribute to endocrine disruption and potential non-monotonic dose responses. Indeed, estrogenic activity was postulated to be a protective mechanism against vinyl chloride-exposed female mice exhibiting diet-induced obesity (Wahlang *et al.* 2020*b*). In addition, benzene, similar to POPs, can alter GH signaling and interfere with downstream GH-related transcriptional activity (Fortunati *et al.* 2017) in an AHR-dependent manner in rat GH-producing pituitary adenoma cells, although the implications of this mechanism on sex-specific liver injury is still not fully understood. Because oxidative stress is a key toxicity endpoint with most VOC exposures, sex differences in response to chemicals that act as oxidative stress inducers should be noted. Indeed, GH interactions with nuclear factor erythroid 2-related factor 2 (NRF2) were demonstrated in mice livers where female mice exhibited higher NRF2 activation while NRF2 activation was attenuated in male livers through GH-STAT5b suppressive actions (Rooney *et al.* 2018). This has been proposed as a plausible mechanism to help explain the enhanced resistance that female mice manifest against chemical-induced oxidative stress. Sex differences in

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Table 2 Selected volatile organic compounds (VOCs) and metals associated with sex-dependent fatty liver disease and related TASH endpoints that were primarily reported.

Chemical/chemical group	Experimental exposure models	Sex-specific toxicity		Epidemiology studies/ toxicological evidence	Sex-specific associations	
		Females	Males		Females	Males
Benzene and xylene	Insulin resistance and metabolic disruption: Sub-acute adulthood or prenatal exposure to benzene in C57BL/6 mice (Debarba *et al.* 2020, Koshko *et al.* 2021)	No metabolic disruption observed with adult exposure and less induction of Cyp2e1 implicated in benzene metabolism; prenatal exposure led to altered hepatic gene expression correlating with inflammation and ER stress in offspring	Insulin resistance and metabolic disruption with adult exposure; prenatal exposure led to severe insulin resistance in offspring, along with hepatic gene expression correlating with inflammation and ER stress	Hepatotoxicity: Scoping review on benzene (Poli *et al.* 2022) Elevated liver enzymes: Cross-sectional analysis on benzene, total xylene, and liver injury in the general Canadian population (Cakmak *et al.* 2020)	Greater risk for hepatotoxicity with benzene exposure and higher rates of benzene metabolization; positive associations for benzene and total xylenes with circulating GGT	Lesser risk for benzene hepatoxicity, no associations with circulating GGT
Other VOCs	TASH, metabolic & endocrine disruption: Sub-chronic exposure to vinyl chloride in diet-induced obese C57BL/6 mice (Wahlang *et al.* 2020*b*)	Decreased susceptibility to diet-induced steatosis, inflammation, and ER stress	Enhanced steatosis and hepatic inflammation, injury, and ER stress	Elevated liver enzymes: Cross-sectiona anlyssi of urinary VOC metabolites and liver injury markers in a residential US population (Wahlang *et al.* 2023)	Positive associations for metabolites of acrolein, acrylamide, acrylonitrile, butadiene, crotonaldehyde, and styrene with ALP	No positive associations with ALP were noted
	Insulin resistance and metabolic disruption : Sub-acute exposure to $PM_{2.5}$ in C57BL/6 mice (Li *et al.* 2020*a*); developmental trichlorethylene exposure in $MRL^{+/+}$ mice (Blossom *et al.* 2020)	Insulin resistance and steatosis with $PM_{2.5}$ exposure; higher liver biomarker levels related to regeneration and repair with trichlorethylene exposure	No significant changes in hepatic lipid profile with $PM_{2.5}$ exposure; less affected by trichlorethylene exposure			
Cadmium	Liver tumors: Sub-chronic and chronic exposures to cadmium in diet in hepatitis B virus expressing transgenic mice (Sell & Ilic 1994)	No difference in tumor incidence with low or high cadmium exposures. Low-cadmium-fed mice had well-differentiated tumors	No difference in tumor incidence with low or high cadmium exposures. High-cadmium-fed mice had poorly differentiated liver tumors	Steatotic and fibrotic indices: Cross-sectional analysis in Korean NHANES participants (Chung *et al.* 2020)	Hepatic steatosis index and fibrosis-4 index positively correlated with blood cadmium levels, and blood cadmium increased the risk for liver fibrosis	Only fibrosis-4 index was positively correlated with blood cadmium levels

(Continued)

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Table 2 Continued.

