Critical Reviews in Toxicology

ISSN: 1040-8444 (Print) 1547-6898 (Online) Journal homepage: http://www.tandfonline.com/loi/itxc20

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To cite this article: Eva Negri, Francesca Metruccio, Valentina Guercio, Luca Tosti, Emilio Benfenati, Rossella Bonzi, Carlo La Vecchia & Angelo Moretto (2017): Exposure to PFOA and PFOS and fetal growth: a critical merging of toxicological and epidemiological data, Critical Reviews in Toxicology, DOI: 10.1080/10408444.2016.1271972

To link to this article: http://dx.doi.org/10.1080/10408444.2016.1271972

CRITICAL REVIEWS IN TOXICOLOGY, 2017
http://dx.doi.org/10.1080/10408444.2016.1271972

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REVIEW ARTICLE

Exposure to PFOA and PFOS and fetal growth: a critical merging of toxicological and epidemiological data

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ABSTRACT

Toxicological and epidemiological evidence on the association between perfluorooctanoic acid (PFOA) or perfluorooctane sulfonic acid (PFOS) and birth/fetal weight was assessed. An extensive search for toxicological information in rats and mice, and a systematic search for epidemiological evidence were conducted. The linear regression coefficient (LRC) of birth weight (BrthW) on PFOA/PFOS was considered, and separate random effects meta-analyses for untransformed (i.e. not mathematically transformed) and log-transformed values were performed.

Toxicological evidence: PFOA: 12 studies (21 datasets) in mice showed statistically significant lower birth/fetal weights from 5 mg/kg body weight per day. PFOS: most of the 13 studies (19 datasets) showed lower birth/fetal weights following *in utero* exposure.

Epidemiological evidence: Sixteen articles were considered. The pooled LRC for a 1 ng/mL increase in untransformed PFOA (12 studies) in maternal plasma/serum was −12.8 g (95% CI −23.2; 2.4), and −27.1 g (95% CI −50.6; −3.6) for an increase of 1 $\log_e$ ng/mL PFOA (nine studies). The pooled LRC for untransformed PFOS (eight studies) was −0.92 g (95%CI −3.4; 1.6), and for an increase of 1 $\log_e$ ng/mL was −46.1(95% CI −80.3; −11.9). No consistent pattern emerged for study location or timing of blood sampling.

Conclusions: Epidemiological and toxicological evidence suggests that PFOA and PFOS elicit a decrease in BrthW both in humans and rodents. However, the effective animal extrapolated serum concentrations are 10^2–10^3 times higher than those in humans. Thus, there is no quantitative toxicological evidence to support the epidemiological association, thus reducing the biological plausibility of a causal relationship.

ARTICLE HISTORY
Received 8 September 2016
Revised 30 November 2016
Accepted 10 December 2016

KEYWORDS
Birth weight; fetal growth; PFOA; PFOS; systematic review; meta-analysis; epidemiology; toxicology; perfluoroalkyls; reproduction; integration; evidence

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Introduction

Perfluoroalkyl acids (PFAA) or their precursors have been used since the 1950s in a wide variety of industrial and consumer applications, due to their water and lipid-repellent properties (Buck et al. 2011). The two most widely used PFAAs are perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS). They are highly persistent global pollutants, and human exposure can occur through ingestion of contaminated water and foods, mainly fish products, as well as through use of commercial products and inhalation. For their capacity to bind primarily to albumin, the blood is the main site of accumulation, reaching the highest concentration in well-perfused tissues such as liver and kidney (Glynn et al. 2012). PFOA and PFOS are widely detected in blood samples of the general human population (Kato et al. 2014). Due to their persistence in the environment and in the human body (half-lives of about 3.5 years for PFOA and 4.8 years for PFOS) and possible health effects, phasing out or restriction/reduction of PFOA and PFOS production and use is taking place in several countries, including the USA and the EU.

Toxicity studies in experimental animals showed that PFOA and PFOS cause weight loss, hepatic toxicity, effects on lipid metabolism, changes in thyroid hormone levels, persistent neurobehavioral, and developmental effects (Lau et al. 2003; Thibodeaux et al. 2003; USEPAa 2003; Luebker et al. 2005a; Chang et al. 2007; Son et al. 2008; Macon et al. 2011; White et al. 2011a). PFOA and PFOS have been shown to interfere with the endocrine system in experimental animals (Olsen & Zobel 2007; Boas et al. 2009). However, in workers exposed to PFOA and/or PFOS, hormone changes have not been consistently found (see e.g. Olsen et al. 2003; Olsen & Zobel 2007; Costa et al. 2009; Raymer et al. 2012).

Since PFOA and PFOS can cross the placenta (Lau et al. 2003; Seacat et al. 2003; Thibodeaux et al. 2003; Inoue et al. 2004; Luebker et al. 2005a; Olsen et al. 2009), concern about their effect on fetal growth and development arose (Olsen et al. 2009; Liew et al. 2014). A systematic review of the toxicological and epidemiological evidence of PFOA effects on fetal growth published up to May 2012 concluded that "developmental exposure to PFOA adversely affects human health based on sufficient evidence of decreased fetal growth in both human and non-human mammalian species" (Johnson et al. 2014; Lam et al. 2014). That review was based on the Navigation Guide systematic review methodology, which has been designed to provide a systematic and rigorous method for research synthesis (Woodruff & Sutton 2014).

An integration of evidence from toxicology and epidemiology for improving causal inference and risk assessment of chemicals has been frequently advocated (Adami et al. 2011; Woodruff & Sutton 2014). The Epid-tox framework has been proposed by a group of epidemiologists and toxicologists for combining toxicological and epidemiological evidence to establish causal inference (Adami et al. 2011). This framework is based on the following five steps: (1) collection of all relevant studies; (2) quality assessment and categorization of studies; (3) evaluation of epidemiological and toxicological weight of evidence; (4) assignment to a scalable conclusion of the biological (toxicological) plausibility and epidemiological evidence; (5) placement of the finding in a causal relationship grid. The framework's aim is to illustrate how epidemiological and toxicological data intersect, provide help in drawing conclusions on causal relationships, and show the influence of potential additional data.

Epidemiologic evidence on the association between PFAAs and fetal growth has been accumulating, and since May 2012, a number of additional studies have been published (Wu et al. 2012; Darrow et al. 2013; Robledo et al. 2015; Bach et al. 2016; Lee et al. 2016). Moreover, although the association of PFOS with fetal growth has been summarized in some articles (Olsen et al. 2009; Bach et al. 2015; Verner et al. 2015), no review similar to that conducted for PFOA has been published. For these reasons, we decided to perform a systematic integrated review of the epidemiological evidence and an extended review of toxicological information on the effect of PFOA and PFOS on fetal growth, taking advantage of the recent developments in methodologies for integrating toxicological and epidemiological results (Adami et al. 2011; Woodruff & Sutton 2014).

The aim of this paper is to quantitatively integrate toxicological and epidemiological data, by estimating effects separately in animals and humans, and – by extrapolating animal plasma concentrations – comparing the estimated dose–response relationship between humans and animals.

Materials and methods

Toxicological evidence

An extensive search for all available toxicological information regarding birth weight (BrthW) in experimental animals administered PFOA or PFOS was undertaken using the following review strategy: (1) identification of evidence and (2) selection of evidence and data collection. Key words used included PFOS/perfluorooctanoate sulfonate/PFOA/perfluorooctanoic acid and birth weight, developmental toxicity, fetal growth, sex ratio, and time to pregnancy.

Identification of evidence

Our objective was to retrieve all developmental/reproductive experimental studies in mammals administered either PFOA or PFOS where a specific adverse pregnancy outcome (BrthW) was investigated.

An extensive literature search was used to retrieve the studies on PFOA and PFOS reporting effect on fetal growth on rodents routinely used for risk assessment (rats and mice). Valuable aspects were kept in mind, like transparency and research reproducibility.

All routes of exposure were considered, and all dose regimes (low, high, single, repeated) of PFOA or its salts, and PFOS or its potassium salt. The treatment included any

Table 1. Summary of PFOA serum concentration in orally exposed mice.

Author	Specie	Exposure period	Oral dose (mg/kg bw)	Serum concentration (ppm)	SD	Sample time
Ngo et al. (2014)	mice	GD 1-17	0.01	0.2	na	GD 18
			0.1	2.2	na	
			3	35.3	na	

SD: standard deviation; bw: body weight; GD: gestation day; na: not applicable.

reproductive/developmental time period (before and/or during pregnancy). Our search strategy was built using separate keywords (e.g. substance AND outcome) or grouped keywords done by combining one or more additional concepts relating to the specific substance-adverse effect (e.g. substance AND outcome AND/OR target).

The search was conducted with Pubmed/Medline and ToxNet databases and was not restricted to any language or publication date. Searching was conducted in titles, abstract, and full article text.

Selection of evidence and data collection

For both PFOA and PFOS, all records identified were merged and duplicates disregarded. Thereafter, a first assessment on the relevance of the study was performed on titles, and abstracts if needed. Non-pertinent studies as well as not relevant ones, were excluded based on the following criteria: (i) epidemiological studies; (ii) analytical studies, (iii) studies not measuring birth/fetal weigh, as indicated in the experimental protocol, (iv) studies where animals were exposed to mixtures of compounds, or (v) studies conducted with species other than rat and mouse, and rabbit (not-found). When the information in the abstract was not detailed enough, a second detailed assessment was performed looking into full text documents. Consequently, studies were disregarded if the above mentioned exclusion criteria were satisfied. The quality of toxicological studies was assessed using the criteria outlined by Klimisch et al. (1997). Only studies falling into category 1 or 2 according to Klimish's criteria were taken into consideration. In addition, the reference list of all selected studies was scrutinized for relevant studies to be included.

Adequate, reliable, and relevant studies were collected in EndNote and significant data were extracted into an *ad hoc* Microsoft Access database. The database was built to allow collection of the following study information: last name of first author, year of publication, study title, aim of the study, effect characterization, doses used, species, exposure route, window of exposure and its duration, and summary of results.

The influence and errors due to data manipulation by the staff operators were kept as low as possible. Where feasible, data were copied and pasted directly from the study into the Access data entry form, and for constrained fields, for which only pre-defined set of values can be used, drop-down menus were compiled. For quality control purposes, a system of metadata was implemented to keep track of the data flow history. When numerical data were available, a tool to draw dose–response curves was also implemented to facilitate the evaluation of results.

Most epidemiological studies provide maternal serum concentrations. Thus, to allow a meaningful quantitative comparison between animal and human data, the relationship between PFOA and PFOS concentration in rodent serum and the administered oral dose was derived by linear extrapolation.

For PFOA, the data from Ngo et al. (2014) (analyses of PFOA serum concentration in mice exposed by oral gavage) have been used to derive the equation to relate oral dose and serum concentration at gestation day (GD) 18 (Table 1). The equation was then applied to the data from the other studies (Table 2).

For PFOS, the data from Ngo et al. (2014) (mice only), Thibodeaux et al. (2003), and Lau et al. (2003) (both mice and rats) have been used to derive the equation to relate oral dose and serum concentration at GD18 (mice) or at GD21 (rats) (Table 3). The equation was then applied to the data from the other studies (Table 4).

Epidemiological evidence

This report follows the meta-analyses of Observational Studies in Epidemiology (MOOSE) and the Preferred Reporting and Items for Systematic Review and Meta-Analysis (PRISMA) guidelines for reporting (Stroup et al. 2000; Liberati et al. 2009).

Search strategy

We conducted a systematic literature search in the Medline and Embase databases of studies published up to November 2015. The search strings (Table 5) combined terms for exposure to perfluorinates compounds (e.g. PFOA, PFOS, perfluoroalkyl substance) and for BrthW and pregnancy complications (e.g. low birth weight, LBW), without restrictions on population, study design, language, or publication date. Two authors (V. G. and E. N.) independently assessed the retrieved articles for inclusion/exclusion criteria. They also checked the reference list of pertinent papers to identify further studies. Abstracts and unpublished studies were not included. No studies were excluded *a priori* for weakness of design or data quality.

Eligibility criteria

We used eligibility criteria built on the basis of the PICOS (participants/population, intervention/exposure(s), comparator(s)/control, outcome(s), and study design) approach (Johnson et al. 2014) as follows: (i) participants; women enrolled before or during pregnancy or at delivery, (ii) exposure: PFOA and PFOS assessed in a biological sample, such as

Table 2. Summary of birth/fetal weight, maternal weight, cesarean section, and pup toxicity findings in mice exposed to PFOA.

