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Exposure to per- and Polyfluoroalkyl Substances and Markers of Liver Injury: A Systematic Review and Meta-Analysis

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Background: Experimental evidence indicates that exposure to certain pollutants is associated with liver damage. Per- and polyfluoroalkyl substances (PFAS) are persistent synthetic chemicals widely used in industry and consumer products and bioaccumulate in food webs and human tissues, such as the liver.

Objective: The objective of this study was to conduct a systematic review of the literature and meta-analysis evaluating PFAS exposure and evidence of liver injury from rodent and epidemiological studies.

Methods: PubMed and Embase were searched for all studies from earliest available indexing year through 1 December 2021 using keywords corresponding to PFAS exposure and liver injury. For data synthesis, results were limited to studies in humans and rodents assessing the following indicators of liver injury: serum alanine aminotransferase (ALT), nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, or steatosis. For human studies, at least three observational studies per PFAS were used to conduct a weighted z-score meta-analysis to determine the direction and significance of associations. For rodent studies, data were synthesized to qualitatively summarize the direction and significance of effect.

Results: Our search yielded 85 rodent studies and 24 epidemiological studies, primarily of people from the United States. Studies focused primarily on legacy PFAS: perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), perfluorononanoic acid (PFNA), and perfluorohexanesulfonic acid. Meta-analyses of human studies revealed that higher ALT levels were associated with exposure to PFOA (z-score = 6.20, $p < 0.001$), PFOS (z-score = 3.55, $p < 0.001$), and PFNA (z-score = 2.27, $p = 0.023$). PFOA exposure was also associated with higher aspartate aminotransferase and gamma-glutamyl transferase levels in humans. In rodents, PFAS exposures consistently resulted in higher ALT levels and steatosis.

Conclusion: There is consistent evidence for PFAS hepatotoxicity from rodent studies, supported by associations of PFAS and markers of liver function in observational human studies. This review identifies a need for additional research evaluating next-generation PFAS, mixtures, and early life exposures. https://doi.org/10.1289/EHP10092

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a public health epidemic.[1] In parallel with the growing obesity epidemic, prevalence of NAFLD has significantly increased in recent years and become one of the most common causes of chronic liver disease globally.[2,3] The prevalence of NAFLD is estimated to be about 25% worldwide, whereas cases in the United States are expected to number 100.9 million, or about one-third of all adults, by 2030.[4] Untreated, NAFLD may progress to more serious liver injury such as nonalcoholic steatohepatitis (NASH), cirrhosis, and end-stage liver disease.[5]

Exposure to environmental chemicals has emerged as a significant contributor to liver disease, including NAFLD. Experimental evidence indicates that exposure to per- and polyfluorinated substances (PFAS), a class of endocrine-disrupting chemicals, has the ability to promote metabolic changes that can result in fatty liver.[6] PFAS are synthetic chemicals widely used in industry and consumer products such as stain-resistant fabric and fire retardants.[7,8] The stable chemical properties that make PFAS ideal for industrial use also allow them to persist and accumulate in the environment,[9] which is of concern because of the potential for long-term human health effects. Recent biomonitoring studies have emphasized the ubiquitous nature of PFAS exposure and have indicated that four congeners of PFAS account for most known human exposure: perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexanesulfonic acid (PFHxS), and perfluorononanoic acid (PFNA).[10,11] Significant sources of exposure include drinking water,[12,13] food,[14,15] indoor and outdoor air,[16,17] and early life placental or breast milk exposure.[18–20] PFAS are detected in the serum of nearly all U.S. adults[21,22] and accumulate in body tissues, such as in the liver.[23–25] This bioaccumulation, coupled with the long half-lives of many PFAS,[26,27] leads to concern about the potential for PFAS to disrupt liver homeostasis should they continue to accumulate in human tissue even if industrial use is abated.

Research evaluating hepatotoxic effects of PFAS has greatly increased in the peer-reviewed literature; however, conclusions remain inconsistent. In animal studies, PFAS have consistently induced steatosis and lipid accumulation in mice,[28] rats,[29] zebrafish,[30]

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chickens,[31] frogs,[32] and primates.[33] Despite this, it is difficult to extrapolate directly from animal results to human health effects in part due to species differences in PFAS elimination and half-lives.[34]

Evaluations of occupationally exposed workers have not consistently reported associations between PFAS exposure and liver enzymes or liver disease,[35–38] although recent analyses of other populations have reported positive associations between PFAS and liver enzymes indicative of liver injury.[39–42] Epidemiological studies have also reported associations between PFAS exposure and cholesterol,[43–47] triglycerides,[38,45,47] bilirubin,[40] and uric acid,[40] further supporting a relationship between PFAS exposure and liver injury given that these are additional biomarkers of metabolic disruption, NAFLD, and advanced liver disease.[48–50]

Indeed, the association between PFAS exposure and NAFLD in humans remains challenging to evaluate given the difficulty in obtaining biopsy-confirmed NAFLD histological data, and thus liver injury is typically assessed using serum biomarkers of hepatotoxicity or imaging assessments of hepatic steatosis.[51] Alanine aminotransferase (ALT) in particular is considered a specific biomarker of liver injury and is widely used in epidemiological studies.[51–53] A recent review summarized the state of the literature regarding toxic effects of PFAS on many adverse health effects, including liver disease, lipid dysregulation, and other metabolic outcomes.[54] Fenton et al.[54] provided an overview of the evidence for hepatoxicity across human and animal studies, as well as a discussion of possible mechanisms underlying this relationship. In contrast, the purpose of the present review is to specifically evaluate the effects of PFAS exposure on NAFLD and markers of NAFLD, with a focus on the liver enzymes commonly used in human epidemiological research. To our knowledge, this is the first systematic review and meta-analysis integrating both the epidemiological (human) and experimental (rodent) evidence for an effect of PFAS exposure on liver enzymes and related markers of liver injury.

Materials and Methods

This review is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. The review protocol was registered in PROSPERO (CRD42020158911).[55]

Search Strategy

We systematically searched two databases, PubMed and Embase, for human and rodent studies evaluating the association between exposure to PFAS and markers of liver injury from earliest available online indexing through 1 December 2021. For PubMed, the search strategy was as follows: (NAFLD OR "nonalcoholic fatty liver disease" OR "nonalcoholic fatty liver disease" OR NASH OR "nonalcoholic steatohepatitis" OR "nonalcoholic steatohepatitis" OR "nonalcoholic fatty liver" OR "fatty liver" OR steatosis OR ALT OR "alanine aminotransferase" OR AST OR "aspartate aminotransferase" OR GGT OR "gamma-glutamyl transferase" OR "gamma glutamyl transferase" OR CK18 OR "cytokeratin 18" OR ALP OR "alkaline phosphatase" OR "liver enzymes" OR "liver damage" OR "liver injury" OR "liver fibrosis" OR "liver weight") AND (Perfluoroalkyl OR Polyfluoroalkyl OR Perfluorinated OR polyfluorinated OR perfluoro* OR polyfluoro* OR PFAS* [tiab] OR PFOS [tiab] OR ((perfluorooctanesulfonic OR perfluorooctane sulfonic) AND acid) OR "perfluorooctane sulfonate" OR PFOA [tiab] OR "perfluorooctanoic" acid OR perfluorooctanoate OR PFHxS [tiab] OR ((perfluorohexane sulfonic OR perfluorohexanesulfonic) AND acid) OR "perfluorohexane sulfonate" OR perfluorohexanesulfonate OR PFNA [tiab] OR "perfluorononanoic acid" OR perfluorononanoate OR GenX [tiab] OR "hexafluoropropylene oxide dimer acid" OR PFOSA [tiab] OR "perfluorooctane sulfonamide" OR PFUnDA [tiab] OR "perfluorodecanoic acid" OR perfluoroundecanoate PFDA OR "perfluorodecanoic acid" OR perfluorodecanoate OR PFBS OR "perfluorobutane sulfonic acid" OR "perfluorobutane sulfonate".

For Embase, the search terms were (NAFLD OR "nonalcoholic fatty liver disease" OR "nonalcoholic fatty liver disease" OR NASH OR "nonalcoholic steatohepatitis" OR "nonalcoholic steatohepatitis" OR "nonalcoholic fatty liver" OR "fatty liver" OR steatosis OR ALT OR "alanine aminotransferase" OR AST OR "aspartate aminotransferase" OR GGT OR "gamma-glutamyl transferase" OR "gamma glutamyl transferase" OR CK18 OR "cytokeratin 18" OR ALP OR "alkaline phosphatase" OR "liver enzymes" OR "liver damage" OR "liver injury" OR "liver fibrosis" OR "liver weight") AND (Perfluoroalkyl OR Polyfluoroalkyl OR Perfluorinated OR polyfluorinated OR perfluoro* OR polyfluoro* OR PFAS*:ab,ti OR PFOS:ab,ti OR ((perfluorooctanesulfonic OR perfluorooctane sulfonic) AND acid) OR "perfluorooctane sulfonate" OR PFOA:ab,ti OR "perfluorooctanoic acid" OR "perfluorooctanoate" OR PFHxS:ab,ti OR ((perfluorohexane sulfonic OR perfluorohexanesulfonic) AND acid) OR "perfluorohexane sulfonate" OR perfluorohexanesulfonate OR PFNA:ab,ti OR "perfluorononanoic acid" OR perfluorononanoate OR GenX:ab,ti OR "hexafluoropropylene oxide dimer acid" OR PFOSA:ab,ti OR "perfluorooctane sulfonamide" OR PFUnDA:ab,ti OR "perfluorodecanoic acid" OR "perfluoroundecanoate PFDA" OR "perfluorodecanoic acid" OR perfluorodecanoate OR PFBS OR "perfluorobutane sulfonic acid" OR "perfluorobutane sulfonate". We also screened the references of recent reviews for eligible studies.

Study Selection

Studies were eligible for inclusion if they met the following criteria: (*a*) were original experimental or observational research published in English (i.e., not a review, meta-analysis, abstract, editorial, letter, or commentary); (*b*) conducted in humans, mice, or rats; (*c*) assessed one or more PFAS; and (*d*) reported data on serum ALT, NAFLD, NASH, or steatosis. ALT was chosen as the biomarker of interest because of its relative specificity to liver disease and use in previous literature on PFAS exposure and NAFLD. Other markers of liver disease—such as bilirubin, alkaline phosphatase, albumin, and uric acid—were not included because alterations in these biomarkers may suggest damage to other organ systems or liver diseases with alternate causes (e.g., cancer, alcoholic fatty liver).[51,56,57] Secondary outcomes were extracted, if available, and included serum aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT), cytokeratin-18 (CK-18), liver histopathology, and relative liver weight (animals only). For the purpose of this review, increases in liver weight were presumed to be adverse, given our focus on additional measures of liver injury (e.g., enzymes, histopathology). However, increases in liver weight alone may be an adaptive response in rodents and do not always indicate that an injury has occurred.[58] Two reviewers (S.R. and E.C.) independently performed an initial screening of titles and abstracts and then evaluated potentially relevant studies based on full-text reviews. Any discrepancies were resolved by discussion with a third reviewer (N.S.).

Data Extraction

In human studies, the following information was extracted from each article: first author, publication year, country, year and method of exposure assessment and outcome assessment, study design, population characteristics, sample size, confounders, and

results [adjusted β coefficients and odds ratios with standard errors (SEs) or 95% confidence intervals]. In rodent studies, the following information was extracted: first author, year, study design, species/strain, sex, sample size, age, exposure, frequency and duration of exposure, administration route, dose, diet, outcome results, and SE. Data were independently extracted by two reviewers (S.R. and E.C.) and compared for accuracy. Any discrepancies were resolved through discussion with a third reviewer (N.S.).

Quality Assessment

Human and rodent study quality was independently evaluated by two reviewers (S.R. and E.C.) using the Office of Health Assessment and Translation (OHAT) Risk of Bias tool,[59,60] with discrepancies resolved through discussion. The OHAT Risk of Bias tool was used to evaluate threats to internal validity and assess the risk of bias. The OHAT tool was chosen for its ability to evaluate cross-sectional studies, which are not considered in other quality rating systems, and applicability to both human and rodent studies.[61,62]

Six of the 10 domains in the OHAT tool were relevant to observational human studies; those pertaining to randomization and blinding were not applicable. Eight domains were relevant to experimental rodent studies; domains that addressed participant selection and confounding were not relevant. For each domain, a study was evaluated for definitely low risk of bias (++), probably low risk of bias (+), probably high risk of bias (–), and definitely high risk of bias (––). In domains where the study did not provide enough information to evaluate bias, an assignment of "probably high" risk was given with the notation "NR" for "not reported." Specific criteria for each domain are described in the section "Description of domains in Office of Health Assessment and Translation (OHAT) Risk of Bias tool" in the Supplemental Material.

Data Synthesis and Meta-Analysis

In human studies, we conducted meta-analyses between exposure to each of the four selected PFAS and serum concentrations of each of three liver enzymes (ALT, AST, and GGT), which were reported in at least three studies of similar design (e.g., cross-sectional, longitudinal). Because of the heterogeneous methodologies (e.g., log-transformation or natural log-transformation of the exposure, the outcome, or both) and noncomparable effect estimates, it was not possible to directly pool effect estimates across studies. For example, the effect estimate from a study that $\log_{10}$-transformed both exposure and outcome cannot be pooled with a study that natural log-transformed only the exposure, and pooling only studies that had similar transformation methodologies may introduce selection bias. Thus, we used a weighted z-scores method to summarize results. z-Scores were calculated using adjusted β coefficients from linear regression analyses of PFAS and their SE.[63] Although the magnitude of the effect cannot be determined using this method, a weighted z-score allows for determination of the statistical significance and direction of the relationship. For each PFAS-liver enzyme relationship, a weighted-average z-score was calculated where weights were the square root of the sample size. Studies in populations <12 years of age (presumed to be either in early stages of puberty or prepubertal based on normal range of puberty in girls and boys)[64,65] were excluded from this calculation to account for developmental effects and included in sensitivity analyses. For different studies with overlapping populations, only the study with the largest population was included. In studies that reported multiple models, we used the effect estimate from the most highly adjusted model. Although the inclusion criteria did not exclude studies with categorical measures, none of the studies in the present review used exclusively categorical measures. The z-score was calculated using the overall β, not those stratified by sex, weight, or other factors, unless an overall β was not available. However, when studies reported stratified analyses by sex in addition to overall population results, we included the stratified results to see whether sex-specific differences might exist when multiple studies are compared. Where possible, additional sensitivity analyses were performed and z-scores were calculated *a*) separately by sex, *b*) after excluding the largest study, *c*) for studies using National Health and Nutrition Examination Survey (NHANES) data, and *d*) including populations <12 years of age. The purpose of these analyses was to determine whether *a*) the relationships differed by sex, *b*) they were driven primarily by a single large study, *c*) the relationship differed between the general population of the United States and populations from other countries or those occupationally exposed, and *d*) including children changed the direction or statistical significance of the relationship.

