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Public health evaluation of PFAS exposures and breastfeeding: a systematic literature review

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Abstract

Per- and polyfluoroalkyl substances (PFAS) are a class of man-made chemicals that are persistent in the environment. They can be transferred across the placenta to fetuses and through human milk to infants. The American Academy of Pediatrics advises that the benefits of breastfeeding infants almost always outweigh the potential risks of harm from environmental chemicals. However, there are few chemical-specific summaries of the potential harms of exposure to PFAS during the neonatal period through breastfeeding. This systematic review explores whether exposure to PFAS through breastfeeding is associated with adverse health outcomes among infants and children using evidence from human and animal studies. Systematic searches identified 4297 unique records from 7 databases. The review included 37 total articles, including 9 animal studies and 1 human study measuring the direct contribution of exposure of the infant or pup through milk for any health outcome. Animal studies provided evidence of associations between exposure to PFOA through breastfeeding and reduced early life body weight gain, mammary gland development, and thyroid hormone levels. They also provided limited evidence of associations between PFOS exposure through breastfeeding with reduced early life body weight gain and cellular changes in the hippocampus. The direct relevance of any of these outcomes to human health is uncertain, and it is possible that many adverse health effects of exposure through breastfeeding have not yet been studied. This review documents the current state of science and highlights the need for future research to guide clinicians making recommendations on infant feeding.

Keywords

PFAS; breastfeeding; nursing; PFOA; PFOS; lactation

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Supplementary data

Supplementary data are available at Toxicological Sciences online.

Declaration of conflicting interests

The authors declare no conflict of interest. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Agency for Toxic Substances and Disease Registry or the views of the Centers for Disease Control and Prevention.

Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic chemicals used for decades in a variety of manufacturing processes and consumer products. They have been used in surface coatings for product packaging, carpets and water-repellant textiles, the manufacturing of non-stick cookware, and as a component of firefighting foams (ATSDR, 2018; EPA, 2019a). PFAS are ubiquitous in the environment and in human matrices (Buck et al., 2011; CDC, 2020).

Perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) are the most well-studied PFAS and can persist in the human body for years (ATSDR, 2018). PFOA and PFOS have been linked to a variety of adverse health effects in human and animal studies, for example liver toxicity, increased risk of kidney and testicular cancers, and pre-eclampsia (ATSDR, 2018, 2022; Sunderland et al., 2019). The major manufacturer of PFOS in the United States voluntarily ceased producing the chemical in 2002, and the major producers of PFOA phased out production from 2006 to 2015 (ATSDR, 2018; Butenhoff et al., 2009; EPA, 2019b). Human serum concentrations of PFOA and PFOS have since trended downward, but exposure continues in part because of their persistence in environmental media such as groundwater (ATSDR, 2018; CDC, 2020).

Other PFAS are replacing PFOA and PFOS in some applications (ATSDR, 2018; Brendel et al., 2018). Besides PFOA and PFOS, perfluorohexane sulfonic acid (PFHxS), and perfluorononanoic acid (PFNA) are the most studied PFAS. There is much less study concerning the health effects associated with emerging PFAS, some of which are being used as replacements for PFOA and PFOS (Brendel et al., 2018; Fenton et al., 2021).

Epidemiological studies have identified associations between early life exposure to certain PFAS with immunotoxicity, small decreases in birth weight, and certain cardio-metabolic outcomes particularly increases in total cholesterol (Abraham et al., 2020; Braun, 2017; Grandjean et al., 2012, 2017; Koshy et al., 2017; Liew et al., 2018; NTP, 2016; Rappazzo et al., 2017). The epidemiological evidence connecting other health effects with early life exposure is evolving, with differences in findings across studies. For instance, of 11 studies included in a systematic review, 4 reported positive findings whereas 7 reported null or inverse findings associating measurements of early life exposure to the most commonly detected PFAS with neurobehavioral outcomes (Braun, 2017). The potential associations between early life exposure and a variety of neurobehavioral outcomes continue to be explored in epidemiologic literature, but a clear, consistent link has not yet been established (Forns et al., 2020; Liew et al., 2018; Vuong et al., 2021; Xie et al., 2022). Five epidemiological studies also had mixed findings concerning potential associations between early life exposure to PFAS and thyroid function (Rappazzo et al., 2017).

Fetuses and neonates can be exposed to PFAS through several potential routes. PFAS can cross the placental barrier and transfer from a pregnant woman to her fetus (Chen et al., 2017; Hanssen et al., 2013; Mamsen et al., 2019). Many PFAS also partition into human milk (Lehmann et al., 2018; Llorca et al., 2010; Tao et al., 2008a; von Ehrenstein et al., 2009). In the United States, legacy PFAS (PFOA and PFOS) are still frequently detected in breastmilk, though concentrations have declined over time (Zheng et al., 2021). Some PFAS currently in use are more frequently detected in breastmilk than they were in the past

(Zheng et al., 2021). Longer duration of breastfeeding is associated with greater levels of most PFAS in infant serum (Grandjean et al., 2017; Koponen et al., 2018; Mogensen et al., 2015; Mondal et al., 2014; van Beijsterveldt et al., 2022). PFAS have also been detected in infant formulas and baby food (Lehmann et al., 2018; Llorca et al., 2010; Macheka et al., 2021; Tao et al., 2008b). Infants may also be exposed when water contaminated with PFAS is used to prepare infant formula.

Both the American Academy of Pediatrics (AAP) and the Dietary Guidelines for Americans recommends exclusive breastfeeding of infants for about 6 months, with continued breastfeeding alongside complementary foods after 6 months (Food Service Guidelines Federal Workgroup, 2017; Meek and Noble, 2022). Breastfeeding is associated with many health benefits for infants, including protection from infection, a 6- to 10-fold reduction in the incidence of necrotizing enterocolitis (severe inflammation of the intestines) in newborns, and protection from sudden infant death syndrome (SIDS), among others (AAP, 2012; Cacho et al., 2017; Duijts et al., 2009; Hauck et al., 2011; Lucas and Cole, 1990; Meek and Noble, 2022). Breastfeeding also benefits mothers, possibly lowering their risk of high blood pressure, type 2 diabetes, and ovarian and breast cancer (AAP, 2012; Jordan et al., 2012; Meek and Noble, 2022; Schwarz et al., 2009; Stuebe et al., 2005, 2009).

The AAP has compiled research and developed recommendations on breastfeeding for mothers exposed to specific pharmaceuticals or environmental chemicals but has not developed guidance specific to PFAS (AAP, 2013). The AAP does advise, however, that the benefits of breastfeeding will almost always outweigh the potential harms of exposure to environmental pollutants through breastfeeding (AAP, 2012).

This systematic review is intended to inform guidelines for breastfeeding mothers who have been exposed to PFAS. There are several existing reviews that document the evidence of health effects of developmental or early life exposure to PFAS, however none of these reviews were designed to specifically explore exposure through breastfeeding (Braun, 2017; Koustas et al., 2014; Lam et al., 2014; Rappazzo et al., 2017).This study seeks to answer 2 questions: What are the adverse health effects that have been linked to exposure to PFAS through breastfeeding from human and animal studies and how strong is the evidence for these effects? This review considers both observational human studies and experimental animal studies in rodents. For consistency, the exposure route of interest is referred to as “breastfeeding” even though rodents nurse rather than breastfeed. The results of this review can be used to ensure recommendations to clinicians for infant feeding reflect the latest science on the effects of exposure to PFAS through breastfeeding.

Materials and methods

Our systematic review followed the criteria outlined by the National Toxicology Program’s Office of Health Assessment and Translation (OHAT) for conducting literature-based assessments of environmental contaminants using experimental animal and observational human studies (OHAT, 2015a). The OHAT Handbook provides a systematic structure for assessing whether a substance may cause adverse health effects using evidence from human and animal studies. Our methods are outlined in more detail in our study protocol, which is

registered with the international prospective register of systematic reviews (PROSPERO [registration number CRD42019130824]). Before conducting the literature search, we outlined a PECO (population, exposure, comparators, and outcome) statement, study questions, and search protocol.

PECO statement

Forming a PECO statement guides the development of the study questions, search strategy, and choice of information to extract from included articles. The PECO statement for this study is outlined in Table 1. The review seeks to document the evidence of health effects attributable to PFAS exposure through breastfeeding. Therefore, our definition for exposure was narrow ("exposure to PFAS through lactation"), but we included any health outcome that could be potentially attributed to that exposure. We excluded health outcomes only if they were highly likely to result from another exposure based on the study parameters. If, for example, in a rodent study, pups were exposed to a PFAS through nursing and then directly dosed with a PFAS after weaning, we excluded outcomes taken after weaning because those may have resulted from exposure after weaning and not from nursing exposure.

Study question

In this manuscript, we describe our efforts to answer the following question: What is the evidence that exposure to PFAS through breastfeeding leads to an adverse health outcome in infants? Specifically, what are the adverse health effects that have been linked to exposure to PFAS through breastfeeding from human and animal studies and how strong is the evidence for these effects?