						Results			
Author	Adverse pregnancy outcome	Species	Exposure Route	Period of exposure	Doses (mg/kg bw/d)	Birth/fetal weight	Maternal weight gain	Cesarean section	Pup toxicity
Abbott et al. (2007)	Birth weight	Mice/WT 129S1/SvImj	Oral (gavage)	GD 1-17	0, 0.1, 0.3, 0.6, 1, 3, 5, 10, 20	X	X	↑ FLR and non-pregnant females from 5 mg/kg bw	↑ open eye delay (only in wild type) from 0.6-1 mg/kg bw
Albrecht et al. (2013)	Fetal weight	Mice/WT Sv/129	Oral (gavage)	GD 1-17	0, 3	X	X	X	X
Lau et al. (2006)	Fetal weight	Mice/CD-1	Oral (gavage)	GD 1-17	0, 1, 3, 5, 10, 20, 40	↓ from 20 mg/kg bw	↓from 20 mg/kg bw	↑ FLR from 5 mg/kg bw	↑ open eye delay ↓ pup survival from 5 mg/kg bw
Wolf et al. (2007)	Birth weight	Mice/CD-1	Oral (gavage)	GD 1-17	0, 3, 5	↓ at 5 mg/kg bw	↑ at 3 and 5 mg/kg bw	↑ litter loss from 5 mg/kg bw	↑ open eye delay from 3 mg/kg bw
				GD 7-17	0, 5	↓ in males	↑		
				GD 10-17	0, 5	↓ in males	↑		
				GD 13-17	0, 5	X	X		
				GD 15-17	0, 5	X	X		
				GD 15-17	0, 20	↓ in males	x		
van Esterik et al. (2015)	Birth weight	Mice/C57BL/6JxFVB	Oral (diet)	GD 1-17	0, 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3	↓ in exposed males at all doses	x	↓ litter size from 1 mg/kg bw	x
Yahia et al. (2010)	Birth weight	Mice/CD-1(ICR)	Oral (gavage)	GD 1-17	0, 1, 5, 10	↓ from 5 mg/kg bw	↓ from 10 mg/kg bw		
	Fetal weight				0, 1, 5, 10	↓ from 5 mg/kg bw		X	↓ pup survival from 5 mg/kg bw
Ngo et al. (2014)	Birth weight	Mice/C57BL/6J-Apc+/+	Oral (gavage)	GD 1-17	0, 0.01, 0.1, 3.0	↓[a] from 0.1mg/kg bw	X	X	↓ pup survival from 3 mg/kg bw
White et al. (2011a, 2011b)	Birth weight	Mice/CD-1	Oral (gavage)	GD 1-17	0, 1, 5	X	X	X	↓ pup survival in P_0 (not in F_1) from 5 mg/kg bw
Suh et al. (2011)	Fetal weight	Mice/CD-1	Oral (gavage)	GD 11-16	2, 10, 25	↓ from 10 mg/kg bw	↓ from 25 mg/kg bw	↓ placenta efficiency from 2 mg/kg bw ↑ implantation loss from 10 mg/kg bw	↓ pup survival from 10 mg/kg bw
White et al. (2007)	Birth weight	Mice/CD-1	Oral (gavage)	GD 1-17	0, 5	↓by 12%	x		x
Hines et al. (2009)	Birth weight	Mice/CD-1	Oral (gavage)	GD 8-17	0, 5	↓by 7%.		X	
				GD 12-17	0, 5	↓by 3%.			
				GD 1-17	0, 0.01, 0.1, 0.3, 1, 5	↓at 5mg/kg bw.	X	No data	X
White et al. (2009)	Birth weight	Mice/CD-1	Oral (gavage)	GD 8-17	5	X	X	X	X

↓/↑: statistically significant change (p values not reported); X: no effect observed.
[a]Pup weight was measured as AUC PND 1–18 (area under the curve at postnatal days 1–18) pooling males and females pups from hybrid strain (C57BL/6J-Min/+) and wild type (Apc+/+).
bw: body weight; GD: gestation day; FLR: full litter resorption.

Table 3. Summary of PFOS serum concentration in orally exposed rodents.

Author	Species	Exposure period	Oral dose (mg/kg bw)	Plasma/ serumconcentration (ppm)	SD	Sample time
Lau et al. (2003)	Rat	GD 2-21	1.07	25	na	LD 8
			0.58	16	na	
Thibodeaux et al. (2003)	Rat	GD 2-20	1	20	2	GD 21
			2	45	2	
			3	72	7	
			5	81	3	
			10	190	7	
Lau et al. (2003)	Mice	GD 1-18	7.02	102	na	LD 6
			3.88	56	na	
Thibodeaux et al. (2003)	Mice	GD 1-17	1	9	na	GD 18
			5	50	na	
			10	179	na	
			15	241	na	
			20	261	na	
Ngo et al. (2014)	Mice	GD 1-17	0.1	1.334	na	GD 18
			3	36.6	na	
			0.01	0.1	na	

SD: standard deviation; bw: body weight; GD: gestation day; na: not applicable.

maternal or umbilical cord serum, plasma or whole blood, or maternal milk; (iii) comparators: newborns exposed to lower levels of PFOA and PFOS; (iv) outcomes: BrthW (primary outcome), LBW (defined as BrthW <2500 g) and small for gestational age (SGA, defined as BrthW $<$ the 10th percentile for gestational age) (secondary outcomes); (v) study design: cross-sectional, case–control, or cohort study.

Data extraction

Data from selected studies were extracted using an *ad hoc* created Excel sheet. Data extraction was undertaken independently by two authors (V. G. and E. N.).

We extracted the following information: last name of the first author, year of publication, location, study design (cross-sectional, case-control or cohort study), number of subjects (cases and controls/non-cases/cohort size), sex, period of enrollment, type of PFAA assessed (PFOA or PFOS), biological matrix used to assess exposure, timing of exposure assessment (at delivery, trimester of pregnancy, and before pregnancy), outcome (BrthW, LBW, and SGA), level of PFAAs (median, mean, standard deviation, and range), exposure and outcome variable used (continuous, categorized, and log-transformed), statistical model and effect estimator used (i.e., beta coefficient, odds ratio, and relative risk), and covariates adjusted for in the analysis.

Risk of bias appraisal

Two researchers (V. G. and E. N.) independently assessed the methodological aspects of each study using a modification of the "Newcastle-Ottawa Quality Assessment Scale" (NOS) (Wells et al. 2000). This scale was originally designed to assess the quality of case-control and cohort studies, and consists of eight items subdivided in three sections: selection of the study groups (four items); comparability of the groups (one item); and ascertainment of either the exposure or outcome of interest for case–control or cohort studies, respectively (three items). For cohort studies, we excluded two items (demonstration that outcome was not present at start of study and whether follow-up was long enough for outcomes to occur), because they were not relevant for the present purpose. For cross-sectional studies, we adapted the NOS for cohort studies by further excluding the item on adequacy of follow-up. On the basis of the results, we defined the risk of bias as low, high or unclear (Higgins et al. 2011). We considered newborn sex, gestational age, maternal age, pre-pregnancy body mass index (BMI), education, parity, and smoking to be the most important potential confounders as these have been shown to be associated with PFAA levels and with outcome, BrthW, LBW, and SGA. The maximum score was allocated for studies who adjusted for all these seven variables.

Chemical assessment quality

An analytical chemist with background in toxicology (EB) evaluated the analytic methodology used to measure PFOA/PFOS levels in the biological matrix, considering the following aspects: (i) quality of the analytical methodology (gas chromatography-mass spectrometry, electron capture detector, inductively coupled plasma mass spectrometry, etc.); (ii) adequacy of the matrix to evaluate PFOA and PFOS and associated effect (urine, serum, plasma, etc.). For the quality of the analytical methodology by three levels (high, medium, and low), we considered the year of publication, and consequent improvements in technology, the introduction of higher standards of quality assurance and control criteria (e.g.: certified reference material, the use of a standardized protocol).

Statistical analysis

We considered the studies as a cohort (or a nested case-control) if women were enrolled before the outcome was measured (i.e. before delivery), and a cross-sectional design if they were enrolled at delivery, and exposure and outcome were measured simultaneously.

For BrthW, we considered the linear regression coefficient (LRC) of the linear regression of BrthW on PFOA or PFOS

Table 4. Summary of birth/fetal weight, maternal weigh, cesarean section and pup toxicity findings in rats and mice exposed to PFOS.

						Results			
Author	Adverse pregnancy outcome	Species	Exposure Route	Period of exposure	Doses (mg/kg bw/d)	Birth/fetal weight	Maternal body weight gain	Cesarean section	Pup toxicity
Grasty et al. (2003)	Birth weight	Rat/SD	Oral (gavage)	GD 2-5	0, 25	↓	↓ at 25 mg/kg bw		
				GD 6-9	0, 25	↓			
				GD 10-14	0, 25	↓			
				GD 14-17	0, 25	X			
				GD 17-20	0, 25	X		No data	↓ pup survival from 25 mg/kg bw
				GD 19-20	0, 25, 50	↓ from 25 mg/kg bw	↓ from 25 mg/kg bw		
Lau et al. (2003)	Birth weight	Rat/SD	Oral (gavage)	GD 2-21	0, 1, 2, 3, 5, 10	Slight decrease at 1 mg/kg bw ↓ at 2, 3, 5 mg/kg bw	No data	No data	↓ growth delay from 2 mg/kg bw ↓ pup survival from 10 mg/kg bw
Thibodeaux et al. (2003)	Fetal weight	Rat/SD	Oral (gavage)	GD 2-20	0, 1, 2, 3, 5, 10	↓at 10 mg/kg bw	↓ from 2 mg/kg bw	X	X
Luebker et al. (2005a)	Birth weight	Rat/Crl:CD(SD)	Oral (gavage)	GD 6-20	0, 0.4, 0.8, 1, 1.2, 1.6, 2	↓ from 0.4 mg/kg bw.	↓ from 1.6 mg/kg bw	X	↓ pup survival from 1.6 mg/kg bw
Luebker et al. (2005b)	Birth weight	Rat/Crl:CD (SD)	Oral (gavage)	GD 1-21	0, 0.1, 0.4, 1.6, 3.2	↓ from 1,6 mg/kg bw	↓ from 3.2 mg/kg bw	↓ viability index from 1.6 mg/kg bw ↓ of implantations from 3.2 mg/kg bw	↓ pup survival from 1.6 mg/kg bw ↑ stillborn from 3.2
Yu et al. (2001)	Birth weight	Rat/Wistar	Oral (diet)	Gestation & Lactation	0, 3.2	X	No data	No data	No data
Chen et al. (2012b)	Birth weight	Rat/SD	Oral (gavage)	GD 1-21	0, 0.1, 2	↓ at 2 mg/kg bw.	No data	No data	No data
Lau et al. (2003)	Birth weight	Mice/CD-1	Oral (gavage)	GD 1-18	0, 1, 5, 10, 15, 20	From 10 mg/kg bw, trend decrease but not statistically significant	No data	No data	Growth delay from 1 mg/kg bw ↓ pup survival from 10 mg/kg bw
Thibodeaux et al. (2003)	Fetal weight	Mice/CD-1	Oral (gavage)	GD 1-17	0, 1, 5, 10, 15, 20	↓ from 10 mg/kg bw	↓ from 2 mg/kg bw	No effect	No effect
Fuentes et al. (2006)	Fetal weight	MiceCD-1	Oral (gavage)	GD 6-18	0, 1.5, 3, 6	X	↓ from 6 mg/kg bw	↑ litters with dead fetuses from 6 mg/kg bw	X
Fuentes et al. (2007)	Birth weight	MiceCD-1	Oral (gavage)	GD 6-18	0, 6	X	No effect	No effect	No effect
Yahia et al. (2008)	Birth weight	Mice/ICR	Oral (gavage)	GD 1-17	0, 1, 10, 20	↓ from 10 mg/kg bw.	↓ at 20 mg/kg bw	↓ live fetuses at 20 mg/kg bw	↓ pup survival from 10 mg/kg bw
	Fetal weight					↓ from 10 mg/kg bw		↑ variation/malformation from 10 mg/kg bw	

↓/↑: statistically significant change (*p* value not reported). X: no effect observed. bw: body weight; GD: gestation day.

Table 5. Search strings used to retrieve epidemiological studies on PFOA/PFOS.