Chemical/chemical group	Experimental exposure models	Sex-specific toxicity		Epidemiology studies/ toxicological evidence	Sex-specific associations	
		Females	Males		Females	Males
	Mortality and liver enzymes: Acute cadmium exposure at different doses to F344 rats (Shimada *et al.* 2012)	Lethality to high-dose cadmium (0% survival rate) and increased ALT with low-dose cadmium in adults. Less lethality in pre-pubertal and ovariectomized rats	Moderate lethality with high-dose cadmium (40% survival rate) in adults	Elevated liver enzymes: Cadmium exposure and liver disease in US NHANES participants (Hyder *et al.* 2013)	Cadmium exposure associated only with hepatic necroinflammation	Cadmium exposure associated with hepatic necroinflammation, NAFLD, and NASH
	Prenatal exposure and metabolic disruption: Gestational exposure to cadmium in CD-1 mice (Jackson *et al.* 2020, 2022)	Impaired glucose tolerance, increased circulating triglycerides, steatosis, and adiposity in adult offspring; decreased fetal hepatic metallothionein	Impaired glucose tolerance in adult offspring			
Mercury, lead, and arsenic	Hepatotoxicity: Acute exposure to mercury in Wistar rats (Hazelhoff & Torres 2018)	Higher mercury-induced hepatotoxicity included elevated liver enzymes and fibrosis (histopathology) and higher liver mercury content	Less liver histopathological changes, and lower liver mercury content attributed to decreased transporter (Oat3) abundance	Steatotic and fibrotic indices: Cross-sectional analysis in Korean NHANES participants (Chung *et al.* 2020)	Hepatic steatosis index and fibrosis-4 index positively correlated with blood lead and blood mercury levels	Only blood mercury levels were associated with hepatic steatosis index
	Epigenetics: Sub-chronic exposure to arsenic in C57BL/6 mice or gestational lead exposure in *a/a* dams (Nohara *et al.* 2011, Svoboda *et al.* 2021)	Arsenic plus methyl-deficient diet increased liver global DNA methylation; peri-natal lead exposure led to sex-specific differentially methylated cytosines in liver of adult mice	Arsenic plus methyl-deficient diet reduced liver global DNA methylation; peri-natal lead exposure led to sex-specific differentially methylated cytosines in liver of adult mice			
	Liver proteomics analysis: Acute arsenic exposure in zebrafish (Carlson *et al.* 2013)	Decreased hepatic lipid accumulation and uptake pathways but increased cell growth	Activation of hepatic fibrosis and liver transport pathways			