In rodent studies, substantial differences in study design (e.g., length of exposure, exposure vehicle, dose) meant that meta-analyses were not feasible. Data were synthesized and displayed graphically. We used strip plots adapted from Thayer et al.[66] to summarize the direction of the effect of PFAS dose (in milligrams per kilogram of body weight or parts per million) on ALT across all eligible studies. Additional plots were used to summarize the effects of PFAS exposure on additional liver enzymes and relative liver weight in those studies that reported secondary outcomes. Some studies provided data on groups treated with PFAS combined with nonstandard diets or supplements; for these, we selected as control the group on standard diet with no PFAS or supplement exposure. PFAS plus experimental diet or supplement were included as exposure groups. All analyses were conducted in R (version 4.0.2; R Development Core Team).

Results

Our search produced 881 articles from PubMed ($n=371$) and Embase ($n=510$), 205 of which were duplicates (Figure 1). After title and abstract screening and full-text review, 109 studies met the eligibility criteria. Two additional studies were identified from review articles (see the section "Review articles screened for additional eligible articles" in the Supplemental Material). Of the 111 total studies, 25 were observational human studies and 86 were experimental rodent studies. Extracted data used in z-score calculations for human studies and in visual data synthesis for

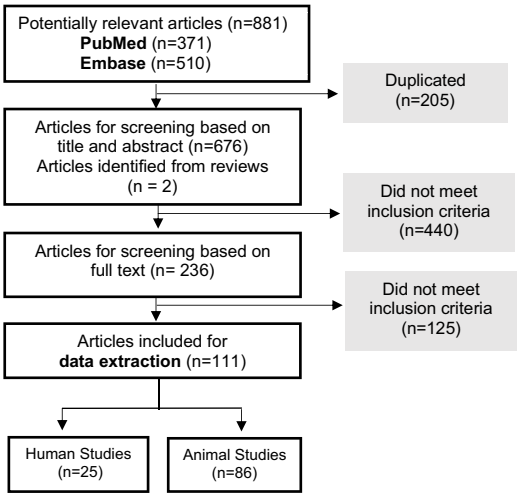


Figure 1. Flow chart of the study selection.

Table 1. Human studies on per- and polyfluorinated chemicals and biomarkers or outcomes of liver injury included for systematic review.

Reference	Population[a]	Year of exposure assessment	Exposure assessment[b]	Year of outcome assessment	Outcome[c]	Confounding	Results
Attanasio[67,169]	NHANES adolescents (USA) $n =$ 353 (M), 305 (F)	2013–2016	Geometric mean (SE) PFOA[d] 1.50 (0.06) ng/mL (M), 1.22 (0.06) ng/mL (F); PFOS[d] 3.68 (0.12) ng/mL (M), 2.76 (0.14) ng/mL (F); PFNA[d] 0.58 (0.03) ng/mL (M), 0.49 (0.03) ng/mL (F); PFHxS[d] 1.31 (0.09) ng/mL (M), 0.88 (0.06) ng/mL (F)	Same as exposure	ALT (U/L),[d] GGT (U/L),[d] AST (U/L)[d]	Adjusted for age, race/ethnicity, weight category, poverty–income ratio, tobacco exposure, and education.	Males: PFOA and PFNA were associated with lower ALT. PFNA was associated with lower AST. There was no association between any PFAS and GGT. Females: PFOA and PFNA were associated with higher ALT. PFOA, PFOS, and PFNA were associated with higher AST. PFOA and PFOS were associated with higher GGT.
Bassler et al.[68]	C8 Health Study adults (USA) $n = 200$	2006	Mean (SE) PFOA[d] 94.6 (183.6) ng/mL; PFOS[d] 26.9 (16.7) ng/mL; PFNA[d] 1.6 (0.7) ng/mL; PFHxS[d] 4.2 (3.9) ng/mL	Same as exposure	CK18 (U/mL)[d]	Adjusted for e-GFR, alcohol consumption category, BMI, age, and sex.	CK18-M30 and CK18-M65 were positively associated with PFOA, PFNA, and PFHxS, and there was a positive trend with PFOS.
Darrow et al.[39]	C8 Health Study adults (USA) $n = 28{,}047$	1951–2006 (cumulative); 2005–2006 (cross-sectional)	PFOA (modeled cumulative exposure) Median PFOA[d] 16.5 ng/mL	2005–2006 (enzymes); 2008–2011 (liver disease)	Liver disease (enlarged liver, fatty liver, or cirrhosis), ALT (U/L),[d] GGT (U/L)[d]	Adjusted for age, sex, BMI, alcohol consumption, regular exercise, smoking status, education, insulin resistance, fasting status, history of working at DuPont plant, and race.	Cross-sectional PFOA and longitudinal (estimated) PFOA were positively associated with ALT. There was no relationship between PFOA and liver disease.
Emmett et al.[69]	Residents (adults and children) of Little Hocking (USA) $n = 371$	Not Specified	Median (IQR) PFOA 354 (181–571) ng/mL	Same as exposure	Liver disease, ALT (U/L), GGT (U/L), AST (U/L)	No adjustment for covariates.	No linear association between PFOA and ALT, GGT, or AST. Having abnormal AST levels was associated with lower PFOA. There was no relationship between liver disease and PFOA.
Gilliland et al.[165]	Male employees of PFOA plant Adults (USA) $n = 115$	1985–1989	Mean (range) Total fluorine 3.3 (0– 26 ppm) (surrogate for PFOA)	Same as exposure	ALT (IU/dL), AST (IU/dL), GGT (IU/dL)	Age, cigarette use, alcohol use, and BMI	Total serum fluorine was not associated with ALT, AST, or GGT. ALT, AST, and GGT levels did not differ by level of fluorine exposure. There was a significant interaction between serum fluorine and BMI: There was a positive association between serum fluorine and both ALT and AST in people with obesity.
Gallo et al.[70]	C8 Health Study adults (USA) $n = 46{,}452$	2005–2006	Median (IQR) PFOA[d] 28.0 (13.5–70.8) ng/mL; PFOS[d] 20.3 (13.7–29.4) ng/mL	Same as exposure	ALT (U/L),[d] GGT (U/L)[d]	Adjusted for alcohol consumption, socioeconomic status, fasting status, race, month of blood sample collection, age, sex, smoking, BMI, physical activity, and insulin resistance.	PFOA and PFOS were positively associated with ALT.
Gleason et al.[40]	NHANES adults and adolescents (USA) $n = 4{,}333$	2007–2010	Median (IQR) PFOA[d] 3.7 (2.5–5.2) μg/L; PFOS[d] 11.3 (7.0–18.0) μg/L; PFNA[d] 1.4 (1.0–2.1) μg/L; PFHxS[d] 1.8 (1.0–3.1) μg/L	Same as exposure	ALT (U/L),[d] GGT (U/L),[d] AST (U/L)[d]	Adjusted for age, sex, race/ethnicity, BMI, poverty, smoking, and alcohol consumption.	PFHxS, PFOA, and PFNA were positively associated with ALT. PFOA and PFNA were positively associated with GGT. PFHxS was positively associated with AST.

Table 1. (*Continued.*)

Reference	Population[a]	Year of exposure assessment	Exposure assessment[b]	Year of outcome assessment	Outcome[c]	Confounding	Results
Jain[71]	NHANES adults (USA) $n = 9{,}523$	2003–2014	PFOA (ng/mL)[e]; PFOS (ng/mL)[e]	Same as exposure	ALT (U/L),[e] GGT (U/L),[e] AST (U/L)[e]	Adjusted for sex, race/ethnicity, smoking status, age, BMI, diabetes status, hypertension status, fasting time, poverty–income ratio, survey year, and alcohol consumption.	PFOA and PFOS were inconsistently associated with ALT, GGT, and AST when stratified by glomerular function stage and obesity status.
Jain and Ducatman[72]	NHANES adults (USA) $n = 2{,}883$	2011–2014	Geometric mean (95% CI) PFOA[e] 2.2 (2.0–2.3) ng/mL (non-obese); 2.0 (1.8–2.1) ng/mL (obese); PFOS[e] 6.3 (5.8–6.8) ng/mL (non-obese); 5.5 (5.0–6.0) ng/mL (obese); PFNA[e] 0.83 (0.76–0.89) ng/mL (non-obese); 0.73 (0.68–0.79) ng/mL (obese); PFHxS[e] 1.41 (1.29–1.54) ng/mL (non-obese); 1.24 (1.13–1.37) ng/mL (obese)	Same as exposure	ALT (U/L),[e] GGT (U/L),[e] AST (U/L)[e]	Adjusted for sex, race/ethnicity, age, age-squared, poverty–income ratio, physical activity, BMI, and serum cotinine.	Positive associations between PFOA, PFHxS, and PFNA and ALT were observed in participants with obesity. In those with obesity, PFOA and PFNA were also positively associated with GGT. Additional PFAS: PFDA was not found to be associated with liver enzymes.
Jin et al.[73]	Children with NAFLD (USA) $n = 74$	2007–2015	Median (IQR) PFOA 3.42 (1.65) ng/mL; PFOS 3.59 (4.46) ng/mL; PFHxS 1.53 (3.17) ng/mL	Same as exposure	Histological severity of NAFLD	—	Higher PFOS, PFOA, and PFHxS concentrations were associated with more severe NAFLD (NASH, fibrosis, lobular/portal inflammation, NAFLD activity score).
Khalil et al.[74]	Dayton Obese Cohort children (USA) $n = 48$	2016	Median (IQR) PFOA 0.99 (0.45) ng/mL; PFOS 2.79 (2.10) ng/mL; PFNA 0.24 (0.15) ng/mL; PFHxS 1.09 (1.41) ng/mL	Same as exposure	ALT (U/L), AST (U/L)	Adjusted for age, sex, race, and multiple testing.	There were no significant relationships between PFAS and ALT or AST.
Lin et al.[75]	NHANES adults (USA) $n = 2{,}216$	1999–2003	Mean (SE) PFOA[e] 4.51 (1.04) ng/mL; PFOS[e] 24.60 (1.04) ng/mL; PFNA[e] 0.79 (1.07) ng/mL; PFHxS[e] 1.98 (1.04) ng/mL	Same as exposure	ALT (U/L), GGT (U/L)[e]	Adjusted for age, sex, race/ethnicity, smoking, alcohol consumption, education level, BMI, HOMR-IR, metabolic syndrome, iron saturation status.	PFOA was positively associated with ALT and GGT, with a stronger effect in those with obesity.

Table 1. (*Continued.*)

Reference	Population[a]	Year of exposure assessment	Exposure assessment[b]	Year of outcome assessment	Outcome[c]	Confounding	Results
Mora et al.[76]	Project Viva children (USA) $n = 508$ (longitudinal); 630 (cross-sectional)	1999–2002 (longitudinal); 2007–2010 (cross-sectional)	Median (IQR) Longitudinal: PFOA (maternal) 5.4 (3.9–7.6) ng/mL; PFOS (maternal) 24.6 (17.9–34) ng/mL; PFNA (maternal) 0.6 (0.5–0.9) ng/mL; PFHxS (maternal) 2.4 (1.6–3.8) ng/mL; Cross-sectional: PFOA (child) 4.3 (3.0–7.0) ng/mL; PFOS (child) 6.2 (4.2–9.7) ng/mL; PFNA (child) 1.5 (1.1–2.3) ng/mL; PFHxS (child) 1.9 (1.2–3.4) ng/mL	2007–2010 (longitudinal, cross-sectional)	ALT (U/L)	Longitudinal: Adjusted for maternal education, prenatal smoking, gestational age at blood draw, sex, race/ethnicity, and age at ALT measurements. Cross-Sectional: Adjusted for maternal education, prenatal smoking, sex, race/ethnicity, and age.	There was an inverse but not statistically significant inverse relationship between maternal PFOS, PFOA, and PFHxS exposure and ALT in girls. Higher childhood PFOA and PFOS concentrations were associated with lower ALT. Additional PFAS: Maternal EtFOSAA and MeFOSAA were not associated with liver enzymes.
Mundt et al.[35]	Employees at a chemical manufacturer (USA) $n = 592$	1976–2003	High, low, no exposure PFNA	1989–2003	ALT (U/L), GGT (U/L), AST (U/L)	Adjusted for age and BMI.	PFNA exposure was not associated with mean ALT, GGT, or AST.
Nian et al.[41]	Adult residents of Shenyang, China $n = 1,605$	2015–2016	Median (IQR) PFOA 6.19 (4.08–9.31) ng/mL; PFOS 24.22 (14.62–37.19) ng/mL; PFNA 1.96 (1.11–3.07) ng/mL; PFHxS 0.73 (0.01–2.68) ng/mL	Same as exposure	ALT (U/L),[d] GGT (U/L),[d] AST (U/L)[d]	Adjusted for age, sex, career, income, education, alcohol consumption, smoking, giblet/seafood consumption, physical activity, and BMI.	PFOA, PFOS, and PFNA were positively associated with ALT. There were also positive associations between PFOA and AST and GGT. Additional PFAS: PFDA was positively associated with ALT.
Olsen et al.[36]	Male employees at two fluorochemical manufacturers (Antwerp, Belgium, and Decatur, Alabama) $n = 178$ (1995); 149 (1997)	1995, 1997	Mean PFOS 1.93 ppm (Antwerp, 1995); 2.44 ppm (Decatur, 1995); 1.48 ppm (Antwerp, 1997); 1.96 ppm (Decatur, 1997)	Same as exposure	ALT (U/L), GGT (U/L), AST (U/L)	Adjusted for age, BMI, alcohol use, and smoking.	PFOS exposure was not associated with ALT, GGT, or AST.
Olsen et al.[37]	Employees at two fluorochemical manufacturers (Antwerp, Belgium, and Decatur, Alabama) $n = 263$ (Decatur), 255 (Antwerp), 174 (longitudinal)	1994–2000 (longitudinal); 2000 (cross-sectional)	Geometric mean (95% CI): PFOA 0.33 (0.27–0.40) ppm (Antwerp); 1.13 (0.99–1.30) ppm (Decatur); PFOS 0.44 (0.38–0.51) ppm (Antwerp); 0.91 (0.82–1.02) ppm (Decatur)	2000	ALT (U/L), GGT (U/L), AST (U/L)	Adjusted for age, BMI, alcohol use, smoking, and location.	Those in the highest quartile of PFOS exposure had higher mean ALT. PFOS was not associated with increased odds of elevated ALT or GGT. There were no associations between PFOS or PFOA and liver enzymes in the longitudinal analysis.
Olsen and Zobel[38]	Male employees at three fluorochemical manufacturers (Antwerp, Belgium; Decatur, Alabama; Cottage Grove, Minnesota) $n = 196$ (Antwerp), 188 (Decatur), 122 (Cottage Grove)	2000	Mean (SD) PFOA[d] 1.02 (1.06) µg/mL (Antwerp); 1.89 (1.61) µg/mL (Decatur); 4.63 (12.53) µg/mL (Cottage Grove)	Same as exposure	ALT (U/L),[d] GGT (U/L),[d] AST (U/L)[d]	Adjusted for age, BMI, and alcohol use.	There were no significant linear associations between PFOA and ALT, GGT, or AST, or between PFOA and elevated liver enzymes.