Database	PubMed
Date	20 November 2015
String	(Fluorocarbon* OR fluorinated OR perfluorinated OR "perfluoroalkyl substance" OR "polyfluoroalkyl substance" OR "perfluoro alkyl acid" OR "polyfluoroalkyl acid" OR PFAS OR "perfluorinated compound" OR PFC* OR "perfluorinated alkyl acid" OR "perfluoroalkyl sulfonic acid" OR PFSA OR "perfluoroalkyl carboxylic acid" OR PFCA OR perfluorosulfonate* OR "perfluorooctane sulfonic acid" OR PFOS OR "perfluorooctane sulfonate" OR perfluorocarboxylate* OR "perfluorooctanoid acid" OR perfluorooctanoate* OR PFOA OR "endocrine disruptor") AND ("Pregnancy Complications"[Mesh] OR miscarriage OR "recurrent miscarriage" OR "preterm delivery" OR "birth defects" OR "gestational age" OR "birth weight" OR "intrauterine growth restriction" OR IUGR OR "adverse pregnancy outcome" OR "fetal development" OR "fetal growth" OR "low birth weight" OR "small for gestational age" OR "adverse pregnancy outcome" OR reproduction* OR "fetal development")
Limits	No limit
Database	Embase
Date	20 November 2015
String	Fluorocarbon OR fluorinated OR perfluorinated OR "perfluoroalkyl substance" OR "polyfluoroalkyl substance" OR "perfluoro alkyl acid" OR "polyfluoroalkyl acid" PFAS OR "perfluorinated compound" OR PFC OR "perfluorinated alkyl acid" OR "perfluoroalkyl sulfonic acid" OR PFSA OR "perfluoroalkyl carboxylic acid" OR PFCA OR perfluorosulfonate OR "perfluorooctane sulfonic acid" OR PFOS OR "perfluorooctane sulfonate" OR perfluorocarboxylate OR "perfluorooctanoid acid" OR perfluorooctanoate OR PFOA OR "endocrine disruptor" AND "pregnancy complications" OR abortion OR "hypertension pregnancy" OR eclampsia OR pre-eclampsia OR "perinatal death" OR "fetal death" OR stillbirth OR miscarriage OR "recurrent miscarriage" OR "preterm delivery" OR "birth defects" OR "gestational age" OR "birth weight" OR "intrauterine growth restriction" OR IUGR OR "adverse pregnancy outcome" OR "fetal development" OR "fetal growth" OR "low birth weight" OR "small for gestational age" OR "adverse pregnancy outcome" OR reproduction* OR "fetal development"
Limits	No limit

levels, and its standard error (SE). When different LRC were reported, we chose the fully adjusted one. Some studies introduced untransformed PFOA/PFOS levels in the regression model, while others used log-transformed values. We performed two separate meta-analyses for actual and log-transformed values. We chose 1 ng/mL and the natural logarithm (ln) of 1 ng/ mL as measurement unit for the LRC for the untransformed and log-transformed analyses, respectively. We rescaled LRCs and SE from models using a different measurement unit, and used the formula for the change of base of logarithms when the PFOA/PFOS values were log-transformed to base 10. One study presented the LRC and SE of the regression of PFOA/PFOS on BrthW and the R2 statistics, from which we derived the LRC and SE of the regression of BrthW on PFOA/PFOS (Monroy et al. 2008).

For all meta-analyses, we used a random effects model based on the inverse variance methods and the DerSimonian and Laird (1986) estimate of the between-study variance. Heterogeneity was quantified by Cochran's χ^2 statistic Q and by the I^2 statistics, i.e. the percentage of variation across studies that is due to heterogeneity rather than chance (Higgins & Thompson 2002). We performed a sensitivity analysis by excluding each study in turn from the pooled analysis, and also evaluated results using a fixed effect model.

Subgroup analyses were conducted by categories of geographic location, adjustment for confounding factors, and medium/timing of PFAA exposure estimation (maternal blood/1st or 2nd trimester; maternal blood 3rd trimester or delivery; umbilical cord blood/delivery) to investigate eventual sources of heterogeneity. We also performed a meta-regression by median/mean level of exposure (weighted by medium/timing of PFAA exposure estimation), to investigate the relation between effect size and dose. Publication bias was investigated by funnel plots (Sterne & Egger 2001) and by the tests proposed by Begg and Mazumdar (1994) and by Egger (Egger et al. 1997). The trim-and-fill method was also used to investigate the potential effect of publication bias on the pooled estimate.

All statistical analyses were performed using the "meta" package v.4.1–0 of R software (Schwarzer 2015).

Results

Toxicological evidence

PFOA

Out of 215 unique records identified, 203 were excluded through title, abstract, and full text screening, resulting in 12 studies describing 21 data sets included in the review. A summary of the study characteristics is provided in Table 2.

The 12 selected studies were conducted on mice: pregnant animals were dosed with PFOA or its salts, and fetal or newborn weight was investigated. The route of exposure was oral, 11 by gavage and 1 *via* diet, and dose range spanned up to about four orders of magnitude from 3 μg/kg body weight (bw) to 40 mg/kg bw per day with 1–8 dose levels in individual studies. For the majority of the studies, *in utero* exposure to PFOA was from GD 1 to 17. The time point of weight measurement varied: near to term of gestation (GD18) or time of birth, postnatal day (PND) 0 or PND4. Parturition monitoring varied, from constant monitoring to daily cage check. Offspring weights were measured individually, grouped per litter, or by sex.

In utero exposure to PFOA was consistently associated with lower birth and/or fetal weight, males being generally more susceptible than females, although in 3/12 studies data for each sex were not reported separately.

Dose–response curves for mean birth/fetal weight were drawn (Figure 1) from all datasets for which numerical data were available for all dose groups. This was not possible e.g. if results were presented as figures only.

Almost all study results presented in Figure 1 show a consistent dose response trend. Both birth (Figure 1(a)) and

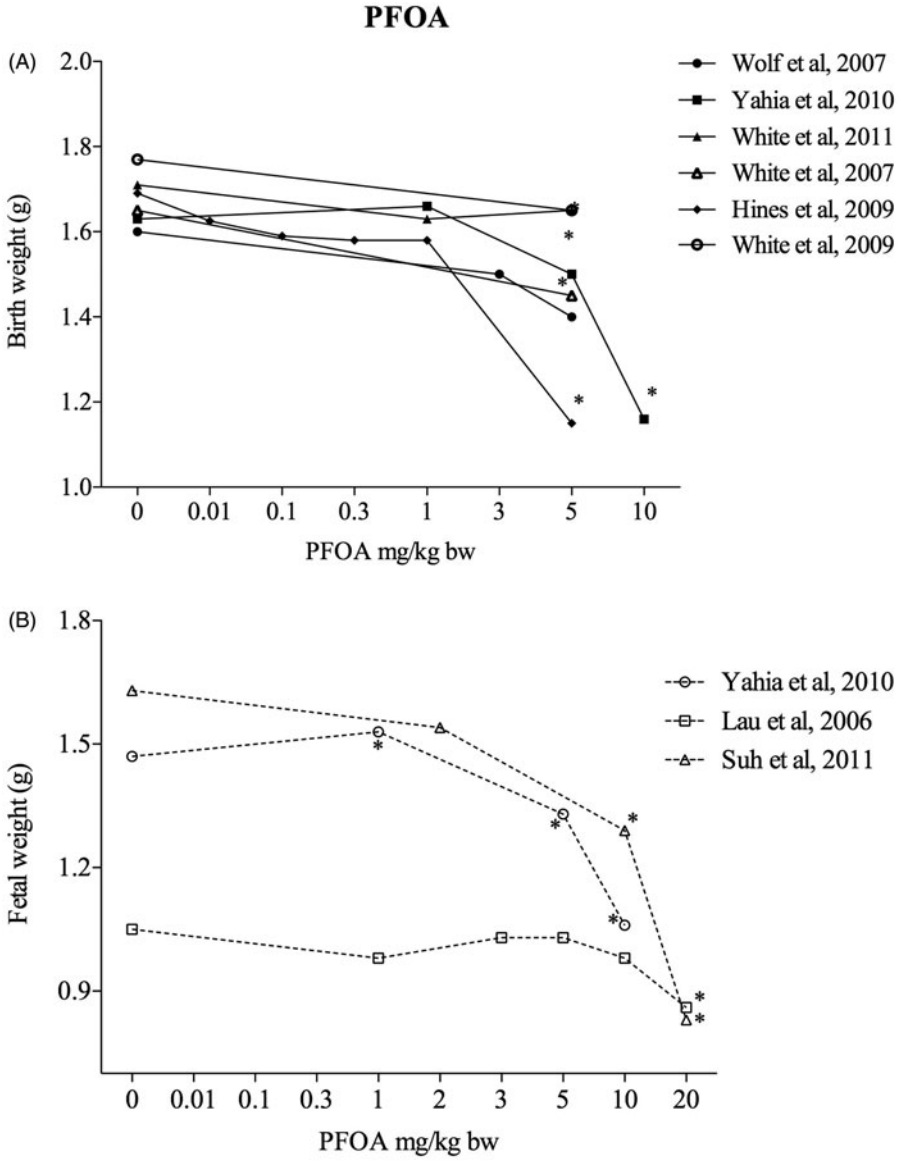

Figure 1. Mice mean birth/fetal weight following *in utero* oral exposure to PFOA. *Statistically significant (*p* values not shown).

fetal (Figure 1(b)) weight decreases start at doses greater than 1 mg/kg bw, with similar slopes up to the highest dose tested, and reach statistical significance starting from 5 mg/kg bw (White et al. 2007; Wolf et al. 2007; Hines et al. 2009; Yahia et al. 2010; Suh et al. 2011). White et al. (2011b) (Figure 1(a)) showed unclear effect (doses range from 1 to 5 mg/kg bw). White et al. (2009) (Figure 1(a)) showed BrthW decrease, although not statistically significant. These findings are in agreement with the few studies not reported in Figure 1 due to lack of original numerical data (only figure available, see study results presented only in Table 2).

Van Esterik et al. (2016) and Ngo et al. (2014) showed the lowest effect dose level (see Table 2). The use of hybrid strains (C57BL/6JxFVB and C57BL/6J-Apc+/+) built to be more susceptible to several *noxae* may explain these differences (Yu et al. 2001; Dolle et al. 2011). Almost all studies employed dose ranges within one order of magnitude. Only one study (Hines et al. 2009) used a broader dose range (more than two orders of magnitude: from 0.01 to 5 mg/kg bw), showing, however, no significant effect at doses lower than 5 mg/kg bw.

Results from Abbott et al. (2007) showed a slight trend in BrthW decrease, although not statistically significant, up to 1 mg/kg bw dose level (Table 2). No data on BrthW were obtained at higher dose levels due to full litter resorption (FLR) early in gestation.

Consistent with the other studies, in the study conducted by Albrecht et al. (2013), no changes in BrthW were evident following a single oral treatment of pregnant mice with 3 mg/kg bw.

In the majority of the studies reporting mean birth/fetal weight reduction, maternal, and/or developmental or pup/fetal toxicity effects were also observed (Table 2) (Lau et al. 2006; Wolf et al. 2007; Yahia et al. 2010; Suh et al. 2011; White et al. 2011b; Ngo et al. 2014).

Results from Lau et al. (2006) showed maternal body weight effect at the same dose level eliciting fetal weight

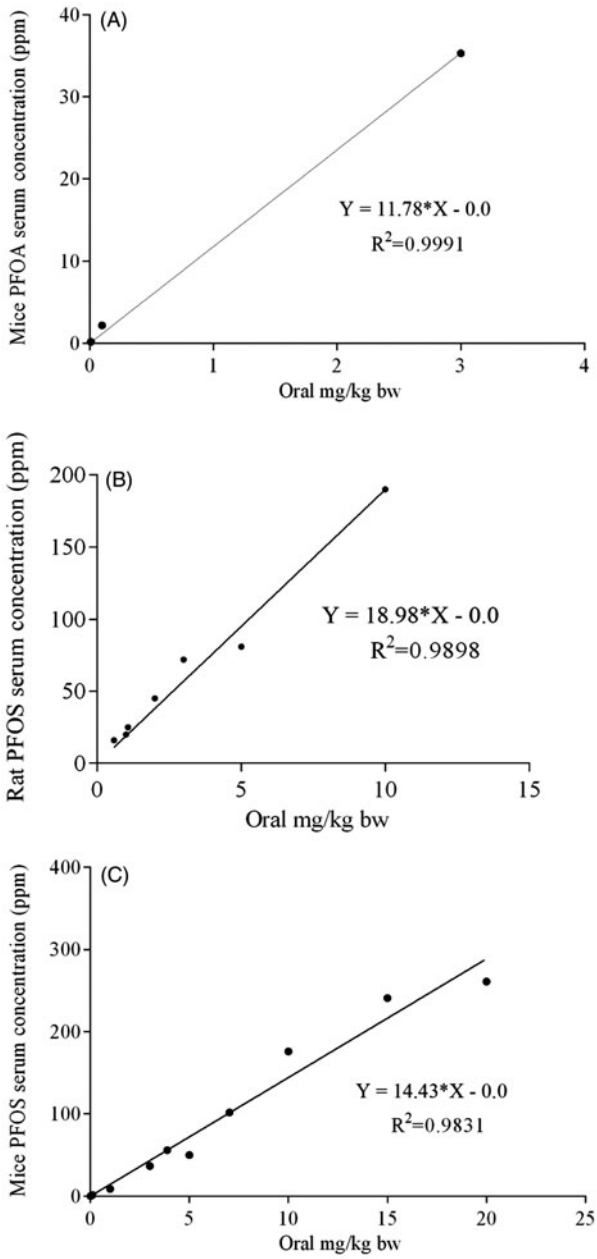

Figure 2. PFOA and PFOS, rat and mice serum concentration interpolation curves.