immune responses have also been established with females tending to be more immune-responsive (Klein & Flanagan 2016), and this could potentially impact VOC modulation on inflammatory and injury endpoints. Due to their more robust immune response, females tend to suffer from auto-immune diseases and inflammatory disorders including auto-immune liver diseases at a greater proportion than males (Whitacre 2001, Schwinge & Schramm 2019). This could also impact the sex-specific toxicity with chemicals including VOCs, and even PFAS that are known to be immune-suppressive (Fenton *et al.* 2021). The sex-specific epidemiologic studies on VOCs and liver injury markers (Cakmak *et al.* 2020, Wahlang *et al.* 2023) call to attention the marked associations between selected VOCs with GGT and ALP, liver enzymes routinely utilized as diagnostic markers for cholestatic liver disease and bile duct obstruction (Lala *et al.* 2022). The study findings strongly suggest that females may be more prone to VOC-mediated liver effects related to cholestasis and bile duct injury including primary biliary cholangitis (PBC). Indeed, PBC, a chronic auto-immune disease is more prevalent in the female sub-population with approximately 90% of PBC patients being either females or females of postmenopausal age (Hirschfield *et al.* 2018) and highlights the estrogenic modulation on immune response across different life stages. Furthermore, glucocorticoid signaling is yet another important aspect that needs consideration when assessing sex-specific hepatic and metabolic toxicity. PM exposure was shown to alter glucocorticoid levels through disruption of the hypothalamus–pituitary–adrenal axis (Li *et al.* 2020*a*). Glucocorticoids play a significant role in metabolic programming of the liver and adipose tissue, and early life exposures could lead to sex-dependent metabolic perturbations in adulthood. Lastly, because VOC exposures in humans are primarily through inhalation, sex differences in inhalation capacity are also worth accounting for when assessing VOC body burdens and toxicity. Male C57BL/6 mice reportedly have a higher breathing frequency than corresponding female mice and may influence toxicological studies assessing VOC inhalational exposures. Further, considerable data have also shown sex differences in murine airway responsiveness to inhalation agents (Card *et al.* 2006, Carey *et al.* 2007, Matsubara *et al.* 2008, Greising *et al.* 2015).

Sex differences in TAFLD associated with metal exposures

Exposures to metal toxicants, particularly cadmium, lead, mercury, and arsenic, have been associated with liver disease prevalence (Nguyen & Kim 2022). Sex-specific associations pertaining to TAFLD endpoints have also been noted (Table 2). Studies analyzing the Korean NHANES (2016–2017) database reported positive correlations between blood cadmium levels and fibrosis only in females and pronounced associations for cadmium, mercury, and lead with steatosis in females vs males (Chung *et al.* 2020). In contrast, a study analyzing the US NHANES (1988–1994) database reported slightly stronger associations between cadmium and NAFLD/NASH endpoints in males (Hyder *et al.* 2013). These counterintuitive findings may be somewhat influenced by differences in (i) mean population age for both sexes, given that blood cadmium levels positively correlate with age, although levels appeared to be higher in females than males (Yamanobe *et al.* 2015, Park *et al.* 2021) and (ii) menopausal status in females given that both progesterone and estrogen can modulate cadmium-mediated toxicity *via* metallothionein expression and metal-complex formation (Sogawa *et al.* 2001, Shimada *et al.* 2012). Prenatal cadmium exposure resulted in compromised metabolic function including insulin insensitivity, dyslipidemia, and steatosis only in female offspring, underscoring its sex-specific delayed metabolic effects (Jackson *et al.* 2020). Similarly, female rats revealed greater susceptibility to mercury-mediated hepatotoxicity than males and higher hepatic mercury accumulation (Hazelhoff and Torres 2018). Moreover, perinatal exposure to lead resulted in sex-specific hepatic epigenetic alterations relevant to lead-mediated metabolic disturbances in adult mice (Svoboda *et al.* 2021). Likewise, dietary arsenic intake in mice led to sex-specific modifications of hepatic global DNA methylation (Nohara *et al.* 2011). Further, short-term arsenic exposure in a zebrafish model also led to distinct hepatic proteomes for both sexes with males portraying activated pathways related to fibrosis while pathways in females were related to hepatic lipid accumulation (Carlson *et al.* 2013).

Sex-specific mechanistic evidence for metals in TAFLD

Mechanisms attributed to the sex-dependent observations in metal hepatotoxicity included sex differences in metal retention and metabolism (Hirayama & Yasutake 1986, Thomas *et al.* 1987, Muhetaer *et al.* 2022). For instance, male rats are reportedly more sensitive to liver damage from arsenic exposures due to higher levels of more toxic arsenic metabolites including monomethylarsonic acid (MMA); however, female rats displayed lower MMA levels but higher levels of dimethylarsinic acid (DMA) in the liver (Muhetaer *et al.* 2022). Human studies have also