Table 1. (*Continued.*)

Reference	Population[a]	Year of exposure assessment	Exposure assessment[b]	Year of outcome assessment	Outcome[c]	Confounding	Results
Rantakokko et al.[77]	Kupio Obesity Surgery Study adult participants (Finland) $n=$ 161	2005–2010	Median (5th, 95th percentile) PFOA[e] 2.56 (1.04, 4.66) ng/mL; PFOS[e] 3.2 (0.89, 10.3) ng/mL; PFNA[e] 0.83 (0.30, 2.19) ng/mL; PFHxS[e] 1.18 (0.54, 2.90) ng/mL	Same as exposure 12 months post (ALT)	ALT (U/L),[e] steatosis, NASH, lobular inflammation, liver cell ballooning	Adjusted for age, fasting insulin, and weight change.	There were no significant associations between PFOA, PFOS, PFNA, or PFHxS and ALT at either baseline or 12 months later. PFOA, PFNA, and PFHxS were inversely associated with lobular inflammation at baseline. Additional PFAS: PFHxA was associated with ALT at 12 months. PFDA and sum of PFCA were associated with lobular inflammation at baseline.
Sakr et al.[44]	Employees at the Washington Works polymer manufacturing site (USA) $n=$ 205	1979–2007	Mean (SD) PFOA 1.13 (2.1) ppm	1980–2007	ALT (U/L), GGT (U/L), AST (U/L)	Adjusted for age, sex, BMI, and decade of hire.	There was a positive association between PFOA and AST.
Sakr et al.[43]	Employees at Washington Works polymer manufacturing site (USA) $n=$ 1,018	2004	Mean (SD) PFOA 0.428 (0.86) ppm	Same as exposure	ALT (U/L),[d] GGT (U/L),[d] AST (U/L)[d]	Adjusted for age, sex, BMI, alcohol consumption, family history of heart attack, and use of lipid-lowering medications.	There was a positive association between PFOA and GGT.
Salihovic et al.[42]	Older adults (Sweden) $n=$ 1,002	2001–2014	Median (IQR) PFOA[d] 3.31 (2.52–4.39) ng/mL; PFOS[d] 13.2 (9.95–17.8) ng/mL; PFNA[d] 0.70 (0.52–0.97) ng/mL; PFHxS[d] 2.08 (1.6–3.42) ng/mL	2006–2014	ALT (ukat/L), GGT (ukat/L)	Adjusted for sex, LDL and HDL cholesterol, serum triglycerides, BMI, fasting glucose levels, statin use, and smoking.	There were positive associations between PFOA, PFOS, PFNA, and PFHxS and ALT. There was also a positive association between PFOA and GGT. Additional PFAS: PFHpA was positively associated with ALT, and PFUnDA was positively associated with GGT.
Sen et al.[79]	Adults undergoing laparoscopic bariatric surgery without other risk factors for NAFLD (Sweden) $n=$ 105	Not Specified	Median (min–max) PFOA 1.89 (0.49–6.36) ng/mL; Br-PFOS 2.13 (0.63–9.71) ng/mL; L-PFOS 2.50 (0.74–11.79) ng/mL; PFNA 0.37 (0.09–1.08) ng/mL; PFHxS 0.60 (0.16–10.58) ng/mL	Same as exposure	NAFLD), NASH), macrosteatosis), necroinflammatory activity), fibrosis	None	Positive associations were observed between PFAS (PFOA, PFOS, PFNA, and PFHxS) and macrosteatosis. PFOA and PFOS were positively associated with necroinflammation and NASH. PFNA was negatively associated with NASH. PFOS was positively associated with fibrosis.
Stratakis et al.[78]	Children in the HELIX cohort (UK, France, Spain, Lithuania, Norway, Greece) $n=$ 1,105	2005–2009 (prenatal)	PFAS mixture Median (IQR) PFOA 2.38 (1.45–3.45) ng/mL; PFOS 6.74 (4.43–10.35) ng/mL; PFNA 0.72 (0.47–1.11) ng/mL; PFHxS 0.59 (0.34–0.93) ng/mL	2014–2015	Liver injury risk (ALT, AST, or GGT levels ≥90th percentile)	Adjusted for cohort, maternal age, maternal education, maternal prepregnancy BMI, child ethnicity, child age, and child sex.	Higher prenatal PFAS exposure was associated with increased ALT, AST, and GGT, and with being at increased risk of liver injury. Additional PFAS: PFUnDA was included in the mixture analysis.

Table 1. (*Continued.*)

Reference	Population[a]	Year of exposure assessment	Exposure assessment[b]	Year of outcome assessment	Outcome[c]	Confounding	Results
Yamaguchi et al.[80]	Japanese residents with no occupational PFAS exposure $n = 608$	2008–2010	Median (IQR) PFOA[e] 2.1 (1.5–3.3) ng/mL; PFOS[e] 5.8 (3.7–8.8) ng/mL	Same as exposure	ALT (IU/L), AST (IU/L), GGT (IU/L)	Adjusted for age, sex, BMI, regional block, and smoking, and alcohol intake.	PFOA and PFOS were significantly positively correlated with ALT and AST. There was also a significant positive correlation with GGT, but not after adjustment for alcohol intake.

Note: —, not available; ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; CI, confidence interval; CK18, cytokeratin 18; eGFR, estimated glomerular filtration rate; EtFOSAA, *N*-ethyl perfluorooctane sulfonamidoacetic acid; F, female; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; HELIX, Human Early Life Exposome; HOMR-IR, Homeostatic Model Assessment of Insulin Resistance; IQR, interquartile range; LDL, low-density lipoprotein; M, male; max, maximum; MeFOSAA, *N*-methylperfluorooctane sulfonamidoacetic acid; min, minimum; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NHANES, National Health and Nutrition Examination Survey; PFAS, per- and polyfluorinated substances; PFDA, perfluorodecanoic acid; PFHpA, perfluoroheptanoic acid; PFHxA, perfluorohexanoic acid; PFHxS, perfluorohexanesulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PFUnDA, perfluoroundecanoic acid; SD, standard deviation; SE, standard error.
[a]Sample sizes given here represent the maximum number of subjects available for at least one of the analyses of interest. Specific analyses may have slightly different sample sizes.
[b]Blood concentration of PFOA, PFOS, PFNA, PFHxS only. Exposure concentrations are reported where available.
[c]Outcomes listed here are limited to liver enzymes (ALT, AST, GGT), NAFLD, NASH, and liver histopathology. Studies may have reported additional outcomes.
[d]Natural log (ln) transformed.
[e]Log_{10} transformed.

animal studies are available in Excel Tables S1 and S2, respectively.

The characteristics of human studies included in this review are shown in Table 1. Eighteen studies included populations from the United States,[35–40,43,44,67–76] 7 included populations from Europe,[36–38,42,77–79] and 2 from Asia.[41,80] Years of PFAS exposure assessment ranged from 1951[39] to 2016.[41,67,74] Sixteen studies were cross-sectional and 6 had a longitudinal design. Two studies, Darrow et al.[39] and Olsen et al.,[37] included both cross-sectional and longitudinal data.

Of the 86 eligible rodent studies, experiments investigating PFOA and PFOS were the most common. Other PFAS included PFNA, PFHxS, perfluorobutyrate (PFBA), perfluorobutanesulfonic acid (PFBS), perfluorodecanoic acid (PFDA), perfluorododecanoic acid (PFDoA), perfluoroundecanoic acid (PFUA), perfluorohexanoic acid (PFHxA), and hexafluoropropylene oxide dimer acid (GenX). Experimental animal study designs varied widely in choice of dosing scheme, duration of exposure, and exposure route (Table 2). Doses ranged from 0.02 to 600 mg/kg body weight and lasted for as little as 1 d to as long as 2 y. The most common route of exposure was oral gavage, although additional studies exposed animals to PFAS through drinking water, diet, inhalation, intraperitoneal injection, or dermal contact. Some study conditions were intended to mimic occupational or environmental human exposure levels (e.g., Blake et al.[81]), whereas others, such as Crebelli et al.[82] or Lieder et al.,[83] chose dose levels based on the no or lowest observed adverse effect level (NOAEL or LOAEL).

Results on OHAT risk of bias ratings are provided in Tables S1 and S2. No studies were excluded based on risk of bias. For human studies, risk of bias was often "definitely low" or "probably low" for all domains, but some were determined to have higher risk of bias because they did not adequately account for confounders related to NAFLD or NASH (e.g., alcohol use, body mass index, smoking). Most animal studies were determined to have "probably high" risk of bias for domains relating to blinding of researchers or concealment of experimental assignments, because most studies were either not blinded or did not report it. Animal studies generally received positive ratings on all other domains.

Exposure to PFOA

Human studies. Eight cross-sectional studies assessing the relationship between PFOA and ALT in adults and adolescents (≥12 years of age) were included in the weighted *z*-score calculation.[38,40,41,43,67,70,72,75] A weighted *z*-score of 6.20 ($p < 0.001$) indicated a positive relationship between PFOA and ALT (Table 3). This positive relationship remained across sensitivity analyses (Table S3). A weighted *z*-score for PFOA and ALT was also calculated for the three available longitudinal studies and was statistically significant (*z*-score = 5.12; $p < 0.001$; Table 3).[39,42,44] Only two studies examined the effect of PFOA exposure on ALT levels in children <12 years of age, reporting no statistically significant associations.[74,76] In adults, there was a positive relationship between PFOA exposure and GGT (*z*-score = 4.13, $p < 0.001$)[38,40,41,43,67,69,70,72,75] (Table S4), and this remained statistically significant after removing the largest study and after restricting the calculation to only NHANES participants (Table S3). There was no statistically significant relationship between PFOA and AST (*z*-score = 1.95, $p = 0.05$) in adults (Table S4).[38,40,41,43,67,69,72] Two longitudinal analyses did not find any associations between PFOA and other liver enzymes.[37,39] One, Salihovic et al.,[42] did find a positive association between PFOA and GGT.

Rodent studies. Thirty-two studies assessed exposure to PFOA in mice and 5 studies assessed exposure to PFOA in rats (Table 2).

Table 2. Animal studies on per- and polyfluorinated chemicals and biomarkers or outcomes of liver injury included for systematic review.

Reference	Exposure	Dose	Species/strain/sex	Exposure route	Duration of exposure	Outcomes	Main findings[a]
Bagley et al.[29]	K^+PFOS, K^+PFOS + CS	100 ppm and 100 ppm + CS	Rats; Sprague Dawley; male and female	Diet	3 wk	Steatosis, ALT, AST, GGT, relative liver weight, liver histopathology	PFOS induces steatosis in male but not female rats, and it was not attenuated by choline supplementation.
Bijland et al.[123]	K^+PFOS, K^+PFHxS, K^+PFBS	PFOS: 3 mg/kg; PFHxS: 6 mg/kg; PFBS: 30 mg/kg	Mice; APOE*3-Leiden. CETP; male	Diet (Western diet)	4–6 wk	Steatosis	PFOS and PFHxS, but not PFBS-induced steatosis.
Blake et al.[81]	NH_4^+PFOA, GenX	PFOA: 1 and 5 mg/kg; GenX: 2 and 10 mg/kg	Mice; CD-1; female (dams)	Gavage	E1.5–11.5, E1.5–17.5	ALT, AST, relative liver weight, liver histopathology	PFOA and GenX exposure resulted in increased liver weights and altered liver histopathology. AST was elevated in the highest PFOA and GenX exposure groups at E17.5.
Botelho et al.[101]	PFOA	0.002%, 0.005%, 0.01%, and 0.02% wt/wt	Mice; C57BL/6; male	Diet	10 d	ALT, relative liver weight, liver histopathology	PFOA exposure increased liver weight in all dose groups. ALT was significantly elevated in the highest dose group. Histopathological alterations were observed after PFOA exposure.
Butenhoff et al.[147]	K^+PFHxS	0.3, 1, 3, and 10 mg/kg	Rats; Sprague Dawley; male and female (F_0 parents, F^1 pups)	Gavage (F_0); prenatal+lactational (F_1)	44 d (F_0 males), 14 d prior to mating–PND 22 (F_0 females); prenatal – PND22 (F_1)	ALT (F_0 only); AST (F_0 only), relative liver weight, liver histopathology	Relative liver weight was increased in F_0 males at the 3- and 10-mg/kg dose levels only. There was no observed effect of PFHxS on liver histopathology or enzymes.
Butenhoff et al.[93]	NH_4^+PFOA, NH_4^+PFBA	PFOA: 30 mg/kg; PFBA, 28 d: 6, 30, and 150 mg/kg; PFBA, 90 d: 1.2, 6, and 30 mg/kg	Rats; Sprague Dawley; male and female	Gavage	28 d (PFOA, PFBA); 90 d (PFBA)	ALT, AST, relative liver weight, liver histopathology	28-d study: In males only, liver weight was increased in 30- and 150-mg/kg PFBA dose groups and after PFOA exposure. ALT was elevated in both sexes after PFOA exposure and returned to normal in males after 21 d of recovery. No change in ALT or AST was observed after PFBS exposure. Histopathological changes were observed in male rats in the 150-mg/kg PFBA and PFOA groups. 90-d study: In males only, liver weight was increased after 30-mg/kg PFBA exposure. There was no change in ALT or AST in either sex. Histological changes were observed in male rats in the 30-mg/kg dose group.
Butenhoff et al.[95]	K^+PFOS	0.5, 2, 5, and 20 ppm	Rats; Sprague Dawley; male and female	Diet	2 y	ALT, AST, relative liver weight, liver histopathology	ALT was increased in the highest dose group at wk 14 and 53, in males only. No changes were observed in AST. PFOA induced histopathological changes and increased liver weight.
Butenhoff et al.[94]	NH_4^+PFOA	30 and 300 ppm	Rats; Sprague Dawley; male and female	Diet	2 y	ALT, AST, relative liver weight, liver histopathology	ALT, AST, and liver weight were elevated in males exposed to PFOA. PFOA also induced histopathological changes, which were more severe in males than in females.
Butenhoff et al.[122]	POSF	30, 100, and 300 ppm vol/vol	Rats; Sprague Dawley; male and female	Inhalation	13 wk (6 h/d, 5 d/wk)	ALT, relative liver weight, liver histopathology	Liver weight increased following exposure. ALT was elevated in male rats but