Database searches

The study team developed search terms designed to capture studies that measured health outcomes and exposure to PFAS through breastfeeding. We included 75 broad and specific PFAS search terms (including terms related to alternative or replacement PFAS). See the Supplementary Material for a complete list of PFAS compounds included in the search. We limited searches by including terms related to breastfeeding exposures. Specific search terms are outlined in the Supplementary Material. Searches were performed in 7 databases: PubMed, Scopus, Embase, CAB Abstracts, Global Health, Medline, and ProQuest Environmental. We first conducted searches in August 2017 and updated them in February 2018. Studies published after January 1, 2000 and before February 2018 were potentially eligible for inclusion. Search terms and database-specific searches are included in the Supplementary Material. We conducted a targeted search using the same terms but restricting to cross-foster animal studies that have been published from February 2018 through August 2022. The search was limited to cross-foster studies because this study design was the most relevant to connecting health outcomes to our exposure of interest. No studies were identified in this targeted search. Although the human study search was not updated after 2018, the authors informally stayed up to date with literature and did not identify additional studies connecting measurements of PFAS in breastmilk with health outcomes in infants.

Literature screening

All articles were screened using a 2-step process that applied the PECO criteria to select articles most suitable to answer the designated questions. Specific inclusion and exclusion criteria are outlined in Tables 2 and 3. First, abstrackr, a free, open source web-based annotation tool designed to assist with screening, was used to randomly assign titles and abstracts of identified publications to research team members for screening and marking abstracts for inclusion or exclusion (Wallace et al., 2012, http://abstrackr.cebm.brown.edu/; last accessed June 7, 2023). At least 2 reviewers independently evaluated each abstract, and neither reviewer could see the other's decision to include or exclude. A third reviewer resolved any disagreements between the 2 initial reviewers. Then, EndNote and Microsoft Excel were used to organize the full-text screen of the publications selected for further review. Two reviewers evaluated each full-text article for relevancy using the PECO criteria, and a third reviewer resolved any disagreements between them.

Risk of bias analysis

We evaluated risk of bias for each included article according to the OHAT risk of bias rating tool (OHAT, 2015b), with the modifications described below. Evaluating risk of bias allows for transparent assessment of the internal validity of the studies included in the review and in some cases may result in exclusion of studies with low internal validity. The tool includes criteria to evaluate risk of bias for both human and animal studies in 10 total domains. In the current review, no articles were rejected due to risk of bias score.

The 10 risk of bias domains (8 for animal studies and 6 for observational human studies) are listed in Table 4. We made several other modifications or additions to the risk of bias protocol outlined in the OHAT tool:

1. Clarifying how information about randomization and allocation concealment could be used in tandem to rate risk of bias in domains 1 and 2;
2. Clarifying how pup mortality would influence risk of bias rating due to attrition (domain 7);
3. Selecting a minimum substance purity (97%) for definitely low risk of bias in domain 8;
4. Clarifying how different combinations of factors affecting confidence in exposure (listed percentage purity, independent verification of purity, tests of administered doses, and exposure administration) may lead to different risk of bias ratings for confidence in exposure characterization (domain 8).

Additional details about these changes are provided in the Supplementary Material.

Two reviewers independently rated each study in every risk of bias domain as *definitely low, probably low, probably high, or definitely high*. Reviewers resolved disagreements through discussion between themselves or with the team.

Studies were rated as tier 1 through 3 based on risk of bias, according to the criteria outlined in Table 5. If there had been studies that received a tier 3 rating, they would have undergone

a further review to determine whether they should be excluded from further analysis due to high risk of bias.

Data extraction

Data were extracted using the Health Assessment Workspace Collaborative (HAWC) web platform (Shapiro et al., 2018). HAWC is a free, open source website designed by researchers from the University of North Carolina Chapel Hill to extract, store, synthesize, and visualize data for assessing the human health effects of chemicals. For the initial phase of extraction, one team member extracted general study details, such as the chemical studied, the species, and the dose groups, and flagged any measured health outcomes that might be eligible for extraction.

This reviewer also categorized each animal study as eligible for extraction limited to all study information and the no observed and lowest observed adverse effect levels (NOAEL and LOAEL, respectively) for all potential health outcomes or full, quantitative extraction of all relevant health outcomes based on whether the study design allowed for separating the contributions of exposure through nursing to health outcomes from the contributions of in utero exposure. In particular, cross-foster animal studies, in which some litters are switched at birth to a dam receiving a different dose than the dam to which they were born, allowed for a direct comparison of pups that were and were not exposed through nursing only. Human studies that did not measure PFAS concentrations in milk were also flagged for limited, narrative extraction. A second reviewer then checked whether all eligible health outcomes from each article were flagged and whether the limited or full extraction categorization was correct.

Qualitative data extraction—For the animal studies flagged for limited extraction, NOAELs, LOAELs, and, where reported, benchmark dose levels (BMDLs) were extracted and plotted for all eligible health outcomes. The purpose of this extraction was to document the range of developmental health outcomes for which exposure through breastfeeding might have played a role and to determine the doses at which these effects occurred.

Quantitative data extraction—For a continuous health outcome to be eligible for full extraction, the article must have reported enough information to calculate a measure of central tendency, a measure of variance, and the number of individuals or litters measured. For categorical outcomes, the article must have reported the number of events and number of individuals or litters measured. For experimental animal studies, health outcomes measured after an animal had further exposure following weaning were considered ineligible for quantitative extraction. All health outcomes excluded due to additional exposure post-weaning had been measured prior to weaning and were extracted at that time point. The study team noted when post-weaning findings differed substantially differed from findings based on outcomes measured pre-weaning.

For studies flagged for full extraction (ie, cross-foster animal studies), eligible health outcomes were extracted at all doses for each dose group. All data were extracted by a first reviewer and checked for accuracy by a second reviewer. Authors generally compared all exposure groups to pups that were not exposed in utero or through nursing using analysis

of variance (ANOVA) tests or similar statistical tests. For continuous outcomes, the study team used the reported data to conduct 2-sided *t* tests comparing pups with no exposure to pups exposed only through nursing, as well as comparing pups with *in utero* exposure to pups with the same in utero exposure and additional lactation exposure.

For a comparison to be considered statistically significant, the *p* value must have been less than .01. We used this more conservative value to mark statistical significance because statistical tests used summary data (usually mean, n, and standard error of the mean) for each dose group rather than raw data. Statistical results could therefore be modestly affected by the precision of the reported summary data. An alpha of $p = .01$ was chosen because an analysis of outcomes measured in Wolf et al. (2007) showed that for some outcomes where *p* is greater than .01 and less than .05, reporting an additional digit would have changed the finding of statistical significance. Health outcomes and results of the statistical tests were displayed on forest plots created in the software package R.

Synthesis and integration of evidence

This review used a systematic, qualitative approach to synthesize evidence. The OHAT handbook provides guidance for rating confidence in bodies of evidence as *high, moderate, low*, or *very low* based on the Grading of Recommendations Assessment, Development and Evaluation (GRADE) framework. Health outcomes from cross-foster animal studies and human cohort studies were grouped into categories based on the types of outcomes outlined in the Agency for Toxic Substances and Disease Registry's (ATSDR's) Draft Guidance for the Preparation of Toxicological Profiles (ATSDR, 2018).

We gave each line of evidence for an adverse human health effect an initial rating based on the study design demonstrating the potential effect. That rating could then be downgraded based on risk of bias, unexplained inconsistency, indirectness, imprecision, and publication bias. The decision to upgrade confidence for consistency was partially informed by findings from studies that did not have a cross-foster design. The rating could be upgraded based on a large magnitude of effect, dose response, consistency of findings, and if there was a strong possibility of an underestimated effect due to residual confounding. A thorough explanation of the criteria we used to upgrade and downgrade study ratings is available in *Handbook for conducting a literature-based health assessment using OHAT approach for systematic review and evidence integration* (OHAT, 2015a). Two reviewers rated confidence for each line of evidence independently. Any disagreements were settled through discussion between reviewers or among the team members. Although outcomes measured in early life (non-cross-foster) animal studies were not directly rated independently, they could influence ratings for consistency of findings for a line of evidence measured in a cross-foster study depending on whether they corroborated findings of cross-foster studies. In addition, if an association between exposure and a health outcome was corroborated across multiple early life animal studies, that line of evidence was less likely to be downgraded for potential publication bias due to few studies measuring the effect.

Confidence ratings were then translated into the level of evidence for a health effect. The level of evidence demonstrating a health effect is the same as the confidence rating, except when the confidence rating in the body of evidence is *very low*; then the level of evidence is

inadequate. Following the OHAT protocol, the rating *evidence of no health effect* is applied only if confidence in the body of evidence is *high*, because of the difficulty of proving a negative.