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reported higher urinary DMA excretion in females vs males (Jansen *et al.* 2016, Torres-Sanchez *et al.* 2016), and the role of estrogens has been credited for the enhanced arsenic metabolism and clearance in females (Tseng 2009). Age is another factor that could define sex-specific toxicity with metal exposures as it impacts bone redistribution, particularly for females, and subsequent metal blood and tissue levels (Vahter *et al.* 2007). Heavy metals often accumulate in bone tissue while also exerting direct toxicity to it, leading to compromised bone health such as osteoporosis (Rodriguez & Mandalunis 2018). As a dynamic tissue, bone undergoes continuous remodeling through different life stages, with estrogens playing a central role. Considerable bone loss after menopause could potentially lead to increased circulating metal concentrations in females of advanced age compared to males. Similar to PFAS that appears to be transporter-dependent for hepatic bioaccumulation, metals also rely on metal ion transporters to mobilize in the liver. Increased expression of metal transporters has been attributed to the increased cadmium (Jackson *et al.* 2022) and mercury (Hazelhoff & Torres 2018) accumulation in female livers. This sex-specific increase in hepatic metal concentrations in female livers consequently led to differences in lipid peroxidation and ROS elimination from exposures to cadmium (Sato & Nagai 1986) and lead (Sobekova *et al.* 2009).

The evidence thus far also underlines the appreciable role of sex hormones in mediating metal toxicity during both developmental growth and adulthood (Sogawa *et al.* 2001, Shimada *et al.* 2012, Yao *et al.* 2019). As with other toxicant classes previously discussed, metals including cadmium, arsenic, and mercury also have the capability to act as EDCs (Haverinen *et al.* 2021). For example, cadmium has been shown to elicit both estrogenic-like and androgenic-like effects *in-vitro* through its ability to form a high-affinity complex with the hormone-binding domain of ER (Wilson *et al.* 2004), as well as its high binding affinity to the AR (Martin *et al.* 2002). Cadmium was also shown to interact with AHR and mediate AHR–ER crosstalk in rat uterus (Kluxen *et al.* 2012). Given that both ER and AR at early life are crucial for liver and metabolic programming, *in-utero* exposure to cadmium in male and female offspring could impact liver and metabolic health in adulthood in distinct ways. Other metals such as mercury and arsenic interfere with pancreatic beta cell function to induce perturbations in insulin production and glucose metabolism (Mimoto *et al.* 2017). Based on known sex differences in basal glucose metabolism and the role of estrogen in enhancing insulin sensitivity, exposures to these metals are bound to exert sex-specific changes in insulin resistance and energy metabolism. Sex hormone regulation of metallothionein, a key protein in the maintenance of metal homeostasis, protection against ROS and buffering heavy metal toxicity, provides further mechanistic evidence for sex-specific metal hepatotoxicity. Both progesterone and 17β-estradiol inhibited cadmium-induced metallothionein expression in ovariectomized mice (Sogawa *et al.* 2001), and increased progesterone has been acknowledged as a factor driving cadmium hepatotoxicity in female rats (Shimada *et al.* 2012), albeit opposite effects observed in male rats (Alese *et al.* 2021). Intriguingly, metallothionein regulated sex-specific diet-induced obesity development by promoting protective estrogenic effects against fat accumulation only in female mice, while augmenting androgenic effects on adipogenesis in male mice (Kawakami *et al.* 2019), and this could be another potential pathway for sex-specific metabolic toxicity with metal exposures. Lastly, sex differences in skin permeability and function are worth mentioning when assessing metal exposures, levels, and toxicity. Environmental exposures to metals including nickel and cadmium can occur through dermal contact, with nickel being the most common cause of contact allergy (Vahter *et al.* 2007). Sex differences in skin properties and function (Dabrowska *et al.* 2018) can influence the amount of metal entering the body through dermal exposures.