Table 2. (*Continued.*)

Reference	Exposure	Dose	Species/strain/sex	Exposure route	Duration of exposure	Outcomes	Main findings[a]
							returned to normal after a 13-wk recovery period.
Chang et al.[146]	K^+PFHxS	0.3, 1, and 3 mg/kg	Mice; CD-1; male and female (F_0 parents, F_1 pups)	Gavage (F_0); prenatal + lactational + gavage (F_1)	42 d (F_0 males); 14 d prior to mating–LD22 (F_0 females);	prenatal–PND21 + 14 days (F_1)	ALT, AST, GGT, relative liver weight, liver histopathology
There was no observed effect of PFHxS on liver histopathology or enzymes in either the F_0 or F_1 generations. Liver weight was increased in F_0 males and females at the 1- and 3-mg/kg dose levels, and in F_1 males and females at PND21 and -36 at the 3-mg/kg dose level.							
Chappell et al.[158]	GenX	0.1, 0.5, and 5 mg/kg	Mice; CD-1; male and female	Gavage	90 d	Steatosis, liver histopathology	Histopathological changes, but no steatosis, were observed in the highest dose group.
Chengelis et al.[155]	PFHxA	10, 50, and 200 mg/kg	Rats; Sprague Dawley; male and female	Gavage	90 d	ALT, AST, liver histopathology	ALT and liver weight were elevated in males at the 200-mg/kg dose level. Histopathological changes were also only observed in males at the highest dose.
Crebelli et al.[82]	PFOA, PFBA	PFOA: 0.1, 1, and 5 mg/kg; PFBA: 5 mg/kg	Mice; C57BL/6; female	Drinking water	5 wk	ALT, AST, liver histopathology	PFOA exposure at 5 mg/kg increased ALT and AST and resulted in histopathological changes. Mild histopathological changes were observed after PFBA exposure.
Cui et al.[170]	PFOA	5 mg/kg	Mice; miR-34a$^{-/-}$ and C57BL/6J (WT); male	Gavage	28 d	ALT, AST, relative liver weight, liver histopathology	PFOA exposure increased ALT, AST, and liver weights in both strains.
Curran et al.[113]	K^+PFOS	2, 20, 50, and 100 mg/kg	Rats; Sprague Dawley; male	Diet	28 d	ALT, AST, relative liver weight	ALT was increased in male rats at the highest dose level and AST in female rats at the highest dose level. Liver weights were increased following PFOS exposure in both sexes.
Das et al.[28]	NH_4^+PFOA, PFNA, K^+PFHxS	10 mg/kg	Mice; Sv/129 (WT) and PPARα-null; male	Gavage	7 d	Steatosis, relative liver weight, liver histopathology	Steatosis was induced after exposure to any PFAS in WT mice and after exposure to PFNA and PFHxS in PPARα-null mice, as well as in control PPARα-null mice. Liver weight increased after all exposures in both strains.
Deng et al.[124]	K^+PFOS	250 mg/kg and 250 mg/kg+PCB126	Mice; C57BL/6; male	Gavage	1 d	Steatosis, ALT, AST, liver histopathology	Coexposure to PCB126 increased lipid droplets and inflammation in the liver. ALT

Table 2. (*Continued.*)

Reference	Exposure	Dose	Species/strain/sex	Exposure route	Duration of exposure	Outcomes	Main findings[a]
							and AST were also elevated in the coexposed group.
Ding et al.[151]	PFDoA	0.02, 0.05, 0.2, and 0.5 mg/kg	Rats; Sprague Dawley; male	Gavage	110 d	Steatosis, ALT, AST, relative liver weight	PFDoA induced steatosis and histopathological changes at doses >0.02 mg/kg. There were no changes to ALT or AST following exposure. Liver weight was increased at all dose levels.
Elcombe et al.[114]	K^+PFOS	20 and 100 ppm	Rats; Sprague Dawley; male	Diet	1, 7, and 28 d	ALT, AST, relative liver weight, liver histopathology	Liver weight was increased in the highest dose group after 7 and 28 d. No changes were observed in ALT or AST. Histopathological alterations increased with duration of treatment.
Elcombe et al.[115]	K^+PFOS	20 and 100 ppm	Rats; Sprague Dawley; male	Diet	7 d	ALT, AST, relative liver weight, liver histopathology	Increases in relative liver weight reduced after 28 d of recovery. ALT and AST were not elevated. Alterations to liver histopathology did not completely resolve after 28, 56, or 84 d of recovery.
Fang et al.[145]	PFNA	0.2, 1, and 5 mg/kg	Rats; Sprague Dawley; male	Gavage	14 d	ALT, AST	PFNA exposure increased ALT and AST in the 5-mg/kg dose group.
Fang et al.[144]	PFNA	0.2, 1, and 5 mg/kg	Rats; Sprague Dawley; male (diabetic)	Gavage	7 d	ALT, AST	PFNA exposure increased ALT levels in the 1- and 5-mg/kg dose groups.
Foreman et al.[149]	PFBA	35, 175, and 350 mg/kg	Mice; Sv/129 (WT), hPPARα, and PPARα-null; male	Gavage	28 d	ALT, relative liver weight, liver histopathology	PFBA induced hepatocellular hypertrophy in WT and hPPARα mice, and focal necrosis in WT. ALT was not elevated in any dose group or strain.
Guo et al.[84]	NH_4^+PFOA	0.4, 2, and 10 mg/kg	Mice; BALB/c; male	Gavage	28 d	ALT, AST, relative liver weight, liver histopathology	ALT and AST increased dose dependently. PFOA exposure increased liver weight and induced histopathological changes.
Guo et al.[90,171]	PFOA, K^+GenX	0.4, 2, and 10 mg/kg	Mice; BALB/c; male	Gavage	28 d	Steatosis, ALT, AST, relative liver weight, liver histopathology	GenX induced mild steatosis in the highest dose group, and PFOA induced steatosis in the 2- and 10-mg/kg dose groups. ALT and AST were elevated in the highest PFOA exposure group. Liver weight increased at all exposure levels.
Hamilton et al.[125]	PFOS	1 mg/kg, 1 mg/kg+HFD, 10 mg/kg, and 10 mg/kg+HFD	Mice; Cyp2b-null and hCYP2B6; male and female	Gavage	21 d	Steatosis, ALT	ALT was increased after 10 mg/kg of PFOS exposure, but less so with coexposure to HFD. Coexposure to HFD exacerbated PFOS-induced steatosis, more so in hCYP2B6 mice.
Han et al.[116]	K^+PFOS	1 and 10 mg/kg	Rats; Sprague Dawley; male	Gavage	28 d	ALT, AST, liver histopathology	ALT and AST levels increased following PFOS exposure. Changes in liver histopathology were observed.
Han et al.[117]	K^+PFOS	1 and 10 mg/kg	Rats; Sprague Dawley; male	Gavage	28 d	ALT, AST, relative liver weight, liver histopathology	ALT and AST levels increased following PFOS exposure. PFOS exposure induced histopathological changes and increases in liver weight.
Huang et al.[126]	PFOS	10 mg/kg and 10 mg/kg+GSPE	Mice; Kunming; male	Gavage	21 d	Steatosis, ALT, AST, relative liver weight, liver histopathology	PFOS induced steatosis, increased ALT and AST levels, and increased liver weight. GSPE supplementation attenuated steatosis, enzyme changes, and liver weight increases in PFOS-exposed mice.
Huck et al.[127]	PFOS	1 mg/kg and 1 mg/kg+HFD	Mice; C57BL/6J; male	Diet	6 wk	Steatosis, relative liver weight, liver histopathology	PFOS induced steatosis in mice fed standard diet. Steatosis did not develop in

Table 2. (*Continued.*)

Reference	Exposure	Dose	Species/strain/sex	Exposure route	Duration of exposure	Outcomes	Main findings[a]
							PFOS+HFD mice. A similar pattern was observed for liver weight
Hui et al.[85]	PFOA	1 and 5 mg/kg	Mice; BALB/c; male	Gavage	7 d	ALT, liver histopathology	PFOA exposure resulted in increased ALT and altered liver histopathology.
Kato et al.[152]	PFDoA	0.1, 0.5, and 2.5 mg/kg	Rats; Sprague Dawley; male and female (dams and nonpregnant females)	Gavage	42 d and 14 d prior to mating–LD5 (dams)	ALT, AST, GGT, relative liver weight, liver histopathology	No changes in ALT or GGT were observed. AST was significantly elevated in nonpregnant females 14 d after exposure ended. Liver weight increased following PFDoA exposure. Histopathological changes were observed in both sexes.
Kim et al.[148]	PFDA	10 mg/kg	Rats; Sprague Dawley; female	Intraperitoneal injection	—	ALT, AST, GGT, relative liver weight	No changes in ALT, AST, or GGT were observed at either Wk 2 or Wk 8. Relative liver weight was increased at both 2 and 8 wk postexposure.
Kim et al.[118]	K^+PFOS	1.25, 5, and 10 mg/kg	Rats; Sprague Dawley; male and female	Gavage	28 d	ALT, AST, GGT, relative liver weight, liver histopathology	AST increased in the highest dose group in males only. Altered liver histopathology was also observed in males. Liver weight increased in the highest dose group for both sexes.
Lai et al.[128]	PFOS	0.3 mg/kg	Mice; CD-1; male and female	Prenatal+DEN postnatally	E1–E18.5	ALT, AST	Elevated ALT and AST was observed in PFOS-exposed offspring after a DEN challenge.
Li et al.[86]	PFOA	1, 2.5, 5, and 10 mg/kg	Mice; Kunming; female	Prenatal	GD1–GD17	ALT, AST, relative liver weight, liver histopathology	ALT, AST, and liver weight were increased on PND21 following prenatal PFOS exposure. Histopathological alterations were observed.
Li et al.[102]	NH_4^+PFOA	1 mg/kg and 1 mg/kg+HFD	Mice; C57BL/6; male	Gavage	2, 8, and 16 wk	Steatosis, ALT, liver histopathology	No change in ALT was observed for PFOA alone, and PFOA+HFD reversed ALT increases and steatosis induced by HFD. PFOA alone and PFOA+HFD increased liver weight.
Liang et al.[141]	PFOS	0.5 and 5 mg/kg	Mice; Kunming; female (dams)	Gavage	E0.5–E20.5	Steatosis, liver histopathology	PFOS-induced histopathological changes and steatosis in dams at the highest dose level.
Lieder et al.[83]	K^+PFBS	60, 200, and 600 mg/kg	Rats; Sprague Dawley; male and female	Gavage	90 d	ALT, AST, relative liver weight, liver histopathology	No changes in ALT, AST, relative liver weight, or liver histopathology were observed after PFBS exposure.
Liu et al.[103]	PFOA	10 mg/kg and 10 mg/kg+GSPE	Mice; Kunming; male	Gavage	14 d	ALT, AST, liver histopathology	PFOA increased ALT and AST levels and altered liver histopathology, but this was attenuated with coexposure to GSPE.
Luo et al.[153]	PFDA	80 mg/kg	Mice; PPARα-null and 129/Sv (WT)	Intraperitoneal injection	One injection	ALT, AST, relative liver weight, liver histopathology	In WT mice, ALT and AST were both elevated 5 d after PFDA exposure. ALT returned to baseline levels 10 d after exposure. There were no changes in ALT or AST in PPARα-null mice, and no changes to liver histopathology in either strain after 5 d. Liver weight increased after PFDA exposure in both strains.
Lv et al.[119]	PFOS	0.5 and 1.5 mg/kg	Rats; Wistar; male and female	Prenatal and lactational	GD0-PND21	Steatosis, liver histopathology	Histopathological changes and steatosis were observed in pups from the highest dose group 19 wk after weaning.

Table 2. (*Continued.*)

Reference	Exposure	Dose	Species/strain/sex	Exposure route	Duration of exposure	Outcomes	Main findings[a]
Lv et al.[129]	PFOS	10 mg/kg and 10 mg/kg+Nar	Mice; strain not reported; male	Gavage	3 wk	ALT, AST, relative liver weight, liver histopathology	Nar coexposure attenuated changes in ALT, AST, liver weight, and histopathology induced by PFOS.
Marques et al.[139]	K^+PFOS	0.0003% wt/wt, 0.0003% wt/wt+HFD, and 0.0003% wt/wt+H-SD	Mice; C57BL/6N; male	Diet	10 wk	Steatosis, relative liver weight, liver histopathology	PFOS exposure induced steatosis in HFD and H-SD groups. PFOS also increased liver weight in all diet groups.
Marques et al.[130]	PFOA, K^+PFOS, K^+PFHxS, PFAS mixture	1 mg/kg and 1 mg/kg+HFD	Mice; CD-1; female (dams) and male and female (pups)	Gavage (dams); prenatal+lactational (pups)	Gestation (GD1–birth) and lactation (birth–PND21)	ALT, relative liver weight	ALT was elevated only in dams fed a standard diet and PFOS. PFOA and PFAS mixture exposure increased liver weights in both diet groups for dams. PFAS exposure generally increased liver weight in pups.
Martin et al.[99]	NH_4^+PFOA, K^+PFOS	PFOA: 20 mg/kg; PFOS: 10 mg/kg	Rats; Sprague Dawley; male	Gavage	1, 3, and 5 d	Steatosis, ALT, liver histopathology	Steatosis and increased liver weight were observed in both treatment groups after 3 and 5 d. Additional histopathological alterations were observed, more frequently after longer exposures. No changes in ALT were observed.
Minata et al.[96]	NH_4^+PFOA	12.5, 25, and 50 mg/kg	Mice; 129S4/SvlmJ (WT) and PPARα-null; male	Gavage	4 wk	Steatosis, ALT, AST, liver histopathology	Dose-dependent increases in ALT and AST were observed following PFOA exposure. Steatosis was present to a greater extent in all PPARα-null mice than in WT mice. Liver weights increased in all exposed mice. Histopathological evaluation suggests that the mode of toxicity is different in PPARα-null and WT mice.
Nakagawa et al.[97]	NH_4^+PFOA	1.0 and 5.0 mg/kg	Mice; Sv/129 (WT), PPARα-null, and hPPARα; male	Gavage	6 wk	Steatosis, ALT, relative liver weight, liver histopathology	Histopathological alterations differed across the three strains. Steatosis was observed in PPARα-null and hPPARα mice. ALT was elevated in all mice at the highest dose. Liver weight was increased in all exposed mice.
Owumi et al.[112]	PFOA	5 mg/kg, 5 mg/kg+NAC (25mg), and 5 mg/kg+NAC (50 mg)	Rats; Wistar; male	Gavage	28 d	ALT, AST, GGT, relative liver weight, liver histopathology	PFOA exposure increased ALT, AST, and GGT, but not when coexposed to NAC. NAC coexposure mitigated histopathological alterations induced by PFOA. There were no changes in relative liver weight.
Pfohl et al.[131]	PFOS, PFNA	3 ppm+LFD and 3 ppm+HFD	Mice; C57BL/6J; male	Diet	12 wk	Steatosis, relative liver weight	Steatosis was present in all treatment groups, but coexposure to HFD mitigated its development. Liver weight was increased in all treatment groups.
Pouwer et al.[87]	NH_4^+PFOA	10, 300, and 30,000 ng/g	Mice; APOE*3-Leiden. CETP; male	Diet	4 and 6 wk	Steatosis, ALT, liver histopathology	ALT and liver weight w ere increased in the highest dose group. Some steatosis was observed in the 10- and 300-ng/g dose groups.
Qazi et al.[104]	PFOA, NH_4^+PFOS	PFOA: 0.002% wt/wt; PFOS: 0.005% wt/wt	Mice; C57BL/6; male	Diet	10 d	ALT, AST, liver histopathology	No changes in ALT or AST were observed for either exposure. Both PFAS-induced histopathological changes.
Qazi et al.[132]	NH_4^+PFOS		Mice; C57BL/6; male	Diet	10 and 28 d		