Results

Database searches and article screening

Figure 1 summarizes article screening, inclusion, and exclusion for the searches conducted August 2017 and February 2018. Results are not shown for the targeted search for cross-foster studies for articles published between February 2018 and August 2022 as no additional articles were included from this search. Database searches identified 4297 unique records. During the title and abstract screening, 4210 articles were excluded because they did not meet the inclusion criteria. This process left 87 articles for full-text review. After at least 2 reviewers screened each article and determined whether the articles met the inclusion criteria for the systematic review, 39 articles were retained for further evaluation.

During the risk of bias evaluation, the team excluded one additional article. Ryu et al. (2014) was initially marked for inclusion because the article appeared to meet the exposure criteria; dams were exposed throughout lactation, and pups were exposed through nursing. However, all outcomes in the article were assessed after post-weaning dietary exposure of pups, resulting in no eligible outcomes and exclusion of the article. One additional article (Stadler et al., 2008) was excluded during data extraction because outcomes were not measured in neonates under relevant exposure conditions.

Risk of bias analysis

The team rated 34 animal study and 3 human study articles for risk of bias and categorized them into 3 tiers. Risk of bias rating is not presented for the study excluded during data extraction.

Risk of bias for animal studies—Ten animal studies were considered tier 1 based on risk of bias rating and 24 were considered tier 2. No studies were rated tier 3 for risk of bias. Ratings for animal studies on each domain are summarized in Figure 2.

Studies were often rated as *probably high* for domain 2 (“Was allocation to study groups adequately concealed?”) because articles did not indicate whether researchers were blinded during study group allocation. Many studies were also rated as *probably high or definitely high* for exposure characterization because of the following factors: A test chemical was less than 97% pure; the dosing solution concentration was not confirmed; and stability and homogeneity of the dosing solution was not confirmed.

Risk of bias for human studies—The risk of bias for the 3 human cohort studies was generally rated as *probably low* or *definitely low* in most areas (Figure 3). No studies were excluded due to high risk of bias (tier 3) rating. None of the risk of bias domains address the question of how directly the exposure relates to the study question. Exposure characterization (domain 8) captured potential for misclassification of exposure and domain 4 allows the study team to check that important confounders were included in the model,

but neither include consideration of how directly or indirectly the exposure captures the exposure of interest. Although all 3 human epidemiologic studies rated similarly in risk of bias, 2 of the studies had exposure measurements that were not directly related to breastfeeding exposure. As discussed in more detail below, only Forns (2015) was carried forward to lines of evidence.

Health outcomes

Cross-foster animal studies—Two studies, White et al. (2009) and Wolf et al. (2007), measured health outcomes in mice exposed to PFOA or a PFOA salt. In both studies, relative liver weight was consistently higher in pups that received additional exposure through lactation, compared with pups that only received in utero exposure or no exposure at all (Figure 4). In both studies, pups with exposure through nursing also tended to have lower body weight gain from birth through postnatal day (PND) 10 or through weaning (PND 22) than pups with comparable in utero exposure and no exposure through nursing. However, this effect was not consistently significant. Neither study measured liver or body weight post-weaning. Other health outcomes showed less consistent patterns with increasing dose through nursing. Eye opening and mammary gland development were considered developmental effects for the purpose of assessing confidence in lines of evidence.

Luebker et al. (2005) examined body weight gain from birth through weaning in rats. The pups that received PFOS exposure only through nursing had lower body weight gains than pups not exposed to PFOS through any route. Rats that received exposure both through nursing and in utero also had less body weight gain than rats exposed only in utero, but the difference was not statistically significant (Figure 5). Yu et al. (2009) looked at absolute body weight in rats at weaning rather than body weight gain, and observed no differences connected to nursing exposure.

Yu et al. (2009) also measured levels of the thyroid hormones (total thyroxine [T4], total triiodothyronine [T3], and reverse T3 [rT3]) in the serum of rats exposed to PFOS in utero and through nursing, beginning at birth and extending post-weaning. Results at weaning are displayed in Figure 5. Total T4 was significantly reduced in the nursing-only exposed group compared with controls, but not in the combined nursing and in utero exposed group compared with in utero only.

In a study of rat pups, relative liver weight at weaning was elevated in both the nursing-only exposed group and the nursing and in utero exposed group compared with their respective controls (Yu et al., 2009). However, that elevation did not rise to the level of statistical significance in either group.

Wang et al. (2015b) conducted a cross-foster study with dams exposed to PFOS. The authors measured cell apoptosis and intracellular free calcium concentration in the hippocampus in rat pups at weaning. They concluded that their observations likely indicated a non-monotonic dose response for cell apoptosis and intracellular free calcium. The article speculated that at higher doses, apoptosis may be suppressed, potentially leading to more serious adverse effects, and calcium signaling may play a role in this cell apoptosis pathway. There are indications of the same non-monotonic dose response pattern when

comparing lactation groups to controls as was observed in the overall findings of the study (Figure 5). Several cross-foster studies measured proteins and mRNA in the hippocampus to explore mechanisms of action potentially leading to neurological outcomes but had limited significant findings and are not visualized in Figure 5. More information on these studies is available in Supplementary Table 6.

One study (Henderson and Smith, 2007) measured exposure to 8-2 fluorotelomer alcohol, which metabolizes to PFOA and PFNA. This study was primarily to explore this metabolism and did not have outcomes that were able to be visualized or statistically analyzed by lactation-treatment groups. No outcomes were available from cross-foster studies exploring exposures to compounds other than PFOA, PFOS, or their salts.

Experimental animal studies with other study designs—In this analysis, developmental animal studies not using a cross-foster design examined all the outcomes measured in cross-foster studies of pups exposed to PFOA or PFOA salts through gestation and lactation. Those studies also examined death, neurological effects, and immunological effects as outcomes. The timing of dosing varied; some studies restricted dosing of the dam to gestation and some extending dosing to span gestation and lactation. A summary of study details is available in Supplementary Table 5. Note that LOAELs reported here are the dose administered to the dam and are not corrected for the exposure duration or timing of the exposure, and therefore do not directly correspond to the dose received by pups. The lowest LOAEL was based on a body weight outcome measured in mice pups where dams were dosed through gestation (GDs 1–17) (Hines et al., 2009, Figure 6). Most cross-foster and general developmental studies measured body weight gain through weaning or absolute body weight at weaning. However, this increase in body weight was in mid-life (20–29 weeks) and was only observed in female mice exposed to the 2 lowest developmental doses (0.01 and 0.03), with no effect at higher doses. The effect did not persist to 18 months. The next lowest LOAEL, a neurological outcome, was based on higher activity at night, as measured by an accelerometer, in male rats born to dams dosed at 0.3 mg/kg/day during gestation (GD 1–21) (Onishchenko et al., 2011).

Similarly, developmental studies in animals exposed to PFOS and PFOS salts include the outcome types studied in cross-foster studies, plus many additional outcome types (Figure 7). The lowest LOAEL observed was an upregulation in the expression of several genes (Bax and cytochrome c) involved in the mitochondrial-mediated cell apoptosis pathway in the heart of rat pups born to dams dosed at 0.1 mg/kg/day during gestation (GD 2–21) (Zeng et al., 2015). None of the effects measured in pups in Zeng et. al (2015) directly correspond to a measurable clinical effect in humans.

Information on the 5 studies that exposed animals to PFAS other than PFOS and PFOA are included in Supplementary Figure 1.

Human cohort studies—Three human cohort studies investigating potential associations between health outcomes in children that have been breastfed and a PFAS exposure as measured in mother’s breastmilk or the serum of the children. Forns et al. (2015) was the only study of the 3 that measured the association between health outcomes and

concentration of PFAS in breastmilk. The study analyzed exposure and outcome data from approximately 850 mother-infant pairs recruited from various centers in Norway between the years 2003 and 2009 within 2 weeks of the mothers giving birth. Researchers measured PFOA and PFOS concentrations in human milk samples that the mothers collected over an 8-day period approximately 1 month after delivery (range 2–177 days, median 32 days after delivery). No information was collected on duration of breastfeeding. Parents completed the Ages and Stages Questionnaire (ASQ-II, a screening test designed to identify children who may be at risk for developmental delays) when the child was at 6 months and at 24 months of age, and the Infant/Toddler Symptoms Checklist (ITSC, used to assess behavioral problems) when the child was at 12 and 24 months of age. The authors did not identify any associations between PFOA or PFOS concentrations in breastmilk and the risk of having an abnormal score on the ASQ at 6 or 24 months, or the risk of scoring high for behavioral problems based on the ITSC at 12 or 24 months.

Oulhote et al. (2016) and Grandjean et al. (2017) were included for narrative study because they both include measurements of prenatal and early childhood exposure to PFAS and their associations with health outcomes. However, because the studies did not attempt to connect any health outcomes directly to exposure through breastfeeding, no outcomes were carried forward from either study for further analysis. The characteristics of these studies are discussed in further detail in the Supplementary Material.