Implications of sex differences in TAFLD, emerging concepts and future directions

The broad implications from the sex-specific TAFLD findings thus far (Tables 1 and 2) greater risk for steatosis, inflammation, and liver injury in females, while males appeared more susceptible to insulin resistance, although the exact toxicity endpoints may vary with the chemical type. The findings also implicated the role of hormones at varying life stages and how aging, together with sex, can dictate these TAFLD outcomes, particularly with increased susceptibility to chemicals observed in postmenopausal females (Tables 1 and 2). In addition, because humans are exposed to a multitude of chemicals over their lifetime, sex-specific TAFLD risks may be different when assessing the effects of pollutant mixtures. While mixture studies maybe more complex with the added investigation of synergism or antagonism of chemical target genes/proteins and other toxicity indices, studies on environmentally relevant mixtures are crucial since they mimic human exposure paradigms.

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The greater majority of sex-dependent studies that have been discussed introduced or projected plausible mechanisms to explain the observable heterogeneity in both exposure biomarker levels and liver disease endpoints, based on sex, and validated the significance and relevance of sex-dependent evaluation in toxicology. Most of these mechanisms centered around inherent hepatic sexual dimorphism regulating the degree of toxicity and chemical disposition in target organs, in addition to the endocrine-disruption status of the chemical. However, other aspects including multi-organ crosstalk and hepatic condition post exposure influence liver susceptibility to other 'hits' or risk modifiers including lifestyle factors such as smoking, alcohol consumption and high caloric diets. Such aspects necessitate consideration when delving into sex-specific disease mechanisms. The 'adipose–liver' axis requires scrutinization, especially for chemicals known to bioaccumulate in fatty tissues and have the ability to elicit adipo-toxicity. Importantly, adipose tissue dysfunction can contribute to hepatic steatosis given that disconnectedness between the adipose and liver can disrupt lipid homeostasis and overall energy metabolism (Duwaerts & Maher 2019). Moreover, sexual dimorphism in adipose gene expression and adipokine secretion has been established for both humans and rodents (Karastergiou & Fried 2017, Anderson *et al.* 2020, Gavin & Bessesen 2020), and thus the adipose–liver axis could be another sex-specific mechanistic pathway in TAFLD. Another holistic approach to understand the comprehensive impact of EDCs/MDCs in hepatic and metabolic health is the assessment of the 'endocrine–gut–liver' axis which is the tri-directional relationship between the gut and its microbiota, the liver, and endocrine hormones. This complex circuit is facilitated by receptor–receptor interactions, gut bacterial translocation, bacterial metabolites, and their role in energy metabolism and molecular signaling pathways. Environmental chemicals have the ability to disrupt one or all components of the axis yielding metabolic outcomes that may be sex-dependent including metabolic-dysfunction associated FLD. Sex-dependent changes in the gut microbiome and induction of gut dysbiosis with toxicant exposures have also been acknowledged (Chi *et al.* 2018, Lim *et al.* 2021), although not fully understood. Specifically, gut microbiome composition and activity closely interact with the host circadian clock for normal function and physiology (Weger *et al.* 2019). GH signaling has been postulated to play an influential role in regulating these circadian rhythm–gut microbiota interactions (Weger *et al.* 2019), and thus it is conceivable that sexual dimorphism will exist. Importantly, toxicant exposures including TCDD and ambient PM are known to interfere with circadian regulation of hepatic energy metabolism in experimental models (Fader *et al.* 2019, Li *et al.* 2020*a*). Future research delving into this mechanistic pathway to understand sex-specific toxicant-associated gut–liver axis disruption, and how gut microbiota can be manipulated for sex-specific intervention and therapeutic strategies is needed.