Table 2. (*Continued.*)

Reference	Exposure	Dose	Species/strain/sex	Exposure route	Duration of exposure	Outcomes	Main findings[a]
		10 d: 0.004% wt/wt and 0.004% wt/wt+ConA; 28 d: 0.0001% wt/wt and 0.0001% wt/wt+ConA				ALT, AST, relative liver weight, liver histopathology	Coexposure of PFOS and Con A increased ALT and AST levels. Histopathological alterations were observed and liver weight increased with PFOS exposure in all study conditions.
Qazi et al.[105]	PFOA	10 d: 0.002% wt/wt+ConA; 28 d: 0.00005% wt/wt and 0.00005% wt/wt+ConA	Mice; C57BL/6; male	Diet	10 and 28 d	ALT, AST, relative liver weight, liver histopathology	Coexposure of PFOS and Con A increased ALT and AST levels in the 10-d study. Substantial histopathological alterations were only observed with PFOS exposure in the 10-d study. Liver weight increased in both exposure groups in the 10-d study, and only in the PFOA group in the 28-d study.
Qin et al.[133]	PFOS	5 mg/kg and 5 mg/kg+HFD	Mice; C57BL/6J; male	Gavage	4 wk	Steatosis, ALT, AST, relative liver weight	PFOS exposure exacerbated steatosis in HFD-fed mice. ALT, AST, and liver weights were increased in both PFOA-exposed groups.
Quist et al.[106]	NH_4^+PFOA	Prenatal: 0.01, 0.1, 0.3, and 1 mg/kg; Postnatal: 0.01 mg/kg +HFD, 0.1 mg/kg +HFD, 0.3 mg/kg +HFD, and 1 mg/kg+HFD	Mice; CD-1; female	Prenatal	GD1–GD17	ALT, AST, relative liver weight, liver histopathology	PFOA did not alter ALT or AST. Histopathological alterations were observed were observed on PND21 and became more severe by PND91 in a dose-dependent fashion. Liver weights were increased at PND21 but not at PND91.
Rigden et al.[92]	PFOA	10, 33, and 100 mg/kg	Rats; Sprague Dawley; male	Gavage	3 d	ALT, AST	Elevated ALT was observed in the 33-mg/kg dose group only 4 d after the end of treatment, and no changes in AST were observed.
Roth et al.[134]	PFAS mixture (PFOS, PFOA, PFNA, PFHxS, GenX)	0.32 mg	Mice; C57BL/6J; male and female	Drinking water	12 wk	ALT, relative liver weight, liver histopathology	ALT and liver weight increased following PFAS exposure in both males and females. PFAS exposure also resulted in alterations to liver histopathology, with more inflammation observed in females.
Schlezinger et al.[98]	PFOA	8 μM	Mice; WT, PPARα-null, and hPPARα; male and female	Drinking water	6 wk	Steatosis, relative liver weight, liver histopathology	Steatosis was present after treatment with PFOA in hPPARα mice, PPARα-null mice, and male WT mice. Liver weights increased in all genotypes.
Seacat et al.[120]	K^+PFOS	0.5, 2.0, 5.0 and 20 ppm	Rats; Sprague Dawley; male and female	Diet	4 and 14 wk	ALT, AST, GGT, relative liver weight, liver histopathology	ALT was increased in females at 4 wk and males at 14 wk in the highest dose group. Liver weight was increased in both sexes at 14 wk. Histopathological alterations were observed in 5- and 20-ppm exposed males and 20-ppm exposed females.
Shao et al.[172]	PFOA	0.05 mg/kg	Mice; CD-1; male (pups)	Prenatal	GD13-delivery	ALT, AST, liver histopathology	ALT and AST were elevated in mice exposed prenatally to PFOA. PFOA induced hepatic inflammation and histopathological alterations.
Shi et al.[173]	PFOA		Mice; C57BL/6J; male	Gavage	1D	ALT, AST, and GGT	ALT, AST, and GGT were increased after PFOA exposure. These increases were

Table 2. (*Continued.*)

Reference	Exposure	Dose	Species/strain/sex	Exposure route	Duration of exposure	Outcomes	Main findings[a]
		300 mg/kg and 300 mg/kg+11 LAB groups					mitigated with LAB exposure. PFOA also increased liver weight, which was not reduced with LAB exposure.
Son et al.[107]	NH_4^+PFOA	2, 10, 50, and 250 ppm	Mice; CD-1; male	Drinking water	21 d	ALT, AST, relative liver weight, liver histopathology	ALT, AST, and liver weight increased dose dependently. Altered liver histopathology was present after PFOA exposure.
Su et al.[135]	PFOS	10 mg/kg, 10 mg/kg+100 mg/kg VC, and 10 mg/kg+200 mg/kg VC	Mice; CD-1; male	Gavage	21 d	Steatosis, ALT, AST, liver histopathology	VC supplementation ameliorated elevations in ALT, AST, and steatosis induced by PFOS. VC supplementation also improved histopathological alterations following PFOS exposure.
Takahashi et al.[156]	PFUA	0.1, .03, and 1.0 mg/kg	Rats; Sprague Dawley; male and female (dams)	Gavage	42 d and 14 d prior to mating–LD4 (dams)	ALT, AST, GGT, relative liver weight, liver histopathology	ALT was increased in males at the 1-mg/kg dose level. Liver weights were elevated in males at dose 0.3 and 1.0 mg/kg and in females at 1.0 mg/kg. PFUA induced histopathological changes at doses >0.1 mg/kg in both sexes.
Tan et al.[108]	PFOA	5 mg/kg+LFD and 5 mg/kg+HFD	Mice; C57BL/6N; male	Diet	3 wk	ALT, AST, relative liver weight, liver histopathology	PFOA exposure increased ALT and liver weight. Coexposure to HFD exacerbated this and induced more severe histopathological changes.
Van Esterik et al.[100]	Na^+PFOA	3, 10, 30, 100, 300, 1,000, and 3,000 μg/kg	Mice; C57BL/6JxFVB; male	Prenatal+lactational	14 d prior to mating–LD21	Steatosis, relative liver weight, liver histopathology	PFOA-exposed offspring fed a HFD after weaning had increased liver weight, and more severe histopathological alterations. Steatosis was observed in the highest dose group.
Wan et al.[136]	PFOS	1, 5 and 10 mg/kg	Mice; CD-1; male	Gavage	3, 7, 14, and 21 d	Steatosis, liver histopathology	PFOS-induced steatosis in a dose- and time-dependent fashion.
Wan et al.[136]	PFOS	1 and 10 mg/kg	Rats; Sprague Dawley; male	Gavage	28 d	ALT, AST, liver histopathology	PFOS exposure increased ALT and AST levels and caused histopathological alterations.
Wang et al.[142]	PFNA	0.2, 1, and 5 mg/kg	Mice; BALB/c; male	Gavage	14 d	ALT, AST, relative liver weight	ALT and AST were elevated in the 5-mg/kg group. Liver weight increased in all dose groups.
Wang et al.[157]	GenX	1 mg/kg	Mice; CD-1; male	Gavage	28 d	ALT, AST, relative liver weight, liver histopathology	GenX exposure resulted in increased liver weight, mild steatosis, and histopathological alterations.
Wang et al.[137]	PFOS	0.3, 3, and 30 mg/kg	Mice; C57BL/6J; male	Gavage	16 d	ALT, AST, GGT, relative liver weight, liver histopathology	PFOS exposure increased ALT levels at all doses and GGT at the highest dose. Histopathology was altered and liver weights increased in all exposure groups.
Wang et al.[154]	PFDA	0.1 mM, 0.1 mM+GTPs, and 0.1 mM+EGCG	Mice; CD-1; male	Drinking water	12 d	Steatosis, ALT, AST, liver histopathology	PFDA induced steatosis. GTPs and EGCG were protective against increases in ALT and AST and against histopathological alterations.
Wang et al.[109]	PFOA	14 d: 3 and 30 mg/kg; 30 d: 2.5, 5, and 10 mg/kg	Mice; C57BL/6J; male	Gavage	14 and 30 d	ALT, AST, GGT, relative liver weight, liver histopathology	PFOA exposure increased ALT levels, altered liver histopathology and increased liver weight.
Weatherly et al.[150]	PFBA	3.75%, 7.5%, and 15% vol/vol	Mice; $B_6C_3F_1$; male and female	Dermal	28 d	ALT, relative liver weight, liver histopathology	There were no observed increases in ALT. Relative liver weight increased after exposure to PFBA.

Table 2. (*Continued.*)

Reference	Exposure	Dose	Species/strain/sex	Exposure route	Duration of exposure	Outcomes	Main findings[a]
Wu et al.[174]	PFOA	5 mg/kg	Mice; Kunming; male	Gavage	1 d	ALT and AST	ALT and AST levels were not significantly increased following exposure.
Wu et al.[91]	PFOA	1 and 5 mg/kg	Mice; Kunming; female	Gavage	21 d	ALT, AST, relative liver weight, liver histopathology	PFOA exposure increased ALT, AST, and relative liver weight in the highest dose group only. Liver histopathology was altered in both dose groups.
Xing et al.[138]	PFOS	14 d: 30, 40, 50, 60, and 70 mg/kg; 30 d : 2.5, 5, and 10 mg/kg	Mice; C57BL/6J; male	Gavage	14 and 30 d	ALT, ASTGGT, liver histopathology	PFOS exposure resulted in histopathological alteration and increased ALT and AST in a dose-dependent fashion.
Yahia et al.[88]	PFOA	1, 5, and 10 mg/kg	Mice; CD-1; female (dams)	Gavage	GD0–GD17/18	ALT, AST, GGT, relative liver weight, liver histopathology	Histopathological alterations and elevated ALT, AST, and GGT were observed in the highest dose group. PFOA exposure increased liver weight in a dose-dependent fashion.
Yan et al.[89]	PFOA, PFOS	PFOA: 0.08, 0.31, 1.25, 5, and 20 mg/kg; PFOS: 1.25 and 5 mg/kg	Mice; BALB/c; male	Gavage	28 d	ALT, AST, relative liver weight	ALT and AST were increased at the highest PFOA and PFOS exposure group. Liver weight increased in all but the lowest dose of PFOA.
Yan et al.[175]	PFOA	5 mg/kg+125 mg/kg 4-PBA and 5 mg/kg+250 mg/kg 4-PBA	Mice; BALB/c; male	Gavage	28 d	ALT, AST, relative liver weight	ALT and liver weight increased in all PFOA-exposed groups. AST increased in the PFOA-only treatment group.
Yang et al.[110]	PFOA	2.5, 5, and 10 mg/kg	Mice; Kunming; male	Gavage	14 d	ALT, AST, relative liver weight, liver histopathology	ALT levels increased in a dose-dependent manner. AST was increased at the two highest dose levels. Histopathological alterations and liver weight increases were seen in all dose groups, and were more severe at the highest dose.
Zhang et al.[140]	K^+PFOS	0.003% wt/wt, 0.003% wt/wt+mMCD, 0.006% wt/wt 0.006% wt/wt+mMCD, 0.012% wt/wt 0.012% wt/wt+mMCD, and 0.003% wt/wt+CS	Mice; C57BL/6; male	Diet	21 d (mMCD) and 6 wk (CS)	Steatosis, ALT, relative liver weight, liver histopathology	PFOS increased ALT and liver weight, and induced histopathological changes and steatosis. Toxicity was exacerbated in the PFOS+mMCD group and attenuated with CS coexposure.
Zhang et al.[143]	PFNA	0.1 mmol/kg	Mice; C57BL/6 (WT), PPARα-null, and CAR-null; male	Intraperitoneal injection	One injection	ALT, relative liver weight, liver histopathology	PFNA increased liver weight in all three strains after 14 d. After 1 wk, ALT was elevated in the WT and CAR-null mice. Alterations in histopathology were observed after 14 and 90 d.
Zou et al.[111]	PFOA	10 mg/kg and 10 mg/kg+Que	Mice; Kunming; male	Gavage	14 d	ALT, AST, liver histopathology	Coexposure to Que decreased PFOA induced ALT and AST levels and ameliorated histopathological changes.

Notes: 4-PBA, 4-phenylbutyrate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAR, constitutive antigen receptor; Con A, Concanavalin A; CS, choline supplementation; DEN, diethylnitrosamine; E, embryonic day; EGCG, epi-gallocatechin-3-gallate; GD, gestation day; GenX, hexafluoropropylene dimer acid; GGT, gamma-glutamyl transferase; GSPE, grape seed proanthocyanidin extract; GTP, green tea polyphenol; HFD, high-fat diet; hPPAR, humanized peroxisome proliferator-activated receptor; H-SD, high-fat diet to standard diet; K^+, potassium ion; LAB, lactic acid bacteria; LD, lactation day; LFD, low-fat diet; mMCD, marginal methionine/choline-deficient diet; NAC, *N*-acetylcysteine; Nar, naringin; NH_4^+, ammonium ion; PCB, polychlorinated biphenyl; PFAS, per- and polyfluorinated substances; PFBA, perfluorobutanoic acid; PFBS, perfluorobutane sulfonate; PFDA perfluorodecanoic acid; PFHxA, perfluorohexanoic acid; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate; PFUA, perfluoroundecanoic acid; PHDoA, perfluorododecanoic acid; PND, postnatal day; POSF, perfluorooctanesulfonyl fluoride; PPAR, peroxisome proliferator-activated receptor; Que, quercetin; SD, standard diet; VC, vitamin C; WT, wild type.
[a]Findings presented here are limited to those related to the markers of liver injury investigated in this review.