Levels of evidence for health effects

Ratings for the strength of lines of evidence demonstrating potential associations between lactation exposure and a health outcome are shown in Table 6. There were 2 lines of evidence for PFOA (neurological and developmental outcomes) and 3 lines of evidence for PFOS (endocrine, hepatic, and neurological outcomes) demonstrating no association between PFOA or PFOS exposure through breastfeeding and a potential health effect (not shown in Table 6). OHAT advises that because of the “inherent difficulties in proving a negative,” lines of evidence demonstrating no effect be rated inadequate unless the study team has high confidence in the line of evidence (OHAT, 2015a). None of these 5 lines of evidence were rated as high confidence, and the evidence demonstrating no effect was therefore considered inadequate for all 5. Supplementary Figure 2 gives a more detailed breakdown of reviewers’ ratings of confidence for all lines of evidence, including lines of evidence demonstrating a lack of association. Since no outcome was measured in more than 2 cross-foster studies and only one human study was carried forward to lines of evidence rating, meta-analysis was not possible. All 5 lines of evidence demonstrating a direct, statistically significant connection between exposure to PFAS through nursing and a health outcome came from cross-foster experimental animal studies.

Discussion

Direct evidence of adverse health effects due to PFAS exposure specifically through breastmilk is primarily limited to animal studies. Only one epidemiologic study measured associations with concentrations in breastmilk with health outcomes. There are several challenges limiting the possibility of drawing causal conclusions about the connection

between breastfeeding exposure to PFAS and health outcomes in infancy and beyond from epidemiologic studies. First, most infants exposed to PFAS through breastfeeding will have been exposed to PFAS in utero. Conflating in utero and breastfeeding exposure is less informative for parents deciding whether or not to breastfeed infants. Second, the ideal comparison group to inform parents is formulafed infants. However, these infants may have some exposure to PFAS and that exposure may be variable, which makes establishing a dose-response relationship of PFAS exposure through breastmilk from epidemiologic literature extremely difficult. These factors made it necessary to rely primarily on animal studies to understand the potential connection of exposure to PFAS through breastfeeding and health.

PFOA and PFOS are the only 2 PFAS for which the direct contribution of exposure through breastfeeding to health outcomes has been studied in animals. Although there is evidence of connection between PFOA and PFOS exposure through nursing in animals and adverse health outcomes, it is not clear how these effects traslate to adverse, measurable outcomes in humans.

The 2 strongest lines of evidence for health effects were a decrease in body weight gain from birth to weaning and an increase in relative liver weight measured in mouse pups at weaning, both due to PFOA exposure through breastmilk (Table 6). Changes in liver weight in rodents without accompanying histological or clinical evidence of liver toxicity are considered potentially adaptive rather than adverse effects and are not directly relevant for human risk assessment (Hall et al., 2012). According to ATSDR's guidance for classifying the severity of a health outcome in toxicological profiles, changes in body weight in developing animals may be considered less serious effects when the change is small in magnitude or transient (ATSDR, 2018). In the 2 cross-foster animal studies that examined change in body weight from birth to weaning in pups exposed to PFOA exposure-related changes in body weight gain were modest; the body weight gain of the lactationally dosed animals varied from slightly greater (2% greater) to moderately less (13% less) than the body weight gain of animals with the same in utero exposure but no lactational exposure. In experimental animal developmental studies included in this review that continued measuring body weight post-weaning, the decrease in body weight gain at weaning was found to be transient, with more complex potential relationships between early life exposure, adiposity, hormone regulation, and body weight for rodents in later life (Hines et al., 2009; van Esterik et al., 2016). The potential of lactation exposure to contribute to adiposity later in life is not clear.

Evidence was present but weaker for a connection between PFOS exposure and body weight gain as well cellular-level changes in the brain. Changes in neurochemistry are also considered less severe unless the changes are large in magnitude (>60%), irreversible, or accompanied by clinical neurological symptoms (ATSDR, 2018).

Of the effects attributable to lactational PFAS exposure in animal studies, effects on mammary gland development are most likely to correspond with a permanent change in an organ system. The effect of perinatal exposure to PFOA on mammary gland development has been observed in studies that were not included in the line of evidence rating because they conflate in utero and lactational exposure (Tucker et al., 2015; White et al., 2007,

2011; Zhao et al., 2010). Perinatal PFOA may cause impairment to pubertal development of breast tissue through interaction with hormone receptors (Fenton et al., 2012; Tucker et al., 2015; Zhao et al., 2010). While rodent and human mammary gland development follow a similar progression, the specific timing of epithelial branching and other processes differs, which may have implications for the importance of neonatal exposure to PFOA (Fenton et al., 2012; Hovey et al., 1999). PFOA is a (Bjork and Wallace, 2009). Although PFOA is a peroxisome proliferator-activated receptor alpha (PPARα) agonist, pubertal effects to mammary gland development from perinatal exposure has been shown to be independent of the PPARα pathway, which increases the possible relevance of this effect in humans (Zhao et al., 2010). White et al. (2011) examined the ability of mice who had been exposed in utero and through breastmilk as fetuses and neonates, respectively (the F1 generation), to feed their young (F2 generation) and found no statistical differences between treatment and control groups, although there was a decrease in milk transfer at higher doses. The authors did not observe any differences in growth and development of F2 pups, indicating that the differences in mammary gland development in the F1 generation did not impair the ability of those animals to provide adequate nutrition to their young. More study is needed to understand the biological significance of this effect and the relevance of the effect to humans.

Human studies have examined the assocation between early life PFOA exposure and effects such as restricting growth in early life, hepatic effects, and effects to reproductive development, but no studies in humans have connected these health effects to exposure through breastfeeding. Estimates from meta-analyses of human epidemiological studies suggest that a 1 ng/mL increase in either maternal serum, maternal plasma, or cord serum PFOA is associated with a small decrease in birth weight ranging from 7 to 19 g (Johnson et al., 2014; Verner et al., 2015). Human studies connecting exposure to PFOA to growth in infancy and childhood have mixed conclusions (Andersen et al., 2010; de Cock et al., 2014; Maisonet et al., 2012; Shoaff et al., 2018). Epidemiological studies in workers and communities near facilities that used or produced PFOA have found inconsistent results concerning the association of PFOA exposure with hepatic serum enzymes and bilirubin levels (summarized in ATSDR, 2018). Two epidemiological studies have been conducted examining the association between maternal serum PFOA at birth and age of menarche in girls; one found no association and the other found a significant association (Christensen et al., 2011; Kristensen et al., 2013).

In spite of the fact that the qualitative search was not as up to date as the targeted cross-foster study search, this review identified a wide number of potential health outcomes assessed in human and animal studies of early life exposure; however, only a small number of health outcomes were identified across 9 cross-foster animal studies, which evaluated the direct contribution of exposure to PFAS through breastmilk. There are many clinically relevant outcomes that could result from exposure to PFAS through breastfeeding that have not yet been studied using a cross-foster design or in epidemiological studies connecting PFAS in human milk to health outcomes. Furthermore, the epidemiological and cross-foster studies included in this review evaluated potential health effects of exposures to only PFOA and PFOS. More research is needed to understand the potential health consequences of breastfeeding exposure to other PFAS and to multiple PFAS simultaneously.

In contrast to the potential health effects of exposure to PFAS in breastmilk, the benefits of breastfeeding for infants have strong evidence for clinically relevant outcomes in human studies (AAP, 2012). In the recent past when serum levels of long-chain PFAS in the U.S. population were higher, studies continued to find health benefits to infants from breastfeeding (Cacho et al., 2017; Duijts et al., 2009; Hauck et al., 2011). Recent systematic reviews on the health effects of environmental chemicals in human milk have concluded that, in general, the benefits of breastfeeding outweigh any potential harms from environmental chemicals in breastmilk at typical levels measured in the U.S. population (LaKind et al., 2018; Lehmann et al., 2018).

One of these reviews also identified studies showing concentrations of PFAS in infant formula, suggesting that ceasing breastfeeding may not eliminate exposure in some cases (LaKind et al., 2018). Even if PFAS were not present in infant formula, ceasing breastfeeding would not address in utero exposures. Infants born to mothers exposed to PFAS have likely already been exposed through in utero transfer (Chen et al., 2017). Reducing exposures to these persistent chemicals before pregnancy would reduce exposure to fetuses through in utero transfer and exposure to infants through breastfeeding without sacrificing the benefits of breastfeeding.

Limitations and research gaps

There remains a critical gap in our understanding of whether there is a dose where the potential harm of exposure to PFAS through breastmilk may outweigh the health benefits of breastfeeding for mothers and infants. Additional research is needed to establish the dose-response relationship between infant exposure to PFAS through breastfeeding and health outcomes.