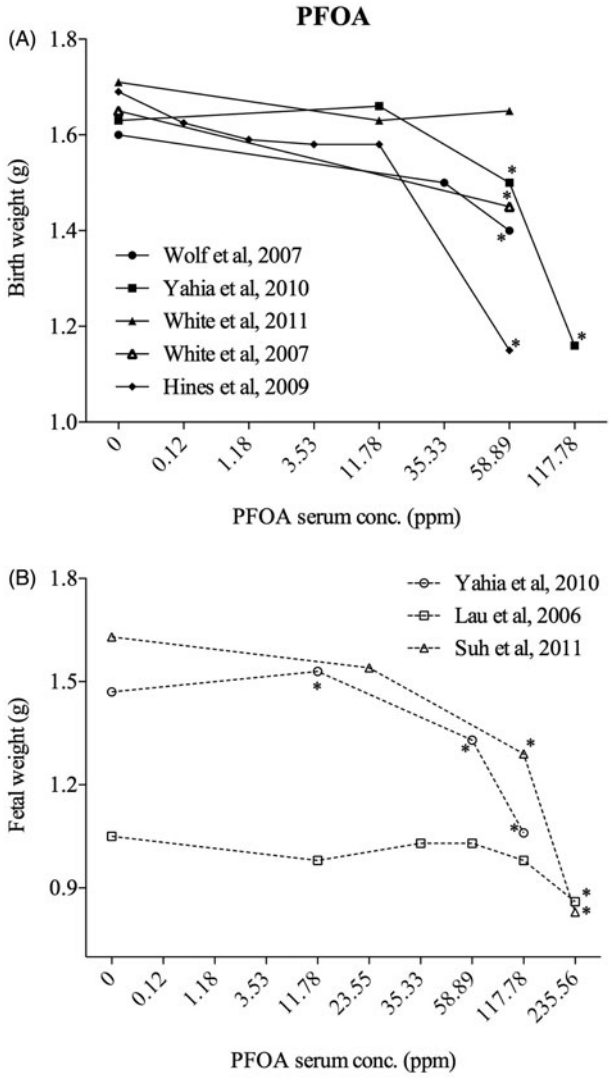

Figure 3. Mice PFOA serum concentration and mean birth/fetal weight. *Statistically significant (p values not shown).

effect, while in Yahia et al. (2010) and Suh et al (2011), maternal body weight effect occurred at dose levels higher than those eliciting birth/fetal weight effect.

In the majority of selected studies, treatment with PFOA resulted in development and/or pup/fetal toxicity effects, mainly occurring in the presence of birth/fetal weigh effect. Developmental effects included increased litter resorption, litter loss, and reduced placenta efficiency. Pup/fetal toxicity effects included reduced pup survival and/or growth delay. In all cases, these effects were present at doses equal or lower than those eliciting birth/fetal weight effect (Table 2).

In few studies, results showed birth/fetal weight effect in the absence of developmental and/or pup/fetal toxicity effects or maternal toxicity (White et al. 2007; Hines et al. 2009; White et al. 2009; van Esterik et al. 2016).

The relationship between PFOA serum concentration during pregnancy and dietary dose was derived by linear extrapolation (Figure 2(a)). This will allow with maternal serum concentrations reported in the epidemiological studies. As indicated in Figure 3, PFOA serum concentration, at given oral doses ranged from 0.12 ppm (equivalent to 0.01 mg/kg) in Hines et al. (2009) to 236 ppm (equivalent to 25 mg/kg) in Suh et al. (2011).

Using estimated serum levels, concentration response curves have been derived (Figure 3) for the above-mentioned studies. Both birth and fetal weight decreases start at serum concentration greater than 12 ppm, with similar slopes up to the highest dose tested, and reach statistical significance starting from 59 ppm (White et al. 2007; Wolf et al. 2007; Hines et al. 2009; Yahia et al. 2010; Suh et al. 2011) (Figure 3).

PFOS

Out of 116 unique records identified, 104 were excluded through title, abstract and full text screening, resulting in 12 studies describing 18 data sets included in the review. A summary of the study characteristics is provided in Table 4.

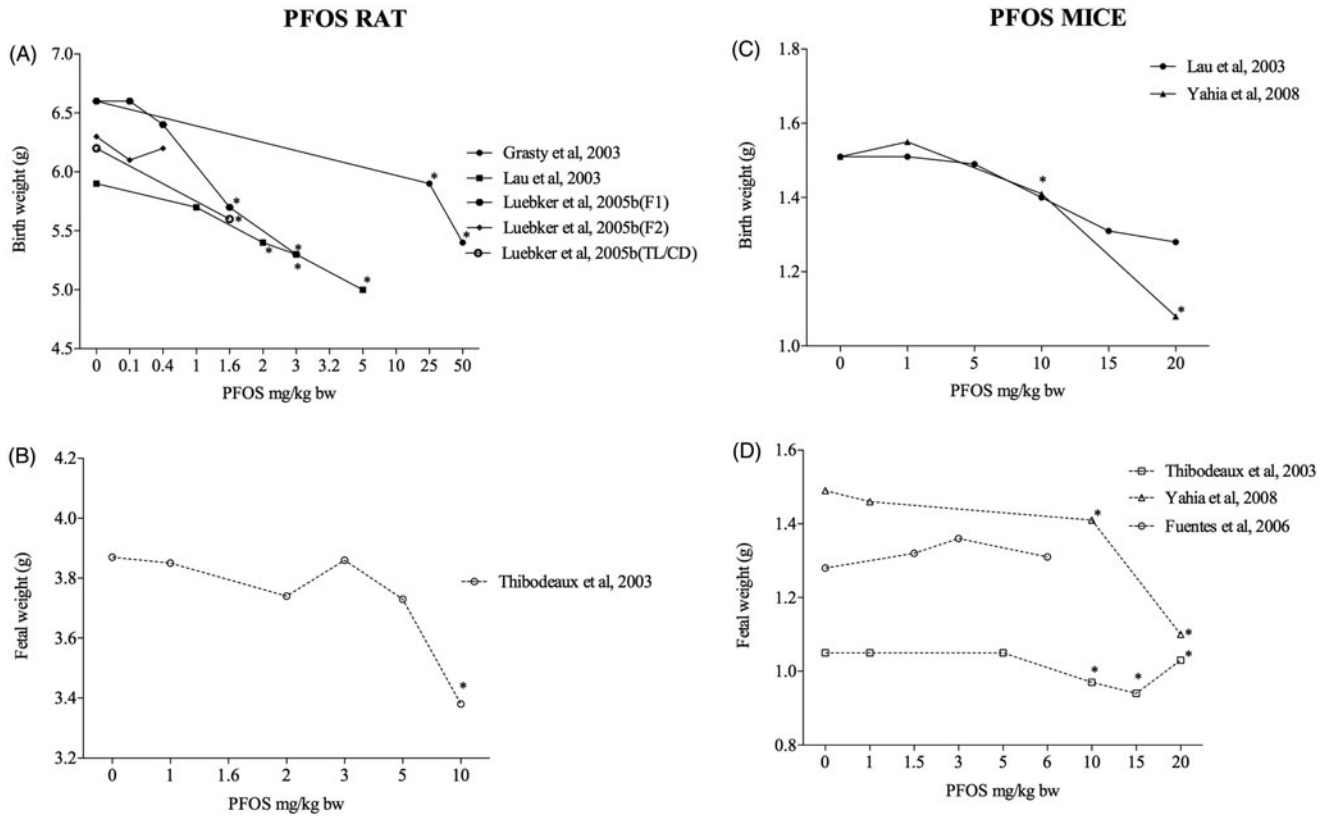

Figure 4. Mean birth weight following *in utero* oral exposure to PFOS in rats (A, B) and mice (C, D). *Statistically significant (*p* values not shown).

Among the 18 selected datasets, 12 were on rats and six on mice. Pregnant animals were dosed with PFOA or its salts, and fetal or newborn weight was investigated. The route of exposure was oral, by gavage in 11 studies (17 datasets) and via the diet in one study (1 dataset); the dose range varied from 0.1 to 20 mg/kg bw per day with 1–6 dose levels in individual studies. In rats, *in utero* exposure to PFOS covered almost the entire period of gestation in all but one study (Grasty et al. 2003) where exposure spanned from 2 to 5 d of exposure, at the beginning, intermediate or end of the gestation period. In mice, exposure varied from 13 d (GD 6–18) to 17 d (GD 1–17). The time point of weight measurement varied between fetal time point near to the term of gestation (GD18) or the time of birth, assessed from PND0 to PND1. Parturition monitoring varied across BrthW studies, from constant monitoring to daily cage check. Offspring weights were measured individually, grouped per litter or by sex.

Figure 4 reports the dose–response curves for birth/fetal weight changes from all datasets for which numerical data were available for all dose groups. This was not possible if results were presented as figures only. In the majority of the datasets, birth and/or fetal weight showed a decrease following *in utero* exposure to PFOS, with rats (Figure 4, panels a and b) being responsive at lower doses than mice (Figure 4, panels c and d).

Rats

Overall, studies conducted in rats showed consistent dose–response trends for both birth and fetal weight (Figure 4, panels a and b), although fetal weight (only one dataset) seems to be less affected compared to BrthW. Data from Grasty et al. (2003) are characterized by a less steep slope. However, the study was designed to investigate several shorter exposure periods during gestation (see Table 4); therefore, the differences could be influenced by the shorter (2 d) exposure compared with longer exposure periods in other studies (in Figure 4(a), exposure during GD 19–20 is reported).

Considering data presented in Figure 4 (panels a and b), BrthW decrease starts at 0.4 mg/kg bw, with similar slopes up to the highest dose tested, and with a statistical significant weight decrease starting from 1.6 mg/kg bw (Grasty et al. 2003; Lau et al. 2003; Luebker et al. 2005b). Luebker et al. (2005a) (Table 4) observed a significant decreased BrthW at all dose tested (0.4, 0.8, 1.0, 1.2, 1.6, and 2.0 mg/kg bw).

Rat fetal weight was investigated only in one study, where the decrease starts at 5 mg/kg bw, reaching statistical significance at 10 mg/kg bw (Thibodeaux et al. 2003). At a first glance, results of the two-generation study from Luebker et al. (2005a) (Figure 4(a)) appear to be inconsistent, since BrthW is affected only in F1 pups. However, dose levels used for the second generation were limited to the two lowest doses due to perinatal mortality in F1 pups at higher dose levels. To investigate whether the perinatal mortality observed in this two-generation study was caused by *in utero* or lactation exposure, the authors performed a follow-up cross foster study, where rats were exposed at 1.6 mg/kg bw

(the highest dose not causing high perinatal mortality) *in utero* and/or during lactation. Data reported in Figure 4(a) correspond to litters exposed *in utero* (TL) and not during lactation (CD); results show a significant BrthW decrease at PND1. Significant BrthW decrease was also observed on PND1 in the group of litters exposed *in utero* and through lactation (data not shown); while litters exposed only via lactation showed no alteration of BrthW up to day PND4 of exposure (data not shown).

In the majority of the studies reporting mean birth/fetal weight reduction, maternal and/or developmental or pup/fetal toxicity effects were also observed (Grasty et al. 2003; Lau et al. 2003; Thibodeaux et al. 2003; Luebker et al. 2005a, 2005b). However, maternal, developmental, and pup toxicity parameters were not investigated in all studies (Table 4).

Results from Grasty et al. (2003) showed maternal body weight effect at the same dose level eliciting fetal weight effect, while in Luebker et al. (2005a, 2005b), maternal body weight effect occurred at higher dose levels eliciting birth/fetal weight effect. In one study only (Thibodeaux et al. 2003), effects on maternal body weight occurred at lower doses than birth/fetal weight effects.

In the majority of studies, treatment with PFOS resulted in developmental and/or pup/fetal toxicity effects, mainly occurring in the presence of birth/fetal weigh effect. Developmental effects included decreased viability and implantation indexes. Pup toxicity effects included reduced pup survival and/or growth delay. These effects were present at doses equal or higher than those eliciting birth/fetal weight effect (Table 4).

Mice

In mice, two studies investigating fetal weight (Thibodeaux et al. 2003; Yahia et al. 2008) and one investigating BrthW (Yahia et al. 2008), showed significant decreased fetal/birth weight starting from 10 mg/kg bw. In Lau et al. (2003), most of the offspring exposed to 10 and 20 mg/kg bw did not survive for 24 h after birth. BrthW at PND0 decreased from 10 mg/kg bw, although not in a statistically significant manner (Figure 4(c)).

In studies from Fuentes et al. (2006, 2007) (Table 4), pregnant mice were exposed to PFOS up to 6 mg/kg bw, resulting in no effect on BrthW. No treatment-related effect on fetal weight was either observed up to 6 mg/kg bw (Fuentes et al. 2006) (Figure 4(d)). These findings are in agreement with the other studies where BrthW was affected by oral treatment with PFOS from 10 mg/kg bw.

In the majority of the studies reporting mean birth/fetal weight reduction, maternal and/or developmental or pup/fetal toxicity effects were also observed (Lau et al. 2003; Thibodeaux et al. 2003; Yahia et al. 2008). However, in Lau et al. (2003), only pup toxicity was investigated (Table 4).

Results from Thibodeaux et al. (2003) showed maternal body weight effect at lower dose level than those eliciting fetal weight effect; while in Yahia et al. (2008), maternal body weight effect occurred at higher dose level than fetal weight effect.

In a few studies, treatment with PFOS resulted in development and/or pup toxicity effects, mainly occurring in the presence of birth/fetal weight effect. Developmental effects included increased variations/malformations and decrease number of live fetuses. Pup toxicity effects included reduced pup survival and/or growth delay. These effects were present at doses equal or lower than those eliciting birth/fetal weight effect. On the contrary, in Fuentes et al. (2006), maternal and developmental effects were seen at the highest dose without any indication of birth/fetal effects (Table 4).

The relationship between PFOS serum concentration and dietary doses in experimental studies derived by linear extrapolation is presented in Figure 2, panels b and c. This will allow a comparison with the maternal serum concentrations reported in epidemiological studies.