Additional avenues of mechanistic evaluations in sex-dependent TAFLD endpoints include chemical-induced alterations in epigenomics and epitranscriptomics. Utilization of high-throughput Omics techniques and adopting multi-Omics platforms can better assist in investigations corresponding to said holistic approaches such as hepatic receptor–sex hormone interactions, organ-–organ crosstalk, and host–microbe communication related to metabolism and inflammation (Hu *et al.* 2021). Such high-throughput, big data approaches can also help recognize molecular initiating events related to adverse outcomes and other elements of the adverse outcome pathways (AOP). Moreover, translational research centered around overcoming sex-specific species differences and employing exposomics in designing exposure models for sex differences evaluation need development and refinement to better translate toxicological findings from experimental research to human population studies. Other emerging aspects of sex-dependent effects in TAFLD include exposure timing and contributions of early life exposures to FLD development in adulthood. Clearly, experimental models have strongly implicated that pre- and peri-natal chemical exposures led to sex-specific disease outcomes in offspring (Tables 1 and 2), suggesting that developmental origins of health and disease are also sex-dependent. Furthermore, while sex differences pertinent to health implications from transgenerational and multi-generational exposures have been reported (Chamorro-Garcia *et al.* 2013, Katz *et al.* 2020, Chamorro-Garcia *et al.* 2021), they are currently understudied and warrant future investigation. Areas for research development also include understanding how biological sex shape interactions between genetic susceptibility and environmental exposures and subsequent implications on FLD and other liver diseases, and sex-specific mechanisms in liver regeneration and repair. Last, but not least, because gender traits and characteristics can also modulate and impact chemical exposures and disease outcomes (Clougherty 2010), integration of sex and gender studies in environmental toxicology and environmental health is essential to comprehend how biological factors intersect with the socio-cultural factors in human health and

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disease outcomes. This may be somewhat challenging given that sex differences are scientifically measurable, while gender is a constantly evolving societal concept that is difficult to measure. Yet, both sex and gender considerations, geared toward achieving inclusivity, are critical in the environmental health sciences and call for multi-disciplinary research platforms to address such complex knowledge gaps.

Summary and conclusions

There is a growing realization and concern that sex differences and women's health are under-represented, particularly in basic science research. The current review highlighted the significance of sex differences in liver toxicology and discussed available sex-dependent studies pertinent to chemical classes that have demonstrated associations with TAFLD, besides identifying potential underlying mechanisms driving these differences (Fig. 1). Thus far, studies have distinguished sex hormones and other endocrine factors as mediators of sex-dependent hepatic effects and emphasized the liver's characteristic as a sexually dimorphic organ contributing to these differences. Current observations and findings suggest that sex differences in TAFLD are governed by the chemical type, as well as the dose, timing, duration, and route of exposure. Moreover, the sex-specific mechanisms and disease progression in TAFLD may not necessarily align with sex-specific NAFLD norms. While the review deliberates on what is currently known and reaffirms sex as a biological factor that can mediate chemical toxicity, it also calls attention to further research on other sex-dependent facets of TAFLD that are still unexplored. Importantly, globalization and industrialization have led to a continuous and rapidly expanding list of environmental toxicants. Although policy changes have lessened the threat of these chemicals to public health