Physiological based pharmacokinetic modeling (PBPK) modeling may be one important component of this additional research. Because of infant growth and changes in PFAS concentration in milk over time during breastfeeding, infants can ingest different daily doses throughout their first year of life. Estimates of postnatal exposure usually depend on measurements such as maternal serum, infant serum, or concentration in breastmilk which only show PFAS concentrations at a snapshot in time. Concentrations and maternal and infant serum also capture both in utero and breastfeeding exposure. The use of PBPK modeling can estimate the contribution of PFAS breastfeeding exposures as Verner et al. (2010) did to estimate the contribution of breastfeeding to polychlorinated biphenol (PCB) exposure and can account for changes in toxicokinetics during infancy. Recent research has demonstrated the utility of PBPK modeling in accounting for lactational exposure and highlighted the critical need for additional data to characterize how PFAS transfer to infants through breastmilk over the course of lactation (LaKind et al., 2022). Future observational research may incorporate PBPK to supplement measured serum or breastmilk PFAS concentrations and advance the understanding of potential dose-response relationships from breastfeeding exposure to PFAS.

Infants may be exposed to PFAS through formula when water contaminated with PFAS is used to prepare infant formula and when PFAS is present in commercially available infant formulas. There has been limited research on the levels of PFAS in infant formula in the

United States. Tao et al. (2008b) examined PFOS, PFOA, PFHxS, PFNA, perfluorobutane sulfonic acid (PFBS), and perfluoroheptanoic acid (PFHpA) in 21 samples of infant formula from 5 manufacturers purchased from retail stores in Washington, District of Columbia and Boston, Massachusetts in 2007. Two of 8 measured PFAS were detected above the LOQ; PFOS was detected in 5 samples and PFHxS in 10 samples. Recent research from other countries suggests that the distribution of PFAS in cow's milk may predict the PFAS that are present in many infant formulas (Macheka et al., 2021). Further, long-chain PFAS are likely to bioaccumulate in cattle, and milk is likely the primary excretion route for PFOS in dairy cows (Death et al., 2021). Taken together, this indicates formula may be an important exposure route. More research is needed to quantify levels of PFAS in infant formula to inform clinical advice related to infant feeding and PFAS exposure.

While the search for cross-foster studies was conducted in August 2022, the search for qualititative, supporting studies has not been updated since February 2018. While this was unlikley to effect our lines of evidence, it may have narrowed the list of health effects identified in early life studies and excluded studies on emerging PFAS which tend to have more recent publication dates.

In spite of the fact that the qualitative search was not as up-to-date as the targeted cross-foster study search, this review identified a wide number of potential health outcomes studied in early life exposure human and animal studies, but a small number of health outcomes identified across 9 cross-foster animal studies that studied the direct contibution of exposure to PFAS through breastmilk. As such, the most clinically relevant outcomes that may result from exposure to PFAS through breastfeeding may not have yet been studied in cross-foster studies or in epidemiolgical studies that connect PFAS in human milk to health outcomes. The outcomes from epidemiological and cross-foster studies included in this review exclusively examined the health effects of PFOA and PFOS. More research is needed to understand the potential health consequences of breastfeeding exposure to other PFAS and to multiple PFAS simultaneously.

While much uncertainty remains about the potential health effects of infant exposure to PFAS through breastfeeding, material has been developed to guide health professionals in communcating with their patients. ATSDR has developed general PFAS clinician guidance with some information specific to PFAS and breastfeeding (ATSDR, 2019). In 2021, the National Academies of Sciences, Engineering and Medicine has appointed an expert committee sponsored by ATSDR and the National Institute of Environmental Health Sciences to "examine the health outcomes associated with PFAS exposure and develop principles clinicians can use to advise patients on exposure reduction" (NASEM, 2021). The committee released their final report in in August 2022, including recommendations related to PFAS exposure during breastfeeding. The report acknowledged that research has not established levels and types of exposure that could be of concern for infants exposed through feeding. ATSDR is now working to revise their PFAS clinician guidance materials to ensure that they reflect the most current scientific knowledge. As scientific knowledge concerning exposure to PFAS through breastmilk and human health evolves, and research continues to fill some of the gaps described here, providing and updating guidance to clinicians will be vitally important.

Conclusion

This systematic review explored the evidence of adverse health effects as a result of PFAS exposure through breastfeeding. A limited number of experimental animal studies provide evidence suggesting a relationship between feeding from a mother exposed to 3–5 mg/kg/day PFOA or PFOA salt and perinatal body weight gain, relative liver weight, and impairment of mammary gland development. The evidence for health effects from lactational exposure to PFOS and other PFAS is too limited to form a conclusion. The evidence is also too limited to form a conclusion about what maternal serum level, if any such level exists, might pose a high enough risk of adverse health outcomes to infants through breastfeeding exposure to recommend ceasing breastfeeding. This review illustrated that even for PFOA and PFOS, more study is needed on the many potential health effects identified in developmental animal studies that have not been measured in cross-foster studies. There is an even more profound research gap for other PFAS, with virtually no information available to make direct inferences about the health consequences of exposure through breastfeeding. Based on the research to date, the demonstrated benefits of breastfeeding for infants are more robust and have a stronger evidence base than the potential health effects of exposure to these environmental chemicals through breastfeeding.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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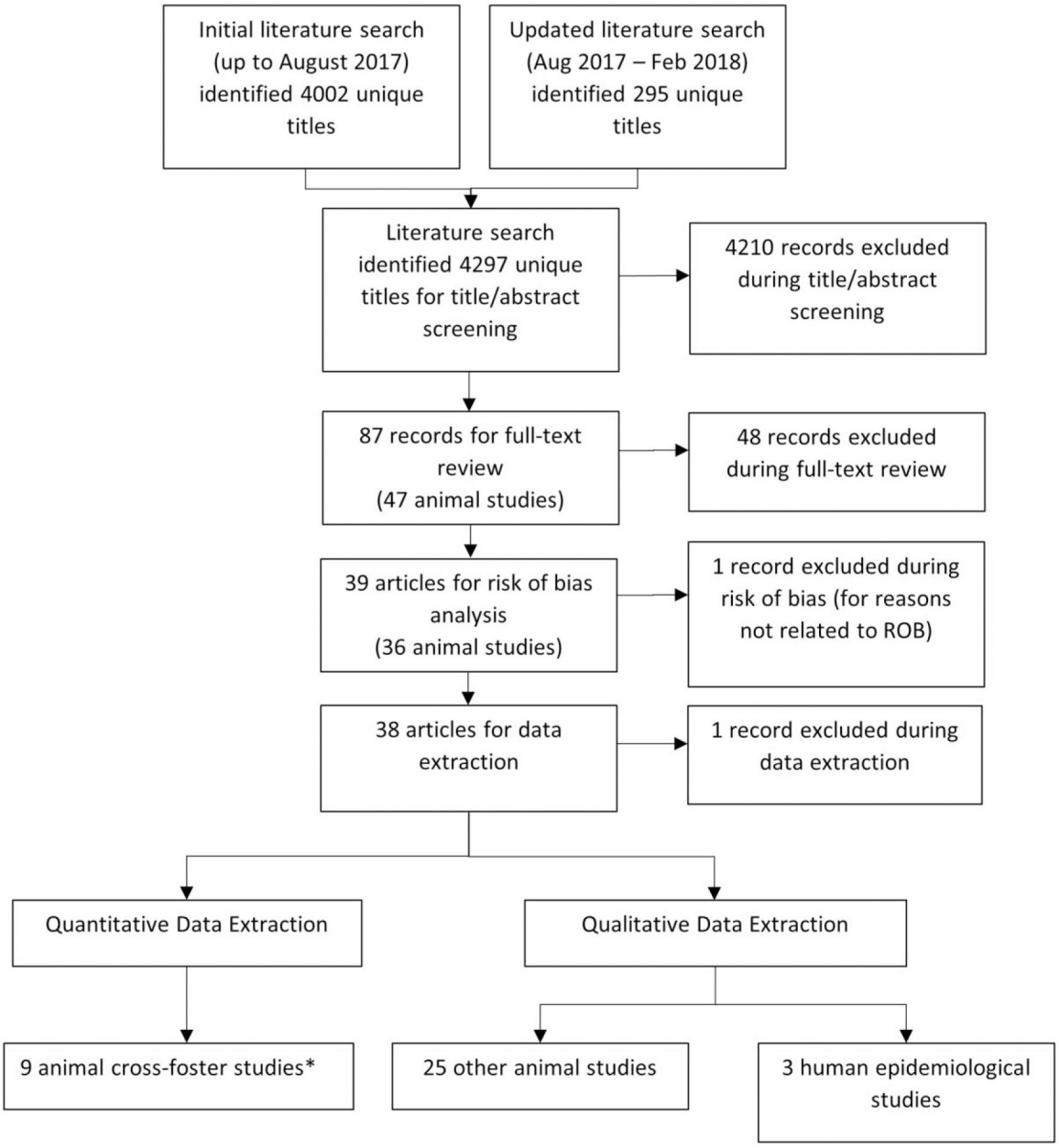

Figure 1.