In rats PFOS derived serum concentrations, ranged from 1.9 ppm (corresponding to 0.1 mg/kg bw in Luebker et al. (2005b) to 949 ppm (corresponding to 50 mg/kg bw in Grasty et al. (2003) (Figure 5).

In mice, PFOS-derived serum concentration ranged from 14.4 ppm (corresponding to 1 mg/kg bw in Lau et al. (2003), Thibodeaux et al. (2003) and Yahia et al. (2008) to 289 ppm (corresponding to 20 mg/kg bw in Lau et al. (2003), Thibodeaux et al. (2003), and Yahia et al. (2008) (Figure 5).

In rats, BrthW decrease apparently starts at 7.6 ppm, reaching statistical significance at 30 ppm, with similar slopes in all studies (Grasty et al. 2003; Lau et al. 2003; Luebker et al. 2005b) (Figure 5, panel a). In the study where fetal weight was investigated, the decrease starts at 95 ppm, with a significance at 190 ppm (Thibodeaux et al. 2003) (Figure 5, panels a and b).

In mice (Figure 5, panels c and d), fetal/birth weight decrease showed statistical significant changes from 144 ppm (Thibodeaux et al. 2003; Yahia et al. 2008). In Lau et al. (2003), BrthW at PND0 decreased from 144 ppm, although not in a statistically significant manner (Figure 5(c)).

Epidemiological evidence

Study selection process

Using the search terms listed in Table 5, a total of 2362 references were obtained: 771 from PubMed and 1591 from Embase (Figure 6). After exclusion of 255 duplicate publications, we screened 2107 records. On the basis of the titles and abstracts, we excluded 2065 articles. We identified two additional publications, by scanning the reference lists of the retrieved articles (Fromme et al. 2010; Kim et al. 2011). For 44 articles, we retrieved the full text, and further excluded 17 articles because they did not report data on the outcomes of interest. Of the remaining 27 publications, we further excluded 10 studies: four studies because the exposure was not assessed in a biological sample (Grice et al. 2007; Nolan et al. 2009; Savitz et al. 2012a, 2012b), one study because separate analyses for PFOA or PFOS were not available (Antignac et al. 2013), and one study because no measure of association between exposure and outcome was reported (Arbuckle et al. 2013). When multiple reports were published on the same study population (Inoue et al. 2004; Fei et al. 2007; Stein et al. 2009; Washino et al. 2009; Andersen et al.

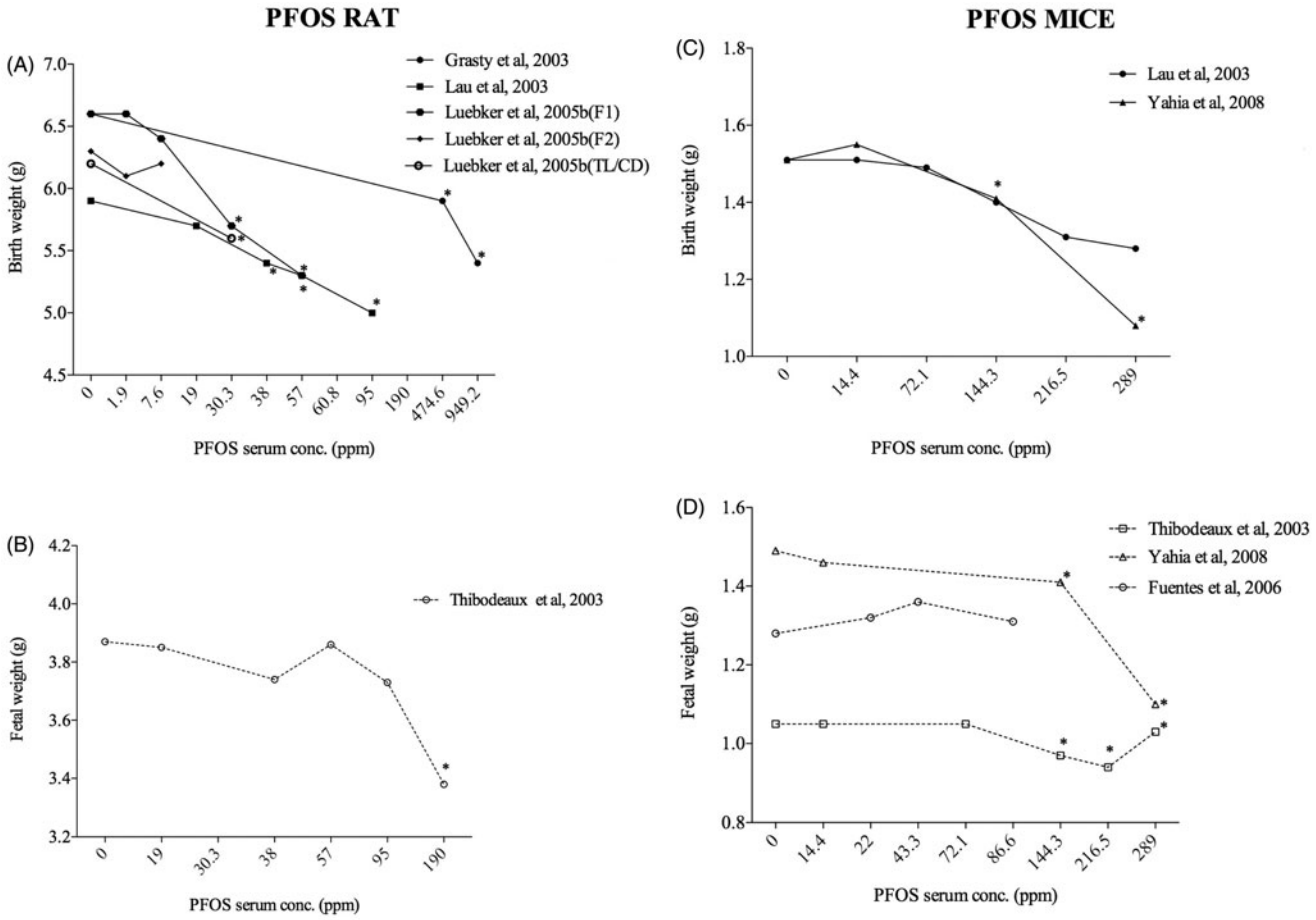

Figure 5. PFOS serum concentration and mean birth/fetal weight in rats (A, B) and mice (C, D). *Statistically significant (*p* values not shown).

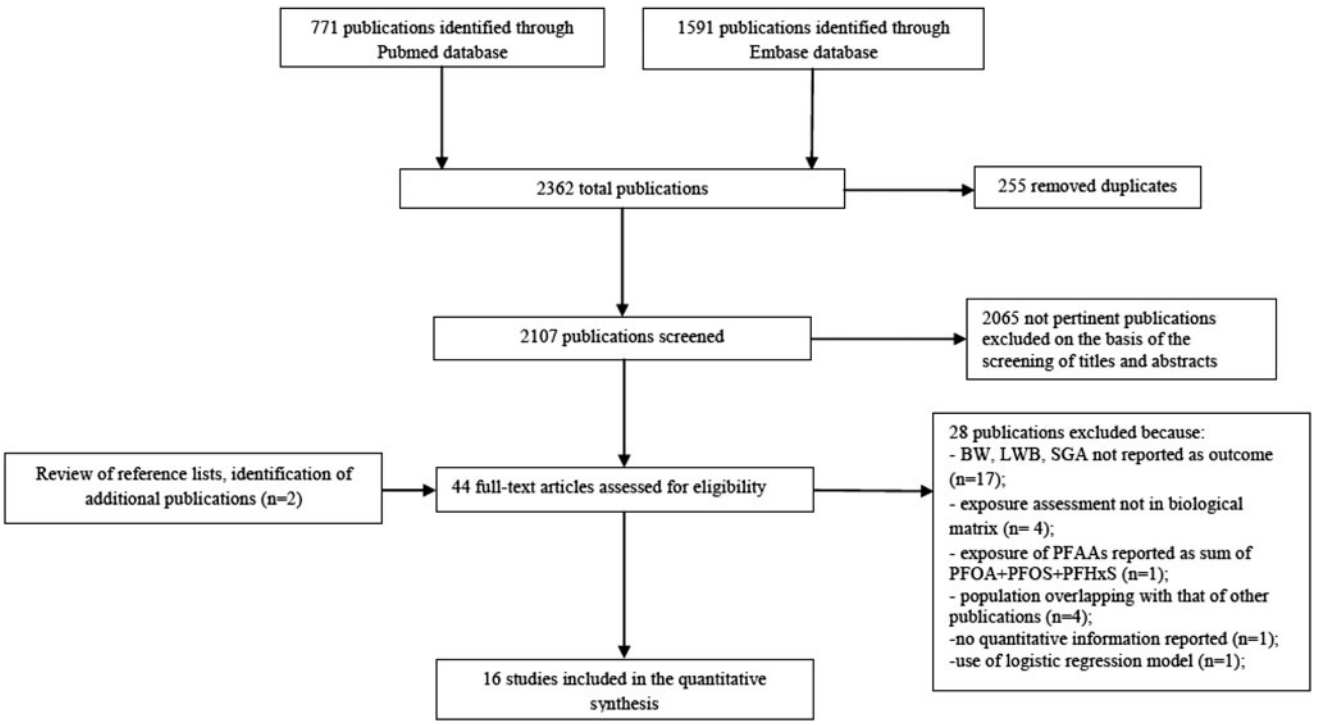

Figure 6. Flow chart for the study selection process. BrthW: birth weight; LWB: low birth weight; SGA: small for gestational age; PFOA: perfluorooctanoate; PFOS: perfluorooctane sulfonate; PFC: perfluorinated compound.

Table 6. Characteristics of the studies on PFOA and PFOS exposure and BrthW, LBW, and SGA risk included in the meta-analysis.

Study authors (year)	Country	Study period	Sample size, sex	Exposure	Biological matrix	Collection timing	Outcomes	Adjustment/matching variables
Cross-sectional studies								
Apelberg et al. (2007) *The Baltimore THREE Study*	USA (Baltimore)	2004–2005	293 B + G	PFOA, PFOS	Umbilical cord serum	Delivery	BrthW	Maternal age, race, pre-pregnancy BMI, height, smoking, parity, net weight gain, diabetes and hypertension, gestational age, baby sex
Kim et al., (2011)[a]	South Korea (Seoul, Cheongju and Gumi)	2008–2009	43 B + G	PFOA	Umbilical cord serum	Delivery	BrthW	Maternal age, parity, gestational age
Wu et al. (2012)	China (Guiyu, e-waste recycling area and Chaonan)	2007	167 B + G	PFOA	Maternal serum	Delivery	BrthW, LBW (computed)	Maternal age, education, smoking, catching cold in pregnancy, premature delivery, parity and spontaneous abortion, gestational age, baby sex
Lee[b] et al. (2013)	South Korea	2011	59 B + G	PFOA, PFOS	Maternal serum Umbilical cord serum	Delivery Delivery	BrthW	Maternal age, gestational age.
Lee et al. (2016)	South Korea (Seoul)	2008	85 B + G	PFOA, PFOS	Umbilical cord serum	Delivery	BrthW	Maternal age, gestational age, baby sex, clinician
Cohort studies								
Fei et al. (2007) *The Danish National Birth Cohot (DNBC)*	Denmark	1996–2002	710 B 690 G	PFOA, PFOS	Maternal plasma	First trimester	BrthW, LBW, SGA	Maternal age, socio-occupational status, pre-pregnancy BMI, smoking, quadratic gestational age, parity, gestational weeks at blood drawing, gestational age, baby sex
Monroy et al. (2008)[c] *The Family Study*	Canada (Ontario)	2004–2005	101 B + G	PFOA, PFOS	Maternal serum Umbilical cord blood	Delivery Delivery	BrthW	No adjustment. Analyzes restricted to term births.
Washino et al. (2009) *Hokkaido Study on Environment and Children's Health*	Japan	2002–2005	198 B 230 G	PFOA, PFOS	Maternal serum	23–35 weeks of gestation (72%) Delivery (28%)	BrthW	Maternal age, education, pre-pregnancy BMI, smoking status during pregnancy, parity, blood sampling period, gestational age, baby sex
Fromme et al. (2010)[a]	Germany	2007–2008	33 B + G	PFOA	Umbilical cord serum	Delivery	BrthW	No adjustment
Hamm et al. (2010)	Canada (Alberta)	2005-2006	252 B + G	PFOA, PFOS	Maternal serum	15–16 weeks of gestation	BrthW, SGA	For SGA: maternal age, race, maternal weight and height smoking, parity. For BW further adjusted for gestational age and baby sex
Chen et al. (2012a) *Taiwan Birth Panel Study (TBPS)*	Taiwan	2004–2005	429 B + G	PFOA, PFOS	Umbilical cord plasma	Delivery	BrthW, LBW, SGA	Maternal age, education, pre-pregnancy BMI, log (Ln)-transformed cord blood cotinine levels, type of delivery, parity, gestational age, baby sex
Maisonet et al. (2012)[c] *The Avon Longitudinal Study of Parents And Children (ALSPAC)*	UK	1991–1992	447 G	PFOA, PFOS	Maternal serum	15 weeks of gestation (median), 10–28 weeks of gestation (IQR)	BrthW	Maternal age, pre-pregnancy BMI, smoking, parity, gestational age. In previous analysis maternal age had no effect, so the authors did not include the confounder in the model.
Whitworth et al. (2012)[c] *Norwegian Mother and*	Norwegian	2003–2004	901 B + G	PFOA, PFOS	Maternal plasma	17 weeks of	BrthW, SGA	Maternal age, education, household income, pre-pregnancy