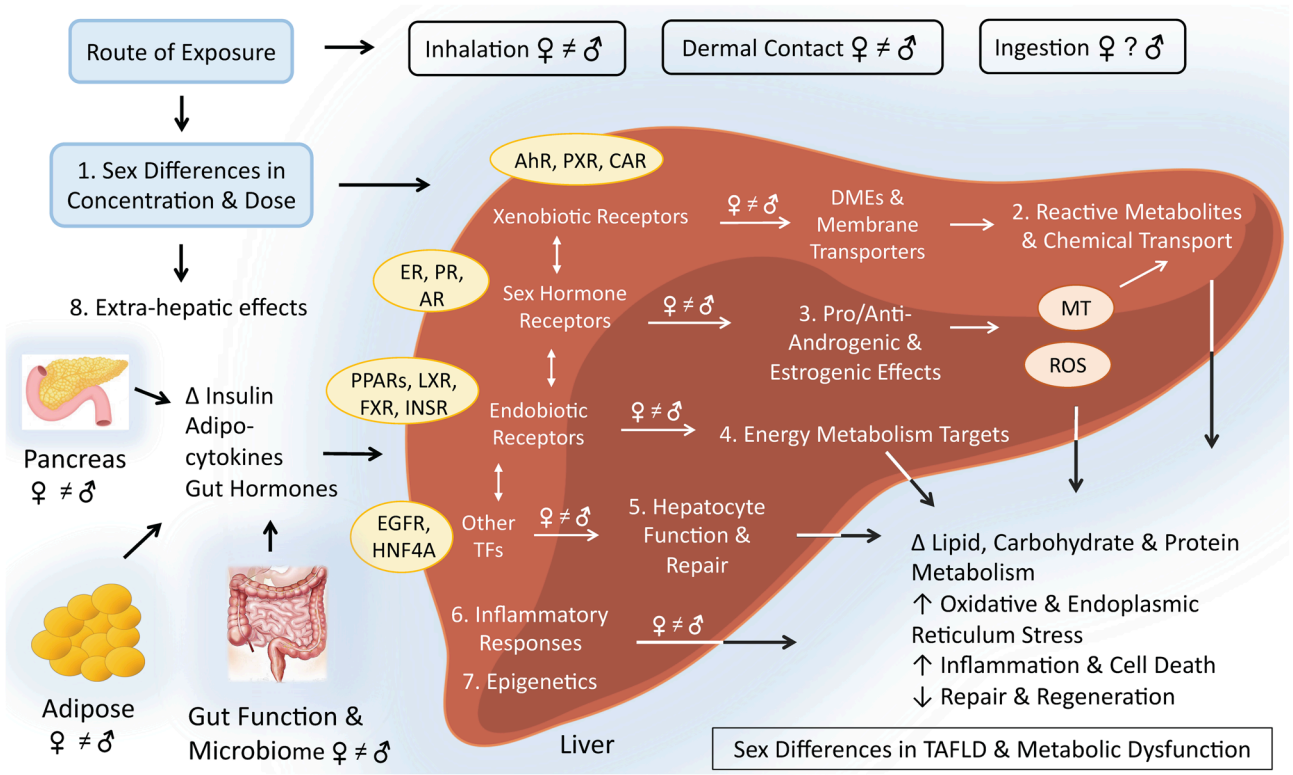

Figure 1
Schematic illustration of potential mechanisms driving sex-specific TAFLD and associated metabolic dysfunction. Sex differences in exposure routes such as inhalation capacity and skin permeability can dictate the levels of chemicals entering the body (1). Sex differences in hepatic receptor expression and activation as well as receptor–receptor crosstalk can result in differences in chemical metabolism, transport, and elimination (2), and differential activation or inhibition of target genes involved in hepatic energy metabolism, and hepatocyte function and repair (4, 5). Chemical interactions with sex hormones and their receptors can also result in varying estrogenic and androgenic effects (3) and subsequent impact on metallothionein complex formation and ROS generation, among other processes. Other mechanisms include sex differences in immune responsiveness to chemical exposures (6), chemical-induced DNA modifications (7) and organ–organ crosstalk (8). DMEs, drug metabolizing enzymes; INSR, insulin receptor; MT, metallothionein; ROS, reactive oxygen species; TF, transcription factors.

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and our ecosystem, one caveat is that such changes frequently result in the emergence of new chemicals serving as substitutes or replacements. Therefore, our current knowledgebases such as the AOP models require elaboration and more robust approaches to better assess chemical safety and toxicity while also accounting for biological, sociological, and lifestyle factors as mediators. Future sex-specific studies are necessitated for other chemical categories and additional disease endpoints including but not limited to cardio-metabolic diseases, neurological and immunological disorders, and cancer. Mechanistic findings from such sex-specific studies will yield better insight into (i) sex-specific exposure and disease biomarkers in toxicant-associated metabolic diseases including TAFLD, (ii) sex-specific intervention approaches to mitigate chemical exposure and attenuate disease risk, in addition to (iii) providing more efficient risk assessment and ability to recognize sub-populations that may be more susceptible to one disease over another. In conclusion, sex is a critical biological variable that dictates the chemical impact on health disorders including TAFLD. More sex-dependent toxicological studies are necessary and will be of paramount value to public health, as they will aid health authorities and policymakers to better distinguish populations vulnerable to such diseases, in part addressing environmental health disparities, while also discovering newer knowledge on more effective prevention, intervention and therapeutic strategies.

Declaration of interest

The author declares no known or potential conflict of interest.

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