Article screening process. *An August 2022 targeted update search did not identify any additional cross-foster animal studies to include in this article

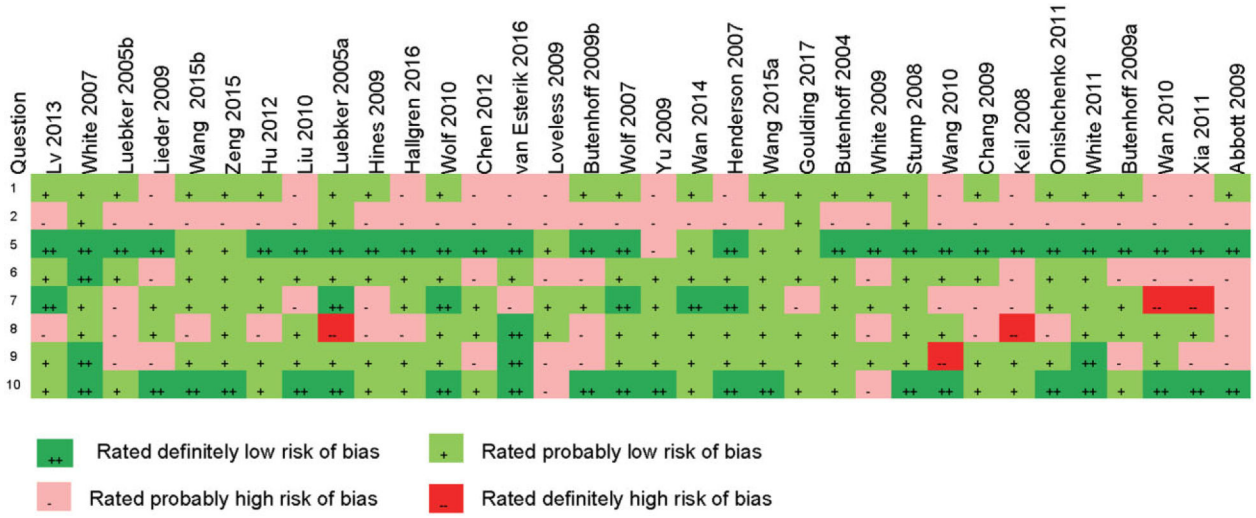

Figure 2.

Risk of bias heatmap for experimental animal studies. The risk of bias domains included the following: 1. Was administered dose or exposure level adequately randomized? 2. Was allocation to study groups adequately concealed? 5. Were experimental conditions identical across study groups? 6. Were research personnel blinded to the study group during the study? 7. Were outcome data complete without attrition or exclusion from analysis? 8. Can we be confident in the exposure characterization? 9. Can we be confident in the outcome assessment (including blinding of outcome assessors)? 10. Were all measured outcomes reported?

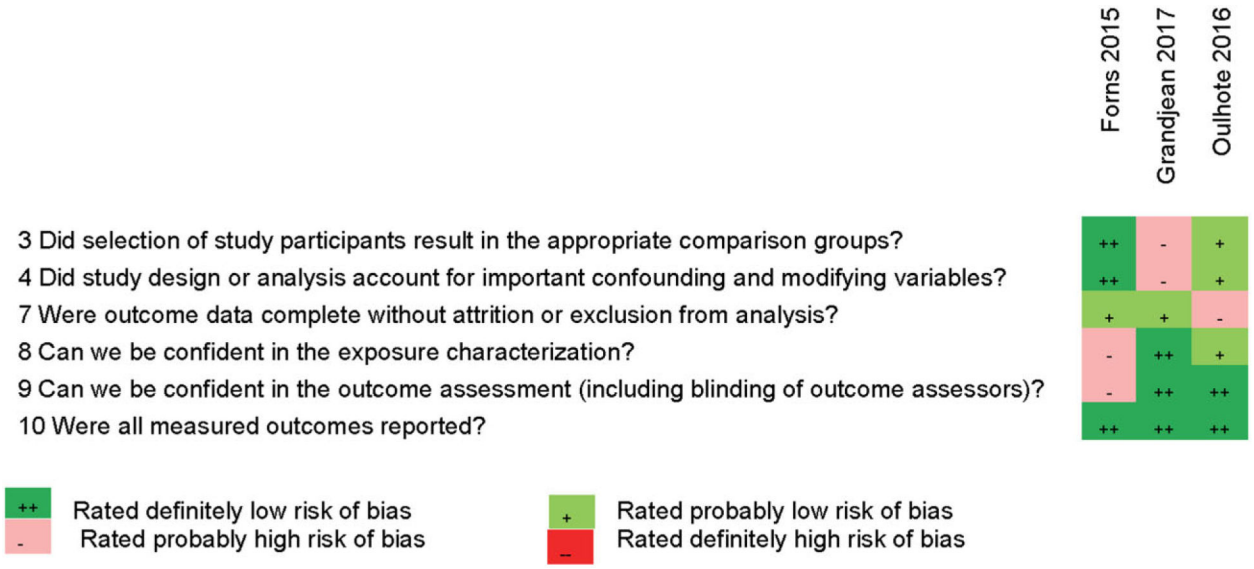

Figure 3.
Risk of bias heatmap for human observational cohort studies.

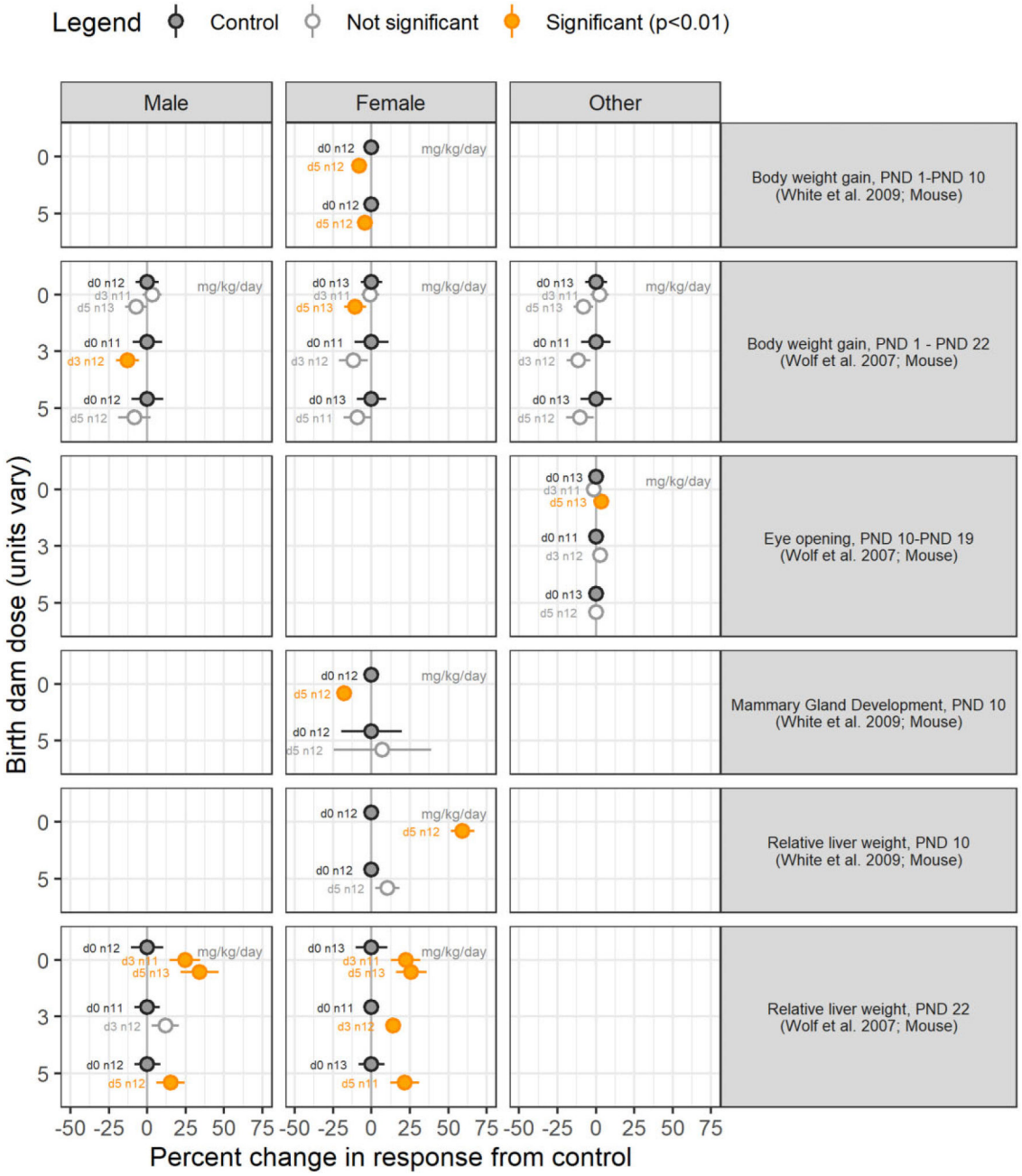

Figure 4.

Plot of outcomes extracted from cross-foster studies in which animals were exposed to PFOA or a PFOA salt. Foster dam dose and study *n* are shown to the left of each point range. Animals with nursing exposures were statistically compared with animals with the same in utero exposure using a 2-sided *t* test.