(*continued*)

Table 6. Continued

Study authors (year)	Country	Study period	Sample size, sex	Exposure	Biological matrix	Collection timing	Outcomes	Adjustment/matching variables
Child Cohort Study (MoBa)						gestation		BMI, pregnancy weight gain at 17 weeks, parity, smoking, alcohol, albumin concentration, interpregnancy interval, quadratic interpregnancy interval, and consumption of lean fish, maternal diabetes, gestational age, baby sex
Darrow et al. (2013)[d] *C8 Health Project*	USA (Ohio and West Virginia)	2005–2010	710 births B + G	PFOA, PFOS	Maternal serum		BrthW, LBW	Maternal age, education, BMI, smoking, parity, diabetes, time between conception and serum measurement. Birth weight also for indicator variables for gestational week
Bach et al. (2015) *The Aarhus Birth Cohort (ABC)*	Denmark	2008–2013	764 B 743 G	PFOA, PFOS	Maternal serum	9–20 weeks of gestation (mostly 12 weeks of gestation)	BrthW	Maternal age, education, parity, pre-pregnancy BMI, gestational age
Lenters et al. (2016) *Three cohort study*	Greenland, Poland, Ukraine	2002–2004	1250 B + G	PFOA, PFOS	Maternal serum	Median weeks of gestation: 25 (Greenland), 33 (Poland) and 23 (Ukraine)	BrthW	Maternal age, study population, pre-pregnancy BMI, maternal height, serum cotinine, parity, alcohol, vitamin D, gestational age, baby sex
Robledo et al. (2015) *The Longitudinal Investigation of Fertility and the Environment (LIFE)*	USA (Michigan and Texas)	2005–2009	113 B 117 G	PFOA, PFOS	Maternal serum	Pre-pregnancy	BrthW	Maternal age, BMI, serum cotinine, maternal and paternal serum lipids, difference in paternal age, the individual and other chemicals, baby sex

B: boys; G: girls; PFOA: perfluorooctanoate; PFOS: perfluorooctane sulfonate; BrthW birth weight; LBW: low birth weight; SGA: small for gestational age; BMI: body mass index; IQR: interquartile range.
[a]Statistical value derived from meta-analysis (Johnson et al., 2014) .
[b]Excluded from the quantitative meta-analysis because a logistic regression model for statistical analysis was used.
[c]Nested case–control design.
[d]We used prospective data only in the quantitative analysis.

Table 7. PFOA levels measured in different biological samples in the studies considered.

Study authors (year)	Biological matrix	Timing collection	Mean ± SD (ng/mL)	Geometric mean ± SD (ng/mL)	Median (IQR) (ng/mL)	Range (minimum–maximum) (ng/mL)
Apelberg et al. (2007)	Umbilical cord serum	Delivery			1.6 (1.2–2.1)	0.3–7.1
Kim et al. (2011)	Umbilical cord serum	Delivery			1.46 (1.15–1.91)	
Wu et al. (2012)	Maternal serum	Delivery	W (Guiyu) 18.32 ± 7.60 W (Chaonan) 9.76 ± 5.05		W (Guiyu)16.95 (14.30–21.50) W (Chaonan) 8.70 (6.30–12)	5.5–58.5 4.4–30
Lee et al. (2016)	Umbilical cord serum	Delivery	1.11 ± 0.48		1.05 (0.83–1.29)	0.34–3.47
Fei et al. (2007)	Maternal plasma	First trimester	5.6 ± 2.5			<1 (LLOQ) – 41.5
Monroy et al. (2008)	Maternal serum	Delivery	2.24 ± 1.61		1.81	1.33–2.64
	Umbilical cord blood	Delivery	1.94 ± 1.54		1.58	1.09–2.37
Washino et al. (2009)	Maternal serum	23–35 weeks of gestation (72%) Delivery (28%)	1.4	1.2	1.3 (0.8–1.8)	<0.5–5.3
Fromme et al. (2010)	Umbilical cord serum	Delivery				0.5–4.2
Hamm et al. (2010)	Maternal serum	15–16 weeks of gestation	2.1	1.3 ± 2.9	1.5	<LOD-18
Chen et al. (2012a)	Umbilical cord Plasma	Delivery		1.84 ± 2.23		
Maisonet et al. (2012)	Maternal serum	15 weeks of gestation (median), 10–28 weeks of gestation (IQR)			3.7	1.0–16.4
Whitworth et al. (2012)	Maternal plasma	17 weeks of gestation			2.2 (1.6–3.0)	
Darrow et al. (2013)	Maternal serum		31 ± 50.5	16.2 ± 2.8		0.6–459.5
Bach et al. (2015)	Maternal serum	9–20 weeks of gestation (mostly 12 weeks of gestation)			2.0 (1.5–2.6)	0.20–15.10
Lenters et al. (2016)	Maternal serum	Median weeks of gestation: 25 (Greenland), 33 (Poland) and 23 (Ukraine)		1.4		
Robledo et al. (2015)	Maternal serum	Pre-pregnancy		3.16 ± 0.43 G 5.00 ± 0.39 B		

B: boys; G: girls; W: women; SD: standard deviation; IQR: interquartile range; LLOQ: lower limit of quantitation; LOQ: limit of detection.

Table 8. PFOS levels measured in different biological samples in the studies considered.

Study authors (year)	Biological matrix	Timing collection	Mean (ng/mL)	Geometric mean ± SD (ng/mL)	Median (IQR) (ng/mL)	Range (minimum–maximum) (ng/mL)
Apelberg et al. (2007)	Umbilical cord serum	Delivery			5 (3.4–7.9)	0.2–34.8
Kim et al. (2011)	Umbilical cord serum	Delivery			2.93 (2.08–4.36)	
Lee et al. (2016)	Umbilical cord serum	Delivery	0.87 ± 0.46		0.76 (0.56–1.02)	0.26–2.58
Fei et al. (2007)	Maternal plasma	First trimester	35.3 ± 13			6.4–106.7
Monroy et al. (2008)	Maternal serum	Delivery	16.19 ± 10.43		14.54	9.19–20.22
	Umbilical cord blood	Delivery	7.19 ± 5.73		6.08	3.92–9.11
Washino et al. (2009)	Maternal serum	23–35 weeks of gestation (72%) Delivery (28%)	5.6	4.9	5.2 (3.4–7.0)	1.3–16.2
Fromme et al. (2010)	Umbilical cord serum	Delivery				0.3–2.8
Hamm et al. (2010)	Maternal serum	15–16 weeks of gestation	9.0	7.4 ± 2.0	7.8	<LOD-35
Chen et al. (2012a)	Umbilical cord plasma	Delivery		5.94 ± 1.95		
Maisonet et al. (2012)	Maternal serum	15 weeks of gestation (median), 10–28 weeks of gestation (IQR)			19.6	3.8–112.0
Whitworth et al. (2012)	Maternal plasma	17 weeks of gestation			13.0 (10.3–16.6)	
Darrow et al. (2013)	Maternal serum		15.6 ± 8.9	13.2 ± 1.9		<0.25–92.9
Bach et al. (2015)	Maternal serum	9–20 weeks of gestation (mostly 12 weeks of gestation)			8.3 (6.0–10.8)	0.28–36.10
Lenters et al. (2016)	Maternal serum	Median weeks of gestation: 25 (Greenland), 33 (Poland) and 23 (Ukraine)		9.4		
Robledo et al. (2015)	Maternal serum	Pre-pregnancy		12.44 ± 0.55 G 21.6 ± 0.57 B		

B: boys; G: girls; W: women; SD: standard deviation; IQR: interquartile range.

2010; Darrow et al. 2013; Kishi et al. 2015), we included only the most informative one (Fei et al. 2007; Washino et al. 2009; Darrow et al. 2013), resulting in the exclusion of additional four reports. Another publication was excluded because a logistic-regression model was used for statistical analysis (Lee et al. 2013).

We identified 16 articles, published from 2007 to 2015, that considered a quantitative relation between PFOA and PFOS levels in biological matrices and BrthW or the risk of LBW and SGA (Table 6). Of these, four were cross-sectional, three nested case–control, and nine were cohort studies. Five studies were conducted in North America, three in the USA (Apelberg et al. 2007; Darrow et al. 2013; Robledo et al. 2015), and two in Canada (Monroy et al. 2008; Hamm et al. 2010); five in Asia, one in China, two in South Korea, one in Japan, and one in Taiwan (Washino et al. 2009; Kim et al. 2011; Chen et al. 2012a; Wu et al. 2012; Lee et al. 2016), and six in Europe, a multicentric study (Lenters et al. 2016), two in Denmark and one each in UK, Germany, and Norway (Fei et al. 2007; Fromme et al. 2010; Maisonet et al. 2012; Whitworth et al. 2012; Bach et al. 2016). In eight studies, PFAA concentrations were measured in maternal serum, in five studies, during the second or third trimester of gestation (Washino et al. 2009; Hamm et al. 2010; Maisonet et al. 2012; Bach et al. 2016; Lenters et al. 2016), in one study at delivery (Wu et al. 2012) and in two studies before pregnancy (Darrow et al. 2013; Robledo et al. 2015). Two studies assessed PFAA levels in maternal plasma, one during the first trimester of gestation (Fei et al. 2007) and one during the second trimester of gestation (Whitworth et al. 2012). Four studies measured levels of PFOA and PFOS in umbilical cord serum (Apelberg et al. 2007; Fromme et al. 2010; Kim et al. 2011; Lee et al. 2016), and one in umbilical cord plasma (Chen et al. 2012a). One study assessed PFAA concentration at delivery in both umbilical cord blood and maternal serum (Monroy et al. 2008). All studies assessed BrthW. Thirteen studies considered both PFOA and PFOS exposures separately, while three considered PFOA exposure only. Only four studies presented data on LBW and three on SGA. Therefore, these outcomes are reported in Table 6, but not further considered.

Tables 7 and 8 show the biological matrix used to assess exposure, the timing of collection, and selected characteristics of the distribution of exposure (mean, median, percentiles, etc.) for PFOA (Table 7) and PFOS (Table 8). For most studies, the mean/median level of PFOA was between 1 and 3 ng/mL (Table 7), with the exception of Wu et al. (2012) (median 8.7 ng/mL in less exposed and 17.0 in heavily exposed women) and Darrow et al. (2013) (mean exposure 31 ng/mL). Exposure levels for PFOS were higher, 5–15 ng/mL in most studies (Table 8), with the exception of Fei et al. (2007) (mean =35.3 ng/mL) and Lee et al. (2016) (mean =0.87 ng/mL in umbilical cord blood).

In Table 9, we present an evaluation of risk of bias based on the NOS. For the four cross-sectional studies, the evaluation ranged between 4 and 5 out of a total of 6 points. Potential bias could emerge from incomplete control of confounding and lack of representativeness of the study

Table 9. Appraisal of risk of bias based on the Newcastle Ottawa scale.

	Selection			Comparability	Outcome		
Study	Representativeness of exposed	Selection of the non exposed	Ascertainment of exposure	Adjustments	Assessment	Adequacy of follow-up	Total
Cross-sectional							
Apelberg et al. (2007)	1	1	1	2	1	NA	6/6
Kim et al. (2011)	–	1	1	1	1	NA	4/6
Wu et al. (2012)	_	_	1	2	1	NA	4/6
Lee et al. (2016)	_	1	1	1	1	NA	4/6
Cohort							
Fei et al. (2007)	1	1	1	2	1	_	6/7
Monroy et al. (2008)	_	1	1	_	1	1	4/7
Washino et al. (2009)	1	1	1	2	1	_	6/7
Fromme et al. (2010)	_	1	1	_	_	1	3/7
Hamm et al. (2010)	_	1	1	2	1	_	5/7
Chen et al. (2012a)	1	1	1	2	1	_	6/7
Maisonet et al. (2012)	1	1	1	1	1	1	6/7
Whitworth et al. (2012)	1	1	1	2	1	1	7/7
Darrow et al. (2013)	1	1	1	2	1	_	6/7
Bach et al. (2015)	1	1	1	1	1	1	6/7
Lenters et al. (2016)	1	1	1	2	1	1	7/7
Robledo et al. (2015)	1	1	1	1	_	_	4/7

NA: not applicable.