Overall, exposure to PFOA in rodents was associated with elevated mean serum ALT (Figure 2). Twenty-one mouse studies observed a statistically significant difference in mean serum ALT in treatment groups relative to unexposed controls. Of these, 10 studies observed a statistically significant positive association at higher doses and no effect at lower doses, suggesting a dose-dependent relationship.[81,82,84–91] However, these results did not reveal an obvious threshold for lowest dose of observed effect. Of the 4 studies in Sprague Dawley rats, 3 found a statistically significant relationship between PFOA exposure and ALT.[92–94] Most studies included only males, and the few studies including both males and females observed no consistent differences by sex in effects on ALT levels.[94,95] Studies also reported elevated AST or liver weight in PFOA-exposed rodents (Figures S1 and S2). PFOA exposure in adult mice and rats frequently induced steatosis.[28,84,87,90,96–99] Only 1 study investigated prenatal PFOA exposure and development of steatosis in adulthood and no association was found.[100] Other reported histopathological alterations included hepatocellular hypertrophy and necrosis in both mice[28,81,82,84,86–88,96,97,101–111] and rats.[93,94,99,112]

Exposure to PFOS

Human studies. Six cross-sectional studies assessing the relationship between PFOS and ALT in adults and adolescents (≥12 years of age) were included in the weighted z-score calculation.[40,41,67,70,72,75] A weighted z-score of 3.55 ($p < 0.001$) suggested a positive association between PFOS and ALT (Table 3). After including two studies in children (<12 years of age),[74,76] the association remained statistically significant (z-score = 3.27, $p < 0.001$); however, the association was no longer statistically significant in sensitivity analyses that removed the largest study[70] (z-score = 1.11, $p = 0.27$) or that restricted the analysis to only those studies using NHANES data[40,67,72,75] (z-score = 0.90, $p = 0.37$) (Table S3). No statistically significant associations between PFOS and ALT were reported in children in either cross-sectional[74,76] or longitudinal[76] analyses. Weighted z-scores did not suggest a relationship between PFOS and GGT when including all eligible studies (z-score = 1.13, $p = 0.26$)[40,41,67,70,72,75] or in sensitivity analyses (Table S3) or between PFOS and AST (z-score = 0.37, $p = 0.72$) in adults (Table S4).[40,41,67,72] One longitudinal analysis reported a positive association with ALT,[42] but none found any relationship between PFOS and other liver enzymes.[37,42]

Rodent studies. Among rodent studies, 13 studies assessed exposure to PFOS in rats[29,95,99,113–122] and 19 assessed PFOS exposure in mice[28,89,104,123–138] (Table 2). PFOS exposure consistently increased serum ALT in mice (Figure 3). This effect was also observed in rats, although several studies did not report any effect of PFOS on ALT levels.[99,114,118] Many mouse studies also observed increases in AST after PFOS exposure (Figure S3), and both mouse and rat studies reported increases in liver weight following PFOS exposure (Figure S4). PFOS exposure was also shown to induce steatosis in mice and rats.[99,118,123,125–127,131,133,139–141] Prenatal exposure also resulted in steatosis in Wistar rats.[119] Hepatocellular hypertrophy and necrosis were also consistently observed after PFOS exposure in both mice[104,129,132,135,137,138] and rats.[29,95,99,113–118,120–122]

Exposure to PFNA

Human studies. Five cross-sectional studies assessing the relationship between PFNA and ALT in adults and adolescents were included in the weighted z-score calculation.[40,41,67,72,75] A weighted z-score of 2.27 ($p = 0.023$) suggested a positive relationship between PFNA and ALT (Table 3). Owing to the limited number of available studies, no sensitivity analyses were performed for this weighted z-score. Mora et al.[76] reported a statistically significant negative association in cross-sectional analyses of PFNA and ALT in boys only, although no statistically significant associations were found for children overall in either cross-sectional or longitudinal analyses by either Mora et al.[76] or Khalil et al.[74] There was no relationship between PFNA and GGT (z-score = 1.45, $p = 0.15$)[40,41,67,72,75] or AST (z-score = 0.95, $p = 0.35$) in adults (Table S4).[40,41,67,72] Mundt et al.[35] found no difference in mean ALT, GGT, or AST between production workers with low, high, or no occupational exposure to PFNA. Salihovic et al. reported a positive association between PFNA and ALT, but no relationship was found between PFNA and GGT in a longitudinal analysis.[42]

Rodent studies. Six studies evaluated exposure to PFNA and markers of liver injury in mice or rats. Results consistently demonstrated elevated ALT, steatosis, and hepatocellular hypertrophy in treatment groups compared with controls in both mice[28,131,142,143] and rats.[144,145]

Exposure to PFHxS

Human studies. Five cross-sectional studies assessing the relationship between PFHxS and ALT in adults and adolescents were included in the weighted z-score calculation.[40,41,67,72,75] A weighted z-score of 1.42 ($p = 0.15$) did not suggest any relationship between PFHxS and ALT (Table 3). No sensitivity analyses were performed for this weighted z-score because of the limited number of available studies. One longitudinal study reported a positive association between PFHxS and ALT.[42] Studies in children reported no relationship between PFHxS and ALT.[74,76] Likewise, weighted z-scores did not indicate a relationship between PFHxS and GGT (z-score = 0.66, $p = 0.52$)[40,41,67,72,75] or between PFHxS and AST (z-score = 1.50, $p = 0.13$) in adults (Table S4).[40,41,67,72]

Rodent studies. Five studies examined the effects of PFHxS on liver outcomes. Two studies in mice[130,146] and one in rats[147] investigated the effects of PFHxS exposure on liver enzymes. No alterations in ALT or AST were observed in adult male rats or rat dams, or in mouse dams or pups.[130,146,147] However, PFHxS-induced steatosis and hepatocellular hypertrophy at doses of >3 mg/kg per day in the one rat and two mouse studies that reported histopathological results.[28,123,147]

Exposure to Other PFAS

Findings among studies assessing exposure to other PFAS (PFDA, PFHxA, PFHpA, PFBS, PFBA, PFDoA, PFHxA, PFDoA, and GenX) were not consistent (Table 1). For instance, Nian et al. observed a positive relationship between PFDA and ALT in humans,[41] whereas several other human studies found no relationship.[42,72,77] Positive associations of human exposure to PFHxA[77] and PFHpA[42] with ALT were observed. Our search identified only one study that evaluated the effects of PFAS as a mixture in humans and found that higher prenatal PFAS exposure was associated with increased risk for livery injury in childhood, based on ALT, AST, and GGT percentiles.[78] This finding suggests that, even if certain individual PFAS exert minor or no effects on the liver, the overall effect of multiple exposures may be detrimental.

No changes in ALT were reported after exposure to PFDA in rats,[148] PFBS in rats,[83] PFBA in mice or rats,[82,93,149,150] or PFDoA in rats.[151,152] Elevated ALT was reported following exposure to PFDA in mice,[153,154] and PFHxA[155] and PFUA[156] in male but not female rats . PFDA[154] and PFDoA[151] exposure was also shown to result in steatosis in mice and rats, respectively, whereas PFBS exposure in mice did not.[123]

Table 3. Strip plots for the *z*-scores of the analyses of PFAS on ALT.

Reference	Population	Age (y)	Sex	Weight	*n*	Exposure	PFAS Blood Conc.	*z*-Score (*p*-value)
PFOA (cross-sectional studies)								
Sakr et al.[43,a]	GHS	≥18	Overall	All	1,024	PFOA	0.428 ppm[b]	1.53 (0.13)
Olsen and Zobel[38,a]	Plant employees	21–67	Male	All	506	PFOA	2,210 ng/mL[b]	−0.59 (0.56)
Emmett et al.[69]	Little Hocking, Ohio	2–90	Overall	All	371	PFOA	354 ng/mL[c]	0.45 (0.67)
Gallo et al.[70,a]	C8HP	≥18	Overall	All	46,452	PFOA	28.0 ng/mL[c]	12.32 (<0.001)
Darrow et al.[39]	C8HP	>20	Overall	All	28,047	PFOA	NS	6.72 (<0.001)
Darrow et al.[39]	C8HP	>20	Male	All	12,364	PFOA	17.1 ng/mL[c]	4.63 (<0.001)
Darrow et al.[39]	C8HP	>20	Female	All	15,683	PFOA	16.0 ng/mL[c]	3.92 (<0.001)
Nian et al.[41,a]	I C8HP	22–95	Overall	All	1,605	PFOA	6.19 ng/mL[c]	4.23 (<0.001)
Lin et al.[75,a]	NHANES 1999–2003	≥20	Overall	All	2,197	PFOA	4.51 ng/mL[b]	2.99 (0.003)
Lin et al.[75]	NHANES 1999–2003	≥20	Male	All	1,063	PFOA	5.05 ng/mL[b]	1.85 (0.064)
Lin et al.[75]	NHANES 1999–2003	≥20	Female	All	1,134	PFOA	4.06 ng/mL[b]	1.65 (0.098)
Gleason et al.[40,a]	NHANES 2007–2010	≥12	Overall	All	4,333	PFOA	3.5 ng/mL[d]	3.10 (0.002)
Jain and Ducatman[72,a]	NHANES 2011–2014	≥20	Overall	Non-obese	1,082	PFOA	2.2 ng/mL[d]	0.22 (0.84)
Jain and Ducatman[72,a]	NHANES 2011–2014	≥20	Overall	Obese	1,801	PFOA	2.0 ng/mL[d]	3.17 (0.002)
Attanasio[67,a]	NHANES 2013–2016	12–19	Male	All	354	PFOA	1.50 ng/mL[d]	−2.29 (0.022)
Attanasio[67,a]	NHANES 2013–2016	12–19	Female	All	305	PFOA	1.22 ng/mL[d]	2.35 (0.019)
Mora et al.[76]	Project Viva	6–11	Overall	All	630	PFOA	4.3 ng/mL[c]	−0.35 (0.74)
Mora et al.[76]	Project Viva	6–11	Male	All	332	PFOA	4.4 ng/mL[c]	−1.18 (0.24)
Mora et al.[76]	Project Viva	6–11	Female	All	298	PFOA	4.2 ng/mL[c]	−1.96 (0.050)
Khalil et al.[74]	DCH	8–12	Overall	Obese	48	PFOA	0.99 ng/mL[c]	1.62 (0.11)
Weighted *z*-score								6.20 (<0.001)
PFOA (longitudinal studies)								
Sakr et al.[44,a]	GHS	≥18	Overall	All	205	PFOA	1.13 ppm[b]	1.06 (0.29)
Darrow et al.[39,a]	C8HP	>20	Overall	All	28,047	PFOA	NS	5.88 (<0.001)
Darrow et al.[39]	C8HP	>20	Male	All	12,364	PFOA	17.1 ng/mL[c]	4.57 (<0.001)
Darrow et al.[39]	C8HP	>20	Female	All	15,683	PFOA	16.0 ng/mL[c]	3.92 (<0.001)
Salihovic et al.[42,a]	Swedish	70	Overall	All	1,002	PFOA	3.31 ng/mL[c]	5.20 (<0.001)
Mora et al.[76]	Project Viva	6–11	Overall	All	508	PFOA	5.4 ng/mL[c]	−1.31 (0.19)
Mora et al.[76]	Project Viva	6–11	Male	All	273	PFOA	5.5 ng/mL[c]	−0.89 (0.38)
Mora et al.[76]	Project Viva	6–11	Female	All	235	PFOA	5.4 ng/mL[c]	−1.31 (0.19)
Weighted *z*-score								5.12 (<0.001)
PFOS (cross-sectional studies)								
Gallo et al.[70,a]	C8HP	≥18	Overall	All	46,452	PFOS	20.3 ng/mL[c]	6.53 (<0.001)
Nian et al.[41,a]	I C8HP	22–95	Overall	All	1,605	PFOS	24.22 ng/mL[c]	2.31 (0.021)
Lin et al.[75,a]	NHANES 1999–2003	≥20	Overall	All	2,216	PFOS	24.6 ng/mL[b]	1.90 (0.057)
Gleason et al.[40,a]	NHANES 2007–2010	≥12	Overall	All	4,333	PFOS	11.3 ng/mL[c]	1.19 (0.24)
Jain and Ducatman[72,a]	NHANES 2011–2014	≥20	Overall	Non-obese	1,082	PFOS	6.3 ng/mL[d]	−1.02 (0.31)
Jain and Ducatman[72,a]	NHANES 2011–2014	≥20	Overall	Obese	1,801	PFOS	5.5 ng/mL[d]	1.26 (0.21)
Attanasio[67,a]	NHANES 2013–2016	12–19	Male	All	354	PFOS	3.68 ng/mL[d]	0.21 (0.85)
Attanasio[67,a]	NHANES 2013–2016	12–19	Female	All	305	PFOS	2.76 ng/mL[d]	1.86 (0.063)
Mora et al.[76]	Project Viva	6–11	Overall	All	630	PFOS	6.2 ng/mL[c]	−1.07 (0.29)
Mora et al.[76]	Project Viva	6–11	Male	All	332	PFOS	6.3 ng/mL[c]	−0.69 (0.50)
Mora et al.[76]	Project Viva	6–11	Female	All	298	PFOS	6.1 ng/mL[c]	−1.21 (0.23)
Khalil et al.[74]	DCH	8–12	Overall	Obese	48	PFOS	2.79 ng/mL[c]	0.16 (0.88)
Weighted *z*-score								3.55 (<0.001)
PFNA (cross-sectional studies)								
Nian et al.[41,a]	I C8HP	22–95	Overall	All	1,605	PFNA	1.96 ng/mL[c]	3.86 (<0.001)
Lin et al.[75,a]	NHANES 1999–2003	≥20	Overall	All	2,216	PFNA	0.79 ng/mL[b]	1.55 (0.12)
Gleason et al.[40,a]	NHANES 2007–2010	≥12	Overall	All	4,333	PFNA	1.2 ng/mL[d]	3.51 (<0.001)
Jain and Ducatman[72,a]	NHANES 2011–2014	≥20	Overall	Non-obese	1,082	PFNA	0.83 ng/mL[d]	0.47 (0.65)
Jain and Ducatman[72,a]	NHANES 2011–2014	≥20	Overall	Obese	1,801	PFNA	0.73 ng/mL[d]	3.53 (<0.001)
Attanasio[67,a]	NHANES 2013–2016	12–19	Male	All	354	PFNA	0.58 ng/mL[d]	−2.49 (0.013)
Attanasio[67,a]	NHANES 2013–2016	12–19	Female	All	305	PFNA	0.49 ng/mL[d]	3.02 (0.003)
Mora et al.[76]	Project Viva	6–11	Overall	All	630	PFNA	1.5 ng/mL[c]	−2.94 (0.003)
Mora et al.[76]	Project Viva	6–11	Male	All	332	PFNA	1.5 ng/mL[c]	−3.92 (<0.001)
Mora et al.[76]	Project Viva	6–11	Female	All	298	PFNA	1.5 ng/mL[c]	−1.31 (0.19)
Khalil et al.[74]	DCH	8–12	Overall	Obese	48	PFNA	0.24 ng/mL[c]	−0.18 (0.86)
Weighted *z*-score								2.27 (0.023)
PFHxS (cross-sectional studies)								
Nian et al.[41,a]	I C8HP	22–95	Overall	All	1,605	PFHxS	0.73 ng/mL[c]	0.39 (0.71)
Lin et al.[75,a]	NHANES 1999–2003	≥20	Overall	All	2,216	PFHxS	1.98 ng/mL[b]	0.40 (0.71)
Gleason et al.[40,a]	NHANES 2007–2010	≥12	Overall	All	4,333	PFHxS	1.8 ng/mL[d]	2.61 (0.009)
Jain and Ducatman[72,a]	NHANES 2011–2014	≥20	Overall	Non-obese	1,082	PFHxS	1.41 ng/mL[d]	0.26 (0.81)
Jain and Ducatman[72,a]	NHANES 2011–2014	≥20	Overall	Obese	1,801	PFHxS	1.24 ng/mL[d]	3.33 (<0.001)
Attanasio[67,a]	NHANES 2013–2016	12–19	Male	All	354	PFHxS	1.31 ng/mL[d]	0.49 (0.64)
Attanasio[67,a]	NHANES 2013–2016	12–19	Female	All	305	PFHxS	0.88 ng/mL[d]	2.35 (0.019)