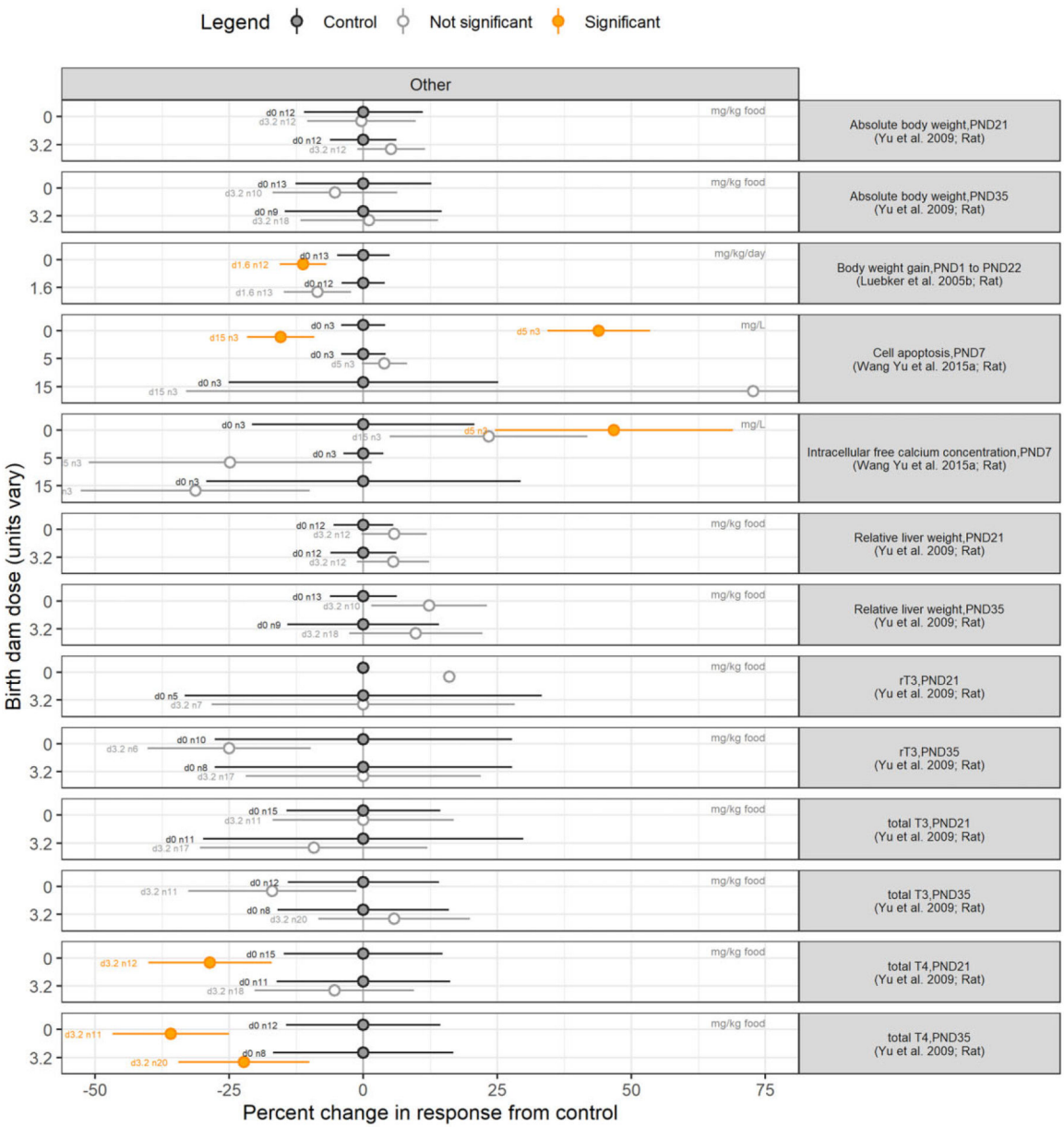

Figure 5.

Plot of outcomes extracted from cross-foster studies where animals were exposed to PFOS or a PFOS salt. Foster dam dose and study *n* are shown to the left of each point range. Animals with nursing were statistically compared with animals with the same in utero exposure using a 2-sided *t* test.

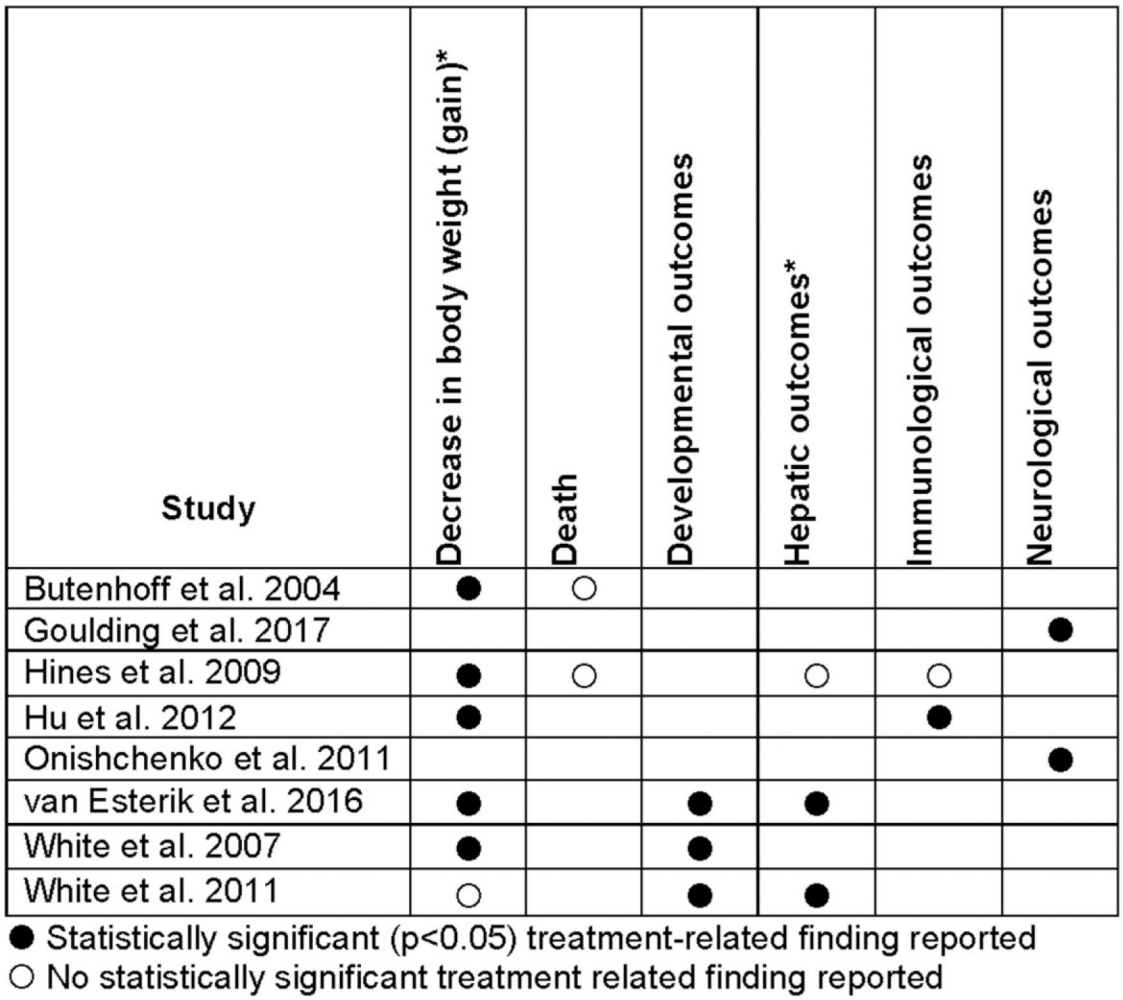

Figure 6.

Outcomes studied in developmental animal studies not using a cross-foster design where the animal was exposed to PFOA or a PFOA salt. Since our interest is in the potential effects of lactation exposure on these outcomes, we report only outcomes measured after PND 5 and not outcomes measured in animals that had other post-developmental exposures. Where the effect was measured multiple times, we report the measurement taken closest to weaning. Some studies measured multiple effects related to the same type of outcome. *Effects may be transitory, and the timing of outcome measurement may influence the significance of the findings.