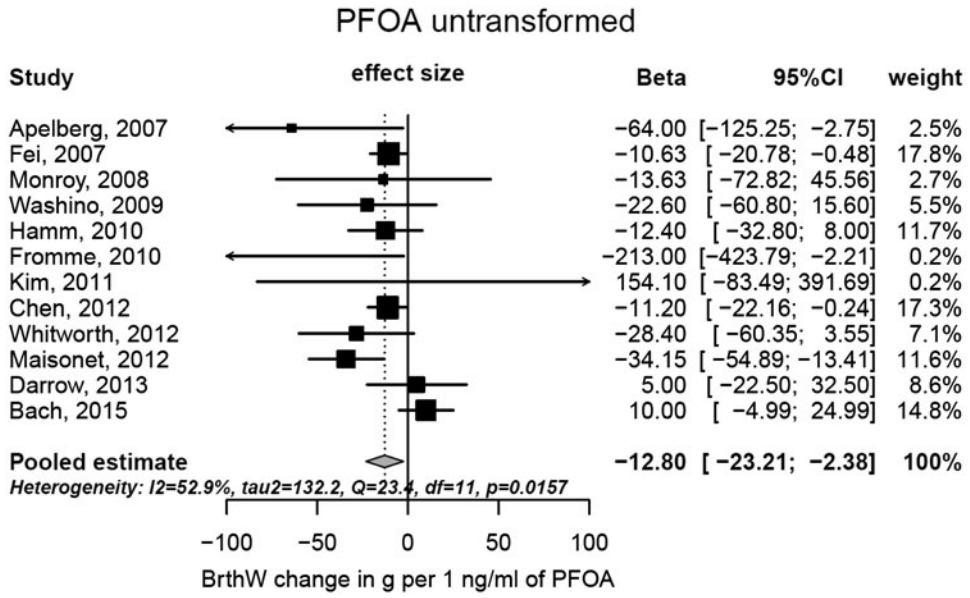

Figure 7. Meta-analysis of BrthW on untransformed PFOA.

population. Among the 12 cohort studies, the evaluation ranged from 3/7 to 7/7. Again, potential risk of bias could mostly derive from lack of control of confounding and unclear adequacy of follow-up of women from recruitment to delivery. For the chemical assessment quality, all biological matrices used to assess PFAA levels (i.e. serum, plasma, whole blood, and umbilical cord) were considered appropriate. Furthermore, analytical methodological procedures used to quantify PFFAs in all studies were well described and considered to have a high quality.

Figures 7 and 8 present the forest plot and the pooled estimate of studies presenting a LRC for the regression of BrthW on untransformed (Figure 7) and log-transformed (Figure 8) PFOA levels. The weights used for combining the individual estimates to obtain the pooled estimate according to the random effect model are also given. The LRC for untransformed PFOA was available for 12 studies, and ranged from −213 to 154 g of BrthW for a change of 1 ng/mL in PFOA level. The pooled estimate was −12.8 g (95%CI −23.2; 2.4), with significant moderate heterogeneity between studies, with an I^2 of 53% (Figure 7). Influence analysis omitting one study in turn gave pooled estimates ranging from −15.9 (omitting Bach et al. 2016) and −9.5 (omitting Maisonet et al. 2012) (data not shown).

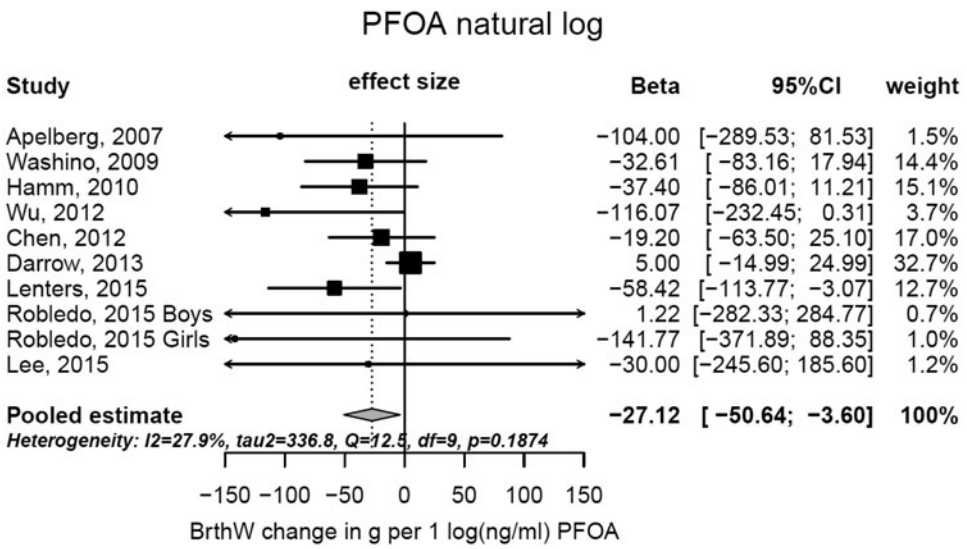

Figure 8. Meta-analysis of BW on log-transformed PFOA.

Table 10. Sensitivity analyzes for PFOA studies.

	Untransformed		Natural log	
	No. of studies	Beta (95%CI)	No. of studies[a]	Beta (95%CI)
Overall				
Random effect model	12	−12.8 (−23.2; −2.4)	10	−27.1 (−50.6; −3.6)
Fixed effect model	12	−10.4 (−16.1; −4.7)	10	−13.9 (−29.1;1.3)
Heterogeneity between studies	$Q = 23.37$, 11 df ($p = 0.016$), I^2=52.9% (9.4%;76.6%)		$Q = 12.48$, 9 df ($p = 0.19$), I^2=27.9% (0%;65.4%)	
Trim-and-fill test	12 + 2	−11.1 (−22.3;0.2)	10 + 5	−7.2 (−32.7;18.1)
Location				
America	4	−11.8(−32.1;8.6)	6	−28.2 (−64.5;8.1)
Asia	3	−12.2 (−27.3;3.0)	4	−31.9(−63.6; −0.2)
Europe	5	−15.5 (−35.4;4.4)	–	
Heterogeneity between group	$Q = 0.09$, 2 df ($p = 0.9$)		$Q = 0.02$, 1 df ($p = 0.9$)	
Adjustment for confounding				
Full	7	−11.8 (−18.3; −5.3)	7	−28.7 (−55.4; −2.0)
Partial	5	−14.1 (−52.5;24.3)	3	−62.6 (−200;75.0)
Heterogeneity between group	$Q = 0.01$, 1df ($p = 0.9$)		$Q = 0.22$, 1df ($p = 0.6$)	
Timing/medium of blood sampling				
Maternal blood/1st-2nd trimester	6	−10.5 (−23.6;2.6)	4	−10.6 (−43.2;22.0)
Maternal blood/3rd trimester-delivery	2	−20.0 (−52.1;12.1)	3	−51.0 (−86.6; −15.5)
Umbilical cord blood/delivery	4	−35.3 (−101;30.7)	3	−24.0 (−66.3;18.2)
Heterogeneity between group	Q = 0.76, 2df, (p = 0.69)		Q = 2.74, 2df (p = 0.25)	

[a]The study by Robledo et al. (2015) was counted as two, since two different estimates were presented for boys and girls.

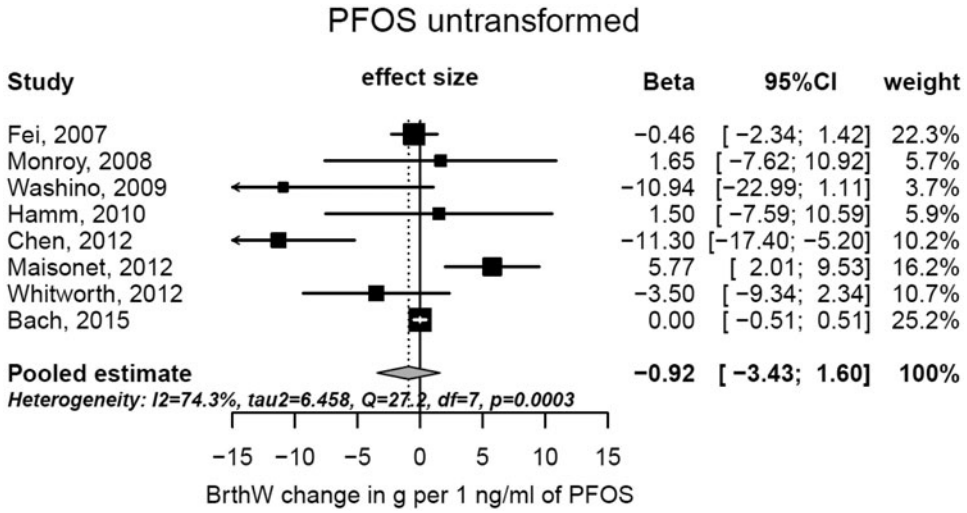

Figure 9. Meta-analysis of BW on untransformed PFOS.

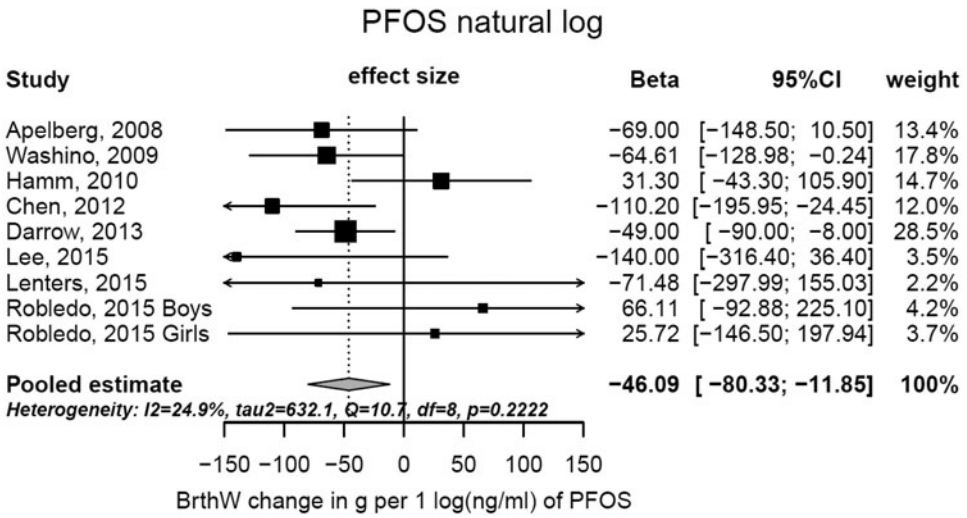

Figure 10. Meta-analysis of BW on log-transformed PFOS.

Table 11. Sensitivity analyzes for PFOS studies.

	Untransformed		Natural log	
	No. of studies	Beta (95%CI)	No. of studiesa	Beta (95%CI)
Overall				
Random effect model	8	−0.9 (−3.4;1.6)	9	−46.1 (−80.3; −11.9)
Fixed effect model	8	0.0 (−0.5;0.4)	9	−47.0 (−73.4; −20.6)
Heterogeneity between studies	$Q = 27.21$, 7 df ($p < 0.001$), I^2=74.3% (47.9%; 87.3%)		$Q = 10.65$, 8 df ($p = 0.22$), I^2=24.9% (0%; 64.7%)	
Trim-and-fill test	8 + 1	−0.1 (−2.6;2.9)	9 + 0	−46.1 (−80.3; −11.9)
Location				
America	2	−1.6 (−4.9;8.1)	6	−25.4 (−66.0; −15.2)
Asia	2	−11.2 (−16.7; −5.8)	3	−85.7 (−135; −36.3)
Europe	4	−0.5 (−1.6;2.7)	–	
Heterogeneity between group	$Q = 16.2$, 2 df ($p < 0.001$)		$Q = 3.41$, 1 df ($p = 0.06$)	
Adjustment for confounding				
Full	5	−4.3 (−9.3;0.7)	6	−50.0 (−77.4; −22.5)
Partial	3	−2.4 (−2.2;6.9)	3	−9.6 (−107;87.8)
Heterogeneity between group	$Q = 3.73$, 1 df ($p = 0.05$)		$Q = 0.35$, 1 df ($p = 0.6$)	
Timing/medium of blood sampling				
Maternal blood/1st-2nd trimester	5	0.6 (−1.4;2.5)	4	−4.0 (−62.3;54.3)
Maternal blood/3rd trim-delivery	2	−4.0 (−16.3;8.2)	2	−65.1 (−127; −3.2)
Umbilical cord blood/delivery	1	−11.3 (−17.4; −5.2)	3	−93.2 (−149; −37.8)
Heterogeneity between group	Q = 13.49, 2df, (p = 0.001)		Q = 4.86, 2df (p = 0.08)	

[a]The study by Robledo et al. (2015) was counted as two, since two different estimates were presented for boys and girls.

Table 12. Half-life (t½) of serum PFOA in mice, rats, and humans.

Species	Sex	*t½*	Reference
Rat	Males	4–6 days	Kemper and Jepson (2003)
	Females	2–4 h	
Mouse	Males	19 days	Lou et al. (2009)
	Females	17 days	
Human		3.3 years[a]	Brede et al. (2010)
		3.5 years[b]	Olsen and Zobel (2007)
		2.9–10 years	Seals et al. (2011)

[a]Average of men, women, children after cessation of exposure from contaminated water.
[b]Geometric mean.