Table 3. (*Continued.*)

Reference	Population	Age (y)	Sex	Weight	*n*	Exposure	PFAS Blood Conc.	*z*-Score (*p*-value)
Mora et al.[76]	Project Viva	6–11	Overall	All	630	PFHxS	1.9 ng/mL[c]	0.00 (1.0)
Mora et al.[76]	Project Viva	6–11	Male	All	332	PFHxS	1.9 ng/mL[c]	−0.65 (0.52)
Mora et al.[76]	Project Viva	6–11	Female	All	298	PFHxS	1.9 ng/mL[c]	0.78 (0.44)
Khalil et al.[74]	DCH	8–12	Overall	Obese	48	PFHxS	1.09 ng/mL[c]	0.08 (0.94)
Weighted *z*-score								1.42 (0.15)

Notes: Both overall and sex-specific results are presented where available. ALT, alanine aminotransferase; C8HP, C8 Health Project; DCH, Dayton Children's Hospital; GHS, General Health Survey; I C8HP, Isomers of C8 Health Project; NHANES, National Health and Nutrition Examination Survey; NS, not specified; PFAS, per- and polyfluorinated substances; PFHxS, perfluorohexanesulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid.
[a]The weighted *z* -score calculation was performed for those ≥12 years of age, using the larger of overlapping cohorts.
[b]Mean.
[c]Median.
[d]Geometric mean.

Few rodent studies evaluated the effects of GenX exposure on liver injury, and no eligible human studies evaluated this relationship. In mice, three studies reported that exposure to GenX resulted in steatosis[90,157] or histopathological changes,[81] although there were no changes in liver enzyme levels . A fourth study in mice did not find any significant histopathological changes or steatosis following GenX administration.[158]

Two studies in mice evaluated the effects of PFAS mixtures. In one, a mixture of PFOA, PFOS, and PFHxS was not found to alter ALT levels in pregnant dams fed either standard or high-fat diet or in their offspring.[130] In the other, a mixture of PFOS, PFOA, PFNA, PFHxS, and GenX was found to increase ALT levels and alter liver histopathology in adult males and females.[134]

Discussion

This systematic review summarizes the body of evidence linking markers of liver injury with exposure to PFOA, PFOS, PFHxS, and PFNA, the most commonly studied PFAS. Meta-analysis in human studies provided convincing evidence that exposure to PFOA, PFOS, and PFNA are associated with higher serum ALT. Rodent studies have consistently demonstrated a positive relationship between exposure to PFOA and PFOS and serum ALT as well as relative liver weight, which may indicate accumulation of excess liver fat. We also found evidence to suggest a positive association between PFNA and ALT. Findings in rodents were largely consistent across studies that differed in exposure routes and duration. Many rodent studies exposed animals to doses far above expected human exposures; this is due to differences in PFAS elimination and half-lives in mice and rats relative to humans[34] and does not preclude comparison with human research. The findings of the present review indicate consistency of results across human and rodent studies, adding support to the idea that associations found in observational human studies may be causal.

Per- and polyfluorinated compounds were first detected in the blood of occupationally exposed workers in the 1970s and in the general population in the 1990s, which brought awareness of their potential health risks.[7] The hydrophobic and oleophobic properties of the carbon–fluorine bond make PFAS ideal for industrial use in flame retardants and surfactants yet also allow them to persist in the environment, with concerning implications for long-term health effects. Although manufacturers started to phase out the production of PFOS and other long-chain PFAS in the early 2000s, the Centers for Disease Control and Prevention still reports widespread PFAS exposure in U.S. adults, demonstrating their persistence in biological systems and the continued public health relevance of the present review.[21,22] Of additional concern, newer PFAS that have replaced the legacy PFAS for industrial use, such as GenX, have similar chemical structure and properties. The limited studies of these replacement PFAS suggest that they may have toxic effects similar to the legacy chemicals.[157,159]

The exact mechanism of PFAS hepatotoxicity remains unresolved. PFAS are thought to promote liver inflammation and triglyceride accumulation through activation of both human and mouse peroxisome proliferator-activated receptor alpha (PPARα) and other receptors given their structural similarities with fatty acids.[28,96–98,143,149,153,158,160] Consequently, altered lipid metabolism has been associated with PFAS exposure in both human[46,54,73,78] and animal studies.[28,32,85,129,136] Although much of the mechanistic research has been done using mouse models, cell-culture studies evaluating comparability of this mechanism in both mouse and human receptors have demonstrated that PFAS similarly activate human PPARα[161–163] However, PFAS-induced liver injury and steatosis may not depend on PPARα alone.[164] Alternate or complementary mechanisms may involve activation of constitutive androstane receptor (CAR),[98,143] down-regulation of nuclear factor erythroid 2-related factor 2 (NRF2),[121,129] and up-regulation of nuclear factor kappa-light-chain-enhancer of activated B cells nuclear factor-kappa B (NF-κB).[145] An additional possibility suggests that PFAS may reduce the bioavailability of choline, leading to steatosis as a result of choline deficiency.[29,140]

Several studies in mice examined the effects of PFAS exposure with coexposure to either a dietary supplement or high-fat diet. Supplementation with antioxidants was consistently found to ameliorate PFAS-induced liver injury.[103,111,126,129,135,137] The effects of PFAS exposure in populations consuming high-fat diets were mixed; studies have found that PFAS exposure in rodents exacerbates the effect of high-fat diets on liver injury,[108,125,139] although others have reported potentially protective effects.[102,127,131] It is possible that the mechanisms by which PFAS induce liver injury are altered when liver homeostasis is already disrupted. These findings have not been replicated or studied extensively in humans, although there is some evidence that the relationship between PFAS and ALT may be mediated by metabolic disease or obesity.[72,165]

The parallel findings in experimental rodent studies identified in the present review address the limitations of observational findings and provide comprehensive evidence to suggest hepatotoxic effects of PFAS exposure. Many human studies, because of limited access to histopathological and imaging data for asymptomatic participants, limit analyses to liver enzymes and other biomarkers than can be easily measured in blood samples. Although levels of ALT and other enzymes are relatively specific indicators of liver injury, the exact nature or severity of the injury cannot be determined without more invasive procedures.[51] However, it is well understood that populations that have higher levels of ALT also experience higher mortality and morbidity related to liver disease, and mild elevations of ALT in individuals may suggest the presence of NAFLD.[57] Animal studies report similar increases in liver enzymes and pathological alterations to the structure and function

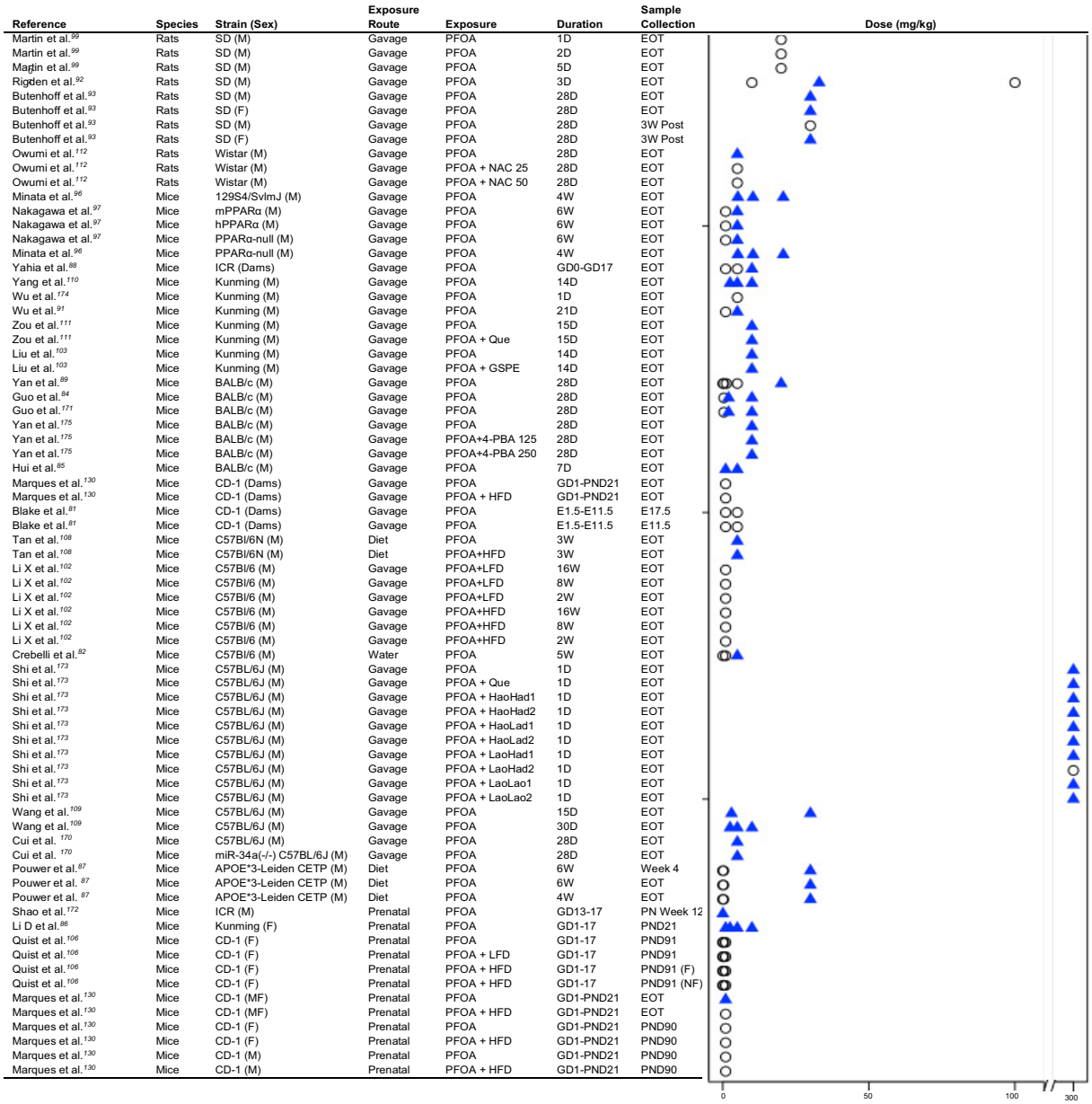
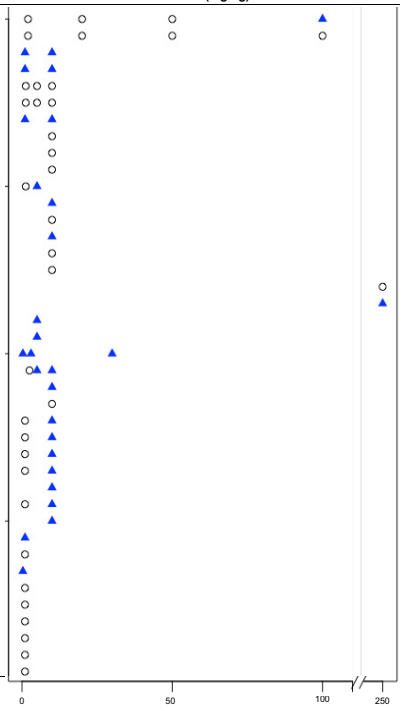


Figure 2. Strip plots for PFOA and ALT in animal studies. Triangles indicate a significant increase in ALT relative to control. Circles indicate no significant change in ALT relative to control. Additional exposures in Shi et al.[173] refer to lactic acid bacterial strains. An accessible version of this figure is available in Table S5. Note: 4-PBA, 4-phenylbutyric acid; ALT, alanine aminotransferase; Con A, concanavalin A; D, day; E, embryonic day; EOT, end of treatment; F, female; GD, gestational day; GSPE, grape seed proanthocyanidin extract; HFD, high-fat diet; hPPAR, humanized peroxisome proliferator-activated receptor; LFD, low-fat diet; M, male; mPPAR, mouse peroxisome proliferator-activated receptor; NAC, *N*-acetylcysteine; PFOA, perfluorooctanoic acid; PND, postnatal day; Que, quecertin; SD, Sprague Dawley; W, week; Y, year.