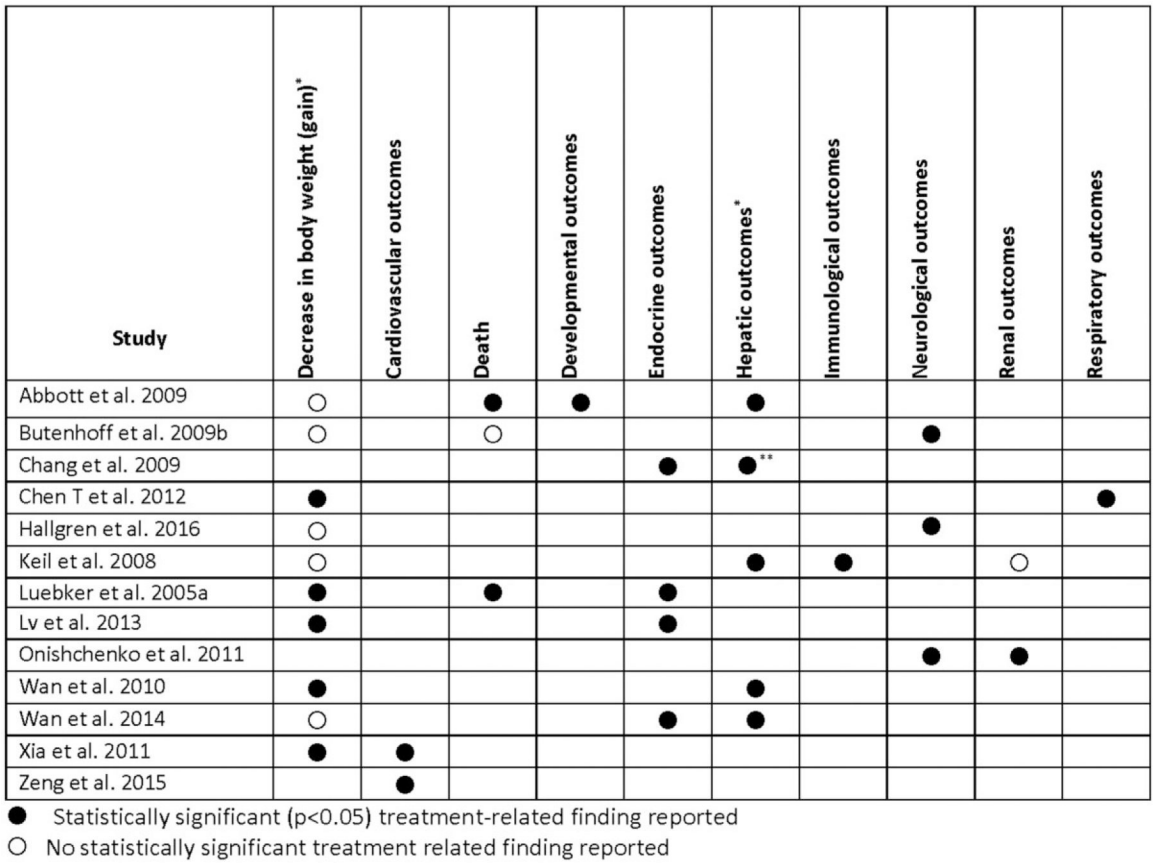

Figure 7.

Outcomes in developmental animal studies not using a cross-foster design where the animal was exposed to PFOS or a PFOS salt. Outcomes measured before PND5 or in animals with post-developmental exposure are not included. Where the effect was measured multiple times, we report the measurement taken closest to weaning. Some studies measured multiple effects related to the same type of outcome. *Effects may be transitory, and the timing of outcome measurement may influence the significance of the findings. **This study measured mRNA transcription of enzymes in the liver that are regulated by the thyroid "as a secondary measure of concordance of the thyroid hormone responses" and to explore potential PPARα-mediated mechanism through which PFOS may cause liver enlargement. This outcome may have limited applicability to humans.

Table 1.

PECO statement

Population	Humans or animals previously or currently exposed to PFAS through breastfeeding or nursing
Exposure	Exposure to PFAS through breastfeeding
Comparators	Humans/animals exposed to lower levels of PFAS (or none) than the more highly exposed breastfed humans/animals
Outcomes	Any health effect not directly attributable to another exposure or health condition

Table 2.

Inclusion and exclusion criteria to determine study eligibility for searches conducted on literature published from 2000 to February 2018 for human studies and experimental animal studies

PECO	Inclusion Criteria	Exclusion Criteria
Population, Human and Animal	Exposure during lactation, no other restrictions	Not exposed during lactation
Exposure Human	Exposure to any PFAS, their salts, or parent compounds based on: **1** Biomonitoring data (eg, urine, blood, or other specimens) and history of breastfeeding, **2** Environmental measures (eg, air, water levels) and history of breastfeeding or **3** Indirect measures (eg, job title) and history of breastfeeding	Exposure not determined or described; no information on breastfeeding exposure
Exposure Animal	Exposure to any PFAS, their salts, or parent compounds through lactation based on administered dose or concentration, biomonitoring data (eg, urine, blood, or other specimens), or environmental measures (eg, air, water levels)	Exposure not determined or described; no information on lactation
Comparators Human	Humans exposed to lower levels (or no exposure/exposure below detection levels), either breastfed or formula fed	No comparator
Comparators Animal	Must include vehicle or untreated control group	No comparator
Outcomes Human	Developmental-related outcomes (eg, neurological, reproductive, etc.), immunological, endocrine, or other health outcome	Health outcome with significant evidence linking it to another exposure or health condition presented in the study and lack of evidence linking it to exposure to PFAS through breastfeeding
Outcomes Animal	Developmental related outcomes (eg, neurological, reproductive, etc.), immunological, endocrine, or other health outcomes	Health outcome that follows exposure to another contaminant or that follows exposure to PFAS that is neither in utero nor through nursing
Publication Type, Human and Animal	Peer-reviewed studies with original data, and abstracts in English language	Studies that have no original data (eg, review, opinion), material that is not peer reviewed[a] (eg, book chapter, dissertation), conference proceedings, and retracted articles

[a]May be used to develop background material, review reference list.

Table 3.

Inclusion and exclusion criteria to determine study eligibility for searches conducted on literature published from February 2018 to August 2022

PECO	Inclusion Criteria	Exclusion Criteria
Population	Experimental animal cross-foster studies, no restrictions based on species	Animal cross-foster studies, no restrictions based on species
Exposure	Exposure to any PFAS, their salts, or parent compounds through nursing in a cross-foster study design	Exposure not determined or described; study does not use a cross-foster design
Comparators	Must include vehicle, untreated control group, and/or group exposed through in utero only that can be compared with a group dosed through in utero and lactation	No appropriate cross-foster groups allowing for comparison that isolates the lactation dose
Outcomes	Developmental-related outcomes (eg, neurological, reproductive), immunological, endocrine, or other health outcomes	Health outcome that follows exposure to another contaminant or that follows exposure to PFAS that is neither in utero nor through nursing
Publication type	Peer-reviewed cross-foster animal studies with original data and abstract in English language	Material that is not peer reviewed (eg, book chapter, dissertation), Conference proceedings and retracted articles

Table 4.

Applicability of risk of bias domains by study type

Risk of Bias Domain	Experimental Animal Studies	Observational Human (Cohort) Studies
1. Was administered dose or exposure level adequately randomized?	✓	×
2. Was allocation to study groups adequately concealed?	✓	×
3. Did selection of study participants result in the appropriate comparisons groups?	×	✓
4. Did study design or analysis account for important confounding and modifying variables?	×	✓
5. Were experimental conditions identical across study groups?	✓	×
6. Were research personnel blinded to the study group during the study?	✓	×
7. Were outcome data complete without attrition or exclusion from analysis?	✓	✓
8. Can we be confident in the exposure characterization?	✓	✓
9. Can we be confident in the outcome assessment (including blinding of outcome assessors)?	✓	✓
10. Were all measured outcomes reported?	✓	✓

Domains that are applicable to the study type are marked with a check.

Table 5.

Risk of bias tiers

Risk of Bias Tier	Criteria
Tier 1	1. Rated *probably low* or *definitely low* risk in all 3 key domains
	2. Two or less *probably high* or *definitely high* risk ratings in all other domains
Tier 2	Did not meet tier 1 or tier 3 criteria
Tier 3	1. Rated *definitely high* or *probably high* risk for all 3 key domains
	2. Had at least 3 additional *probably high* or *definitely high* risk ratings in other domains

Domains 7, 8, and 9 were key domains.

Table 6.

Rating of strength of evidence from cross-foster animal studies for potential associations between exposure to PFAS through breastfeeding and health outcomes in pups

Finding	Chemical	Studies	Level of Evidence	Factors Decreasing Confidence	Factors Increasing Confidence
Decreased body weight gain from birth to weaning	PFOA	White et al. (2009) and Wolf et al. (2007)	High	Publication bias	Consistency
Increased relative liver weight	PFOA	White et al. (2009) and Wolf et al. (2007)	High	Imprecision	Dose-response
Lower mammary gland development score	PFOA	White et al. (2009)	Moderate	Publication bias	
Decreased body weight gain from birth to weaning	PFOS	Luebker et al. (2005)	Low	Risk of bias Inconsistency	
Non-monotonic dose-response for percent of apoptotic cells and calcium concentration fold change in hippocampus	PFOS	Wang et al. (2015b) [a]	Low	Indirectness Publication bias	

Only outcomes with statistical associations with the chemical are reported here. A list of all outcomes evaluated is available in the Supplementary Material.

[a]Supporting information on neurological outcomes from PFOS exposure is available in Liu et al. (2010) and Wang et al. (2010, 2015a,b).