Reference	Species	Strain (Sex)	Exposure Route	Exposure	Duration	Sample Collection
Curran et al.[113]	Rats	SD (M)	Diet	PFOS	28D	EOT
Curran et al.[113]	Rats	SD (F)	Diet	PFOS	28D	EOT
Han et al.[116]	Rats	SD (M)	Gavage	PFOS	28D	EOT
Han et al.[117]	Rats	SD (M)	Gavage	PFOS	28D	EOT
Kim et al.[118]	Rats	SD (M)	Gavage	PFOS	28D	EOT
Kim et al.[118]	Rats	SD (F)	Gavage	PFOS	28D	EOT
Wan et al.[121]	Rats	SD (M)	Gavage	PFOS	28D	EOT
Martin et al.[99]	Rats	SD (M)	Gavage	PFOS	1D	EOT
Martin et al.[99]	Rats	SD (M)	Gavage	PFOS	2D	EOT
Martin et al.[99]	Rats	SD (M)	Gavage	PFOS	5D	EOT
Yan et al.[69]	Mice	BALB/c (M)	Gavage	PFOS	28D	EOT
Lv et al.[129]	Mice	- (M)	Gavage	PFOS	21D	EOT
Lv et al.[129]	Mice	- (M)	Gavage	PFOS + Nar	21D	EOT
Su et al.[135]	Mice	ICR (M)	Gavage	PFOS	21D	EOT
Su et al.[135]	Mice	ICR (M)	Gavage	PFOS + VC100	21D	EOT
Su et al.[135]	Mice	ICR (M)	Gavage	PFOS + VC200	21D	EOT
Deng et al.[124]	Mice	C57BL/6 (M)	Gavage	PFOS	1D	2D Post
Deng et al.[124]	Mice	C57BL/6 (M)	Gavage	PFOS + PCB126	1D	2D Post
Qin et al.[133]	Mice	C57BL/6J (M)	Gavage	PFOS	4W	EOT
Qin et al.[133]	Mice	C57BL/6J (M)	Gavage	PFOS + HFD	4W	EOT
Wang G et al.[137]	Mice	C57BL/7 (M)	Gavage	PFOS	16D	EOT
Xing et al.[138]	Mice	C57BL/7 (M)	Gavage	PFOS	30D	EOT
Huang et al.[126]	Mice	Kunming (M)	Gavage	PFOS	21D	EOT
Huang et al.[126]	Mice	Kunming (M)	Gavage	PFOS + GSPE	21D	EOT
Hamilton et al.[125]	Mice	hCYP2B6-Tg (M)	Gavage	PFOS	3W	EOT
Hamilton et al.[125]	Mice	Cyp2b-null (M)	Gavage	PFOS	3W	EOT
Hamilton et al.[125]	Mice	hCYP2B6-Tg (F)	Gavage	PFOS	3W	EOT
Hamilton et al.[125]	Mice	Cyp2b-null (F)	Gavage	PFOS	3W	EOT
Hamilton et al.[125]	Mice	hCYP2B6-Tg (M)	Gavage	PFOS + HFD	3W	EOT
Hamilton et al.[125]	Mice	hCYP2B6-Tg (F)	Gavage	PFOS + HFD	3W	EOT
Hamilton et al.[125]	Mice	Cyp2b-null (F)	Gavage	PFOS + HFD	3W	EOT
Marques et al.[130]	Mice	CD-1 (dams)	Gavage	PFOS	GD1-PND21	EOT
Marques et al.[130]	Mice	CD-1 (dams)	Gavage	PFOS + HFD	GD1-PND21	EOT
Lai et al.[128]	Mice	C57BL/7 (MF)	Prenatal	PFOS + DEN	E0-E18.5	EOT
Marques et al.[130]	Mice	CD-1 (MF)	Prenatal	PFOS	GD1-PND21	EOT
Marques et al.[130]	Mice	CD-1 (MF)	Prenatal	PFOS + HFD	GD1-PND21	EOT
Marques et al.[130]	Mice	CD-1 (F)	Prenatal	PFOS	GD1-PND21	PND90
Marques et al.[130]	Mice	CD-1 (F)	Prenatal	PFOS + HFD	GD1-PND21	PND90
Marques et al.[130]	Mice	CD-1 (M)	Prenatal	PFOS	GD1-PND21	PND90
Marques et al.[130]	Mice	CD-1 (M)	Prenatal	PFOS + HFD	GD1-PND21	PND90



Reference	Species	Strain (Sex)	Exposure Route	Exposure	Duration	Sample Collection
Seacat et al.[120]	Rats	SD (F)	Diet	PFOS	14W	EOT
Seacat et al.[120]	Rats	SD (M)	Diet	PFOS	14W	EOT
Elcombe et al.[115]	Rats	SD (M)	Diet	PFOS	7D	1D Post
Elcombe et al.[115]	Rats	SD (M)	Diet	PFOS	7D	28D Post
Elcombe et al.[115]	Rats	SD (M)	Diet	PFOS	7D	56D Post
Elcombe et al.[115]	Rats	SD (M)	Diet	PFOS	7D	84D Post
Elcombe et al.[114]	Rats	SD (M)	Diet	PFOS	1D	EOT
Elcombe et al.[114]	Rats	SD (M)	Diet	PFOS	7D	EOT
Elcombe et al.[114]	Rats	SD (M)	Diet	PFOS	28D	EOT
Butenhoff et al.[95]	Rats	SD (F)	Diet	PFOS	52W	W4
Butenhoff et al.[95]	Rats	SD (F)	Diet	PFOS	52W	W14
Butenhoff et al.[95]	Rats	SD (F)	Diet	PFOS	52W	W27
Butenhoff et al.[95]	Rats	SD (F)	Diet	PFOS	52W	EOT
Butenhoff et al.[95]	Rats	SD (M)	Diet	PFOS	52W	W4
Butenhoff et al.[95]	Rats	SD (M)	Diet	PFOS	52W	W14
Butenhoff et al.[95]	Rats	SD (M)	Diet	PFOS	52W	W27
Butenhoff et al.[95]	Rats	SD (M)	Diet	PFOS	52W	EOT
Bagley et al.[29]	Rats	SD (M)	Diet	PFOS	2D	EOT
Bagley et al.[29]	Rats	SD (M)	Diet	PFOS + CS	2D	EOT
Bagley et al.[29]	Rats	SD (M)	Diet	PFOS	9D	EOT
Bagley et al.[29]	Rats	SD (M)	Diet	PFOS + CS	9D	EOT
Bagley et al.[29]	Rats	SD (M)	Diet	PFOS	16D	EOT
Bagley et al.[29]	Rats	SD (M)	Diet	PFOS + CS	16D	EOT
Bagley et al.[29]	Rats	SD (M)	Diet	PFOS	23D	EOT
Bagley et al.[29]	Rats	SD (M)	Diet	PFOS + CS	23D	EOT
Bagley et al.[29]	Rats	SD (F)	Diet	PFOS	2D	EOT
Bagley et al.[29]	Rats	SD (F)	Diet	PFOS + CS	2D	EOT
Bagley et al.[29]	Rats	SD (F)	Diet	PFOS	9D	EOT
Bagley et al.[29]	Rats	SD (F)	Diet	PFOS + CS	9D	EOT
Bagley et al.[29]	Rats	SD (F)	Diet	PFOS	16D	EOT
Bagley et al.[29]	Rats	SD (F)	Diet	PFOS + CS	16D	EOT
Bagley et al.[29]	Rats	SD (F)	Diet	PFOS	23D	EOT
Bagley et al.[29]	Rats	SD (F)	Diet	PFOS + CS	23D	EOT
Butenhoff et al.[122]	Rats	SD (M)	Inhalation	PFOS	13W*	EOT
Butenhoff et al.[122]	Rats	SD (M)	Inhalation	PFOS	13W*	4W Post
Butenhoff et al.[122]	Rats	SD (F)	Inhalation	PFOS	13W*	EOT
Butenhoff et al.[122]	Rats	SD (F)	Inhalation	PFOS	13W*	4W Post
Qazi et al.[104]	Mice	C57BL/6 (M)	Diet	PFOS	10D	EOT
Qazi et al.[132]	Mice	C57BL/6 (M)	Diet	PFOS	10D	EOT
Qazi et al.[132]	Mice	C57BL/6 (M)	Diet	PFOS + Con A	10D	EOT
Qazi et al.[132]	Mice	C57BL/6 (M)	Diet	PFOS	28D	EOT
Qazi et al.[132]	Mice	C57BL/6 (M)	Diet	PFOS + Con A	28D	EOT
Zhang et al.[140]	Mice	C57BL/6 (M)	Diet	PFOS	2W	EOT
Zhang et al.[140]	Mice	C57BL/6 (M)	Diet	PFOS + mMCD	2W	EOT
Zhang et al.[140]	Mice	C57BL/6 (M)	Diet	PFOS	6W	EOT
Zhang et al.[140]	Mice	C57BL/6 (M)	Diet	PFOS + CS	6W	EOT

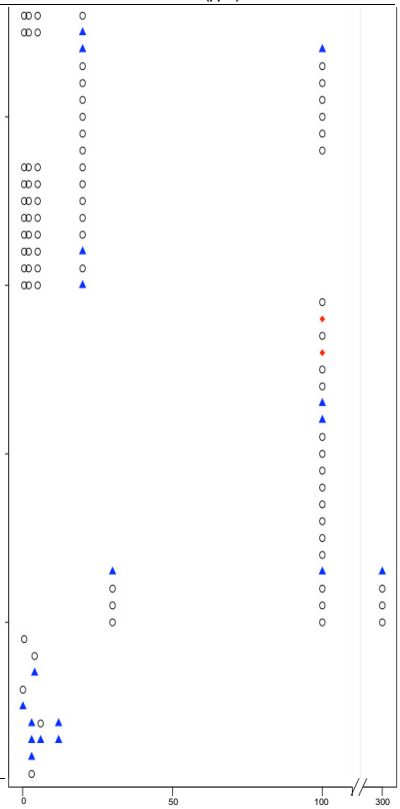


Figure 3. Strip plots for PFOS and ALT in rodent studies. Triangles indicate a significant increase in ALT relative to control. Diamonds indicate a significant decrease in ALT relative to control. Circles indicate no significant change in ALT relative to control. Plots are ordered by species and strain. In the study by Butenhoff et al.[122], atmospheric exposure occurred for 5 h/d, 5 d/wk. An accessible version of this figure is available in Table S6. Note: ALT, alanine aminotransferase; CS, choline supplementation; Con A, concanavalin A; D, day; DEN, diethylnitrosamine; EOT, end of treatment; F, female; GD, gestational day; GSPE, grape seed proanthocyanidin extract; HFD, high-fat diet; M, male; mMCD, marginal methionine/choline-deficient diet; Nar, naringin; PCB, polychlorinated biphenyl; PFOS, perfluorooctanesulfonic acid; PND, postnatal day; SD, Sprague Dawley; VC, vitamin C; W, week.

of the liver. Indeed, changes to serum biomarkers of liver function following PFAS exposure are often accompanied by histopathological changes or steatosis in rodents,[126,135,140] suggesting that associations between PFAS and ALT, AST, and GGT may be indicative of liver disease. However, only one study in humans reported both histological and liver enzyme data.[77] Some rodent studies reported histological alterations without associated changes in liver enzymes,[29,81,99,151] demonstrating the limitations of liver enzymes as markers of liver health. Recently, metabolomics[79,131] and mixtures[78,130,134] methods have emerged as more focused approaches to uncovering the relationship and mechanism between PFAS and liver injury and account for realistic exposure conditions, which may address this limitation. Most human studies identified by this review were cross-sectional, which precludes causal conclusions, and were conducted using different methods of data transformation and control of potential confounders. Far more studies have been conducted in rodents, and these findings, in conjunction with the limited number of longitudinal human studies, support a direct effect of PFAS on liver injury.

Still, there are a number of understudied factors in both epidemiolocal and experimental studies that require evaluation to elucidate the relationship between PFAS and liver injury. In this review, we have identified few studies in humans or rodents that evaluated sex-specific histological effects of PFAS exposure. Attanasio[67] reported positive associations between PFAS and ALT in female adolescents and negative associations in male adolescents, whereas studies in adults did not observe any sex-specific differences.[39,75] Some evidence for sex-specific differences was also reported in rats, with elevated ALT observed more frequently in male rats following PFAS exposure,[29,94,95,120,122] and sex-specific differences in the elimination half-lives of PFOA and PFOS have also been reported for rodents.[34] Many rodent studies were limited to males alone, which narrows the scope of findings and potential for mechanistic understanding. PFAS have been found to exert differential health effects by sex among other disease outcomes,[166,167] and thus, the sex specificity of PFAS toxicity merits further investigation. Early life exposure to PFAS is another potentially significant factor that requires additional investigation. In humans, Stratakis et al. [78] reported that prenatal PFAS exposure was significantly associated with elevated liver enzymes in childhood; however, Mora et al. [76] observed modest inverse associations between maternal PFAS concentration and child ALT levels. In rodents, *in utero* and perinatal PFAS exposure was associated with elevated liver enzymes and liver weight, steatosis, and other histopathological alterations.[86,100,106,119,128] These significant findings in rodent studies warrant the need for further consideration in humans. In both human and rodent studies, we found that most studies focused on the relationship between a single PFAS exposure and liver injury. As NHANES and other surveillance programs have indicated, multiple PFAS can regularly be detected in individuals and these exposures are highly correlated.[21,40,67,72,75] Research suggests that effects of PFAS mixtures, as well as the interaction between PFAS and other environmental exposures (e.g., diet, polychlorinated biphenyls) may exert synergistic or antagonistic effects.[168] Only two animal studies appear to have investigated this possibility to date, and the study designs differ in vehicle and duration of exposure, as well as in life stage and PFAS mixture composition, making it difficult to draw conclusions or extrapolate to humans.[130,134] Only one study in humans investigated the liver effects of PFAS mixtures rather than single exposures and found convincing evidence for synergistic effects.[78] Rapidly evolving methods for assessing exposure–mixture effects in population studies have potential to unravel the complex relationships between environmental exposures and liver injury.

To our knowledge, this is the first systematic review of the literature on PFAS exposure and liver injury and one of few reviews to consider both observational human and experimental rodent evidence for the effects of environmental exposures on health. We focused on ALT as a specific indicator of liver injury in occupationally exposed and general human populations and have followed PRISMA guidelines to limit the risk of bias in data synthesis and reporting of results. We found significant heterogeneity in the analyses, which limited our ability to perform a traditional meta-analysis and obtain a pooled effect estimate for human studies. However, evidence from experimental rodent studies consistently supported the results from human studies and indicates that PFAS exposure may contribute to markers of liver injury such as elevated liver enzymes, steatosis, and histopathological alterations.

Conclusion

Data from human studies consistently demonstrate an association between PFOA, PFOS, and PFNA and markers of liver injury: ALT, AST, and GGT. Complementary evidence from experimental rodent studies provides biological plausibility that this association may be causal. Insufficient evidence in both human and rodent studies exists to conclude that PFHxS and other PFAS have hepatoxic effects, possibly due to the low number of available studies. That there are positive associations between PFAS and ALT levels in humans suggests that PFAS exposure may contribute to the growing NAFLD epidemic. Future research should evaluate the full spectrum of NAFLD (including inflammation, hepatocellular injury, steatosis, and fibrosis) through histopathology or imaging, as well as consider additional investigation on lesser studied PFAS and PFAS mixtures to elucidate potential synergistic effects.

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