Nine studies presented results for the regression of BrthW on log-transformed PFOA levels. Robledo et al. (2015) presented data for boys and girls that were included separately in the pooled analysis (Figure 8). The estimated LRC ranged from −142 to 5 g for an increase of 1 $\log_e$ ng/mL PFOA, i.e. for an increase of approximately 2.7 times in PFOA-untransformed levels. The pooled estimate was −27.1 (95% CI −50.6; −3.6), with low heterogeneity ($I^2 = 28\%$). Influence analysis omitting one study in turn gave pooled estimates ranging from −40.0 (omitting Darrow 2013, Darrow et al. 2013) and −19.6 (omitting Wu et al. 2012 or Lenters et al. 2016) (data not shown).

Table 10 presents various additional analyses conducted using the fixed effect model, the trim and fill test, and subgroups analyses by location, adjustment for confounding and timing/medium of blood sampling. The pooled estimate for analyses based on untransformed PFOA did not materially change when a fixed effect model, or when correction for publication bias using the trim-and-fill test, was performed. Also, no significant difference emerged between the various strata considered, which did not explain heterogeneity between studies. Conversely, the pooled estimate for the log transformed PFOA became not significant and closer to zero when a fixed effect model was used (−13.9 g) or when the trim-and-fill test for publication bias was performed (−7.2 g). Again subgroup analyses did not explain the observed

Table 13. Half-life (t½) of serum PFOS in mice, rats, and humans.

Parameter	Species	Sex	Dose		Reference
	Rat		SOD 2 mg/kg bw	SOD 15 mg/kg bw	Chang et al. (2012[a], 2012b)
$T_{1/2}$ (days)		Males	38.31	41.19	
		Females	62.3	71.13	
AUC (μg day/mL)		Males	90	1342	
		Females	371	3234	
	Mouse		SOD 1 mg/kg bw	SOD 20 mg/kg bw	
$T_{1/2}$ (days)		Males	42.81	36.42	
		Females	37.8	30.45	
AUC (μg day/mL)		Males	212	4000	
		Females	210	3363	
$T_{1/2}$ (years)	Human		4.8 years[a]		Olsen and Zobel (2007)

SOD: single oral dose.
[a]Geometric mean.

heterogeneity. The meta-regression coefficient by median exposure level was non-significant for untransformed PFOA, while it was positive and significant ($p = 0.03$) for the log-transformed analysis (data not shown). The latter result was driven by one large study with high median exposure levels (Darrow et al. 2013) which did not find an association between PFOA levels and BrthW.

Figures 9 and 10 present the forest plot and the pooled estimate of studies presenting a LRC for the regression of BrthW on untransformed (Figure 9) and log-transformed (Figure 10) PFOS levels. The weights used for combining the individual estimates to obtain the pooled estimate according to the random effect model are also given. The LRC for untransformed PFOS was available for eight studies, and ranged from −11.3 to 5.8 grams of BrthW for a change of 1 ng/mL in PFOS level. The pooled estimate was −0.92 g (95%CI −3.4;1.6), with, however, high heterogeneity between studies, with an I^2 of 74% (Figure 9). Influence analysis omitting one study in turn gave pooled estimates ranging from −2.0 (omitting Maisonet et al. 2012) and 0.6 (omitting Whitworth et al. 2012) (data not shown).

Eight studies presented results for the regression of BrthW on log-transformed PFOS levels. Robledo et al. (2015) presented data for boys and girls that were included separately in the pooled analysis (Figure 10). The estimated LRC ranged from −140 to 66.1 g for an increase of 1 $\log_e$ ng/mL PFOS, i.e. for an increase of approximately 2.7 times in PFOS-untransformed levels. The pooled estimate was −46.1 (95% CI −80.3; −11.9), with low heterogeneity (I^2=25%). Influence analysis omitting one study in turn gave pooled estimates ranging of −58.2 (omitting Hamm et al. 2010) and −41.7 (omitting Washino et al. 2009) (data not shown).

Table 11 presents various additional analyses using the fixed effect model, the trim-and-fill test and subgroups analyses by location, adjustment for confounding, and timing/medium of blood sampling. The pooled estimate for analyses based on untransformed and log-transformed PFOS did not materially change when a fixed effect model was used, or when correction for publication bias using the trim-and-fill test was performed. Studies from Asia showed a significantly stronger inverse association as compared with studies from North America or Europe, studies with "full" adjustment for confounding also showed stronger associations. There was also significant heterogeneity according to timing and medium of blood sampling, with stronger association for sampling taken later in the pregnancy or at delivery, and even stronger, when PFOS levels were measured in the umbilical cord blood. The meta-regression coefficient by median exposure level was non-significant for either untransformed or log-transformed PFOS (data not shown). For the outcomes LBW and SGA, there were too few studies to combine the results (Table 6).

Discussion and conclusions

Conclusions on toxicological data

Most studies indicated that both birth and fetal weights decreased following oral exposure of pregnant animals to PFOA (mice) or PFOS (mice and rats).

In mice, exposure to PFOA induced birth and fetal weight decrease at lower doses (5 mg/kg bw) when compared with PFOS (10 mg/kg bw). In rats, PFOS induced BrthW decrease at doses lower than in mice.

In only a few cases, effects on maternal body weight were evident at doses lower or equal to those eliciting birth/fetal weight effect. Taken together, it is unlikely that birth/fetal weight effect is maternally mediated. In most studies, maternal body weight effect was not observed or was evident at doses higher than doses eliciting birth/fetal weight (see Tables 2 and 4).

In mice, *in utero* exposure to PFOA decreased birth and fetal weight at estimated serum concentrations of 59 ppm and higher (White et al. 2007; Wolf et al. 2007; Hines et al. 2009; Yahia et al. 2010; Suh et al. 2011) (Figure 3).

In rats, *in utero* exposure to PFOS decreased BrthW at an estimated serum concentration of 30 ppm and higher levels (Grasty et al. 2003; Lau et al. 2003; Luebker et al. 2005b), while fetal weight decreased at estimated serum concentration of 190 ppm in the only data set available (Thibodeaux et al. 2003) (Figure 5, panels a and b).

In mice, *in utero* exposure to PFOS (Figure 5, panels c and d), fetal/birth weight started to decrease at an estimated serum concentration of 144 ppm (Thibodeaux et al. 2003; Yahia et al. 2008).

In rats, PFOA is readily absorbed after oral exposure and mainly found in the liver, kidneys, and blood. It can easily cross the blood–placenta border and enter the fetuses where it can be found in the liver (EFSA 2008). It is not metabolized and its elimination occurs mainly via urine in female rats,

while in males, the excretion occurs both through urine and feces. Renal elimination in humans seems to be negligible. Species differences in elimination half-lives are summarized in Table 12.

The half-life varies greatly among species, and between genders in rats. Elimination half-lives are a few hours in female, and 4–6 d in male rats (Kemper & Jepson 2003; Johnson et al. 2014). In both mice genders, it is around 20 d (Lou et al. 2009), and in humans about 3.3 years (Brede et al. 2010). It is believed that these different excretion rates are due to differences in organic anion transporters, which are responsible for the active transport (excretion and reabsorption) of many organic anions, including xenobiotics, across membranes in the kidney and other organs (Weaver et al. 2010; Han et al. 2012). Because PFOA is rapidly excreted in female rats (2–4 h), it does not reach steady state after repeated once daily dosing. For this reason, female rat seems not to be the best experimental model to investigate PFOA potential human developmental effects. Therefore, these toxicokinetic differences might be the reason why studies have been performed in mice only.

After oral exposure, PFOS is readily absorbed in rats and is found in the liver, kidneys, and blood. It can cross the blood-placental barrier and enter the fetuses, where it can be found in the liver (EFSA 2008). No *in vivo* data are available for PFOS metabolism. As for its salt, PFOS is mainly eliminated via urine and to a lesser extent in feces. Renal elimination in humans seems to be negligible. Species differences elimination half-lives are summarized in Table 13. There are large differences on elimination rates between rodents and humans. In rodent species, the elimination half-lives are in the order of 1–2 months (Chen et al. 2012b), while in humans, it has been estimated to be 4.8 years (Olsen & Zobel 2007).

Perfluorinated alkyl acids, including PFOA and PFOS, are structurally similar di-free fatty acids and thus have the ability to interact with membranes and nuclear receptor, peroxisome proliferator-activated receptor (PPARα), as well as other nuclear receptors such as constitutive androstane receptor (CAR) and pregnane X receptor (PXR) (Rosen et al. 2008a, 2008b; Elcombe et al. 2010). PPARs regulate important physiological processes such as cell proliferation, lipid homeostasis, adipogenesis, steroidogenesis, reproduction, and carcinogenesis. This family of nuclear receptors is expressed in many tissues both in fetuses and adults of human and rodents (Abbott 2009). However, the possible role on development following interaction of PFOA or PFOS has not been clarified. Abbott (2009) reports that in KO mice developmental effects of PFOA, but not of PFOS, depend on the PPARα expression. Given the fact that interaction with human PPARα has different consequences than in rodents; e.g. it does not lead to cytotoxicity in humans as it does in rodents (Gonzalez & Shah 2008; Ross et al. 2010), there is no indication on whether there is any role of PFOA/PFOS interaction with PPARα and effects on development in humans.

Conclusions on epidemiological data

Sixteen and 13 studies were included in the meta-analysis for PFOA and for PFOS, respectively. PFOA levels were significantly inversely related to BrthW in studies presenting the regression of BrthW on either untransformed or log-transformed PFOA levels. For PFOS, the combined estimate of studies using log-transformed levels was significantly inversely related to BrthW, but no evidence emerged from studies using untransformed levels.

Risk of bias

We considered baby sex, gestational age, maternal age, pre-pregnancy BMI, education, parity, and smoking to be the most important potential confounders of the relationship between PFAA and BrthW, as they have been shown to be associated with both exposure and outcome. However, when we restricted the analysis to studies with full adjustment for these confounders, results were similar to those including all studies.

On one hand, another important potential confounder, related to both exposure and outcome, is glomerular filtration rate (GFR). Some studies have shown that women whose GFR fails to rise sufficiently during pregnancy tend to have smaller babies (Verner et al. 2015). On the other hand, GFR is likely to influence the urinary excretion of xenobiotics like PFAA. Indeed, higher blood PFAA levels have been observed in people with lower GFR (Verner et al. 2015). As renal elimination in humans seems to be negligible and no study adjusted for GFR, the influence of GFR on the results remains undefined.

On one hand, fish consumption is another potential confounder since fish contains considerable amounts of PFAAs (Brantsaeter et al. 2013). On the other hand, fish intake has been suggested to have a favorable role on fetal growth (Brantsaeter et al. 2013). One study only (Whitworth et al. 2012) included lean fish intake in the regression model, and its results were not statistically heterogeneous with the pooled estimates of the other studies (data not shown).

Besides confounding, other sources of bias must be considered, and for systematic reviews publication bias specifically. Among studies that were excluded because they did not report the outcome of interest, it is possible that some found a null association. However, the statistical tests we applied did not suggest that publication bias did occur.

Exposure

A strength of our meta-analysis is that we included only studies that measured PFOA and PFOS concentrations in biological samples. The included studies were conducted in different countries, some in low and some in highly exposed populations, thus including a variety of exposure levels. A meta-regression model did not find a relation between median exposure level and effect estimate, with the exception of the log-transformed PFOA, where the result was mainly driven by a study focused on a highly exposed population in the Mid-Ohio Valley living near a chemical manufacturing in West Virginia (Darrow et al. 2013). This study found little evidence of association of PFOA serum levels with LBW or BrthW. Another study was conducted in China, and compared presumably high exposed (to PFOA) pregnant women living in an e-waste recycling area to pregnant women living

in a control area with no e-waste recycling (Wu et al. 2012). That study found a strong inverse relation of BrthW with PFOA. Anyway, sensitivity analysis excluding each study in turn showed that no single study had a strong influence on the overall result. Studies carried out in Asia tended to find a stronger relationship as compared with North American or European studies, regardless of median exposure levels.

The included studies were conducted in different years and temporal declines in serum PFAA levels have been reported, following decreasing environmental exposure due to the phase out or regulation of production of these chemicals in some areas of the world (Canada 2010, USA 2000, Europe 2006) (Bach et al. 2016) in the last decade. This was shown in two studies from Denmark conducted in 1996–2002 (Fei et al. 2007) and 2008–2013 (Bach et al. 2016), where median levels of PFOA in maternal plasma decreased from 5.2 to 2 ng/mL, and PFOS levels from approximately 35 to 8.3 ng/mL.

We combined studies that measured PFOA and PFOS at different times during pregnancy (I, II, or III trimester of gestation) or at delivery, and in different biological matrices (umbilical cord serum, umbilical cord plasma, maternal serum, and maternal plasma). Concerning the use of serum or plasma samples, a study showed that the ratio of PFOA and PFOS levels between serum and plasma is 1:1 (Ehresman et al. 2007). The timing of sample collection relative to pregnancy may instead influence the results, as PFAA levels appear to decrease during pregnancy. This decline is associated with decreased serum albumin levels due to dilution effect, caused by the increasing blood volume in pregnancy (Glynn et al. 2012).

Differences in PFAA levels also exist between maternal blood collected during pregnancy or at delivery and umbilical cord blood, with higher level in maternal blood (Glynn et al. 2012). The ratio between the median maternal and the cord serum concentration at delivery was around 1:1 for PFOA but around 2:1 for PFOS in a study from the US (Kato et al. 2014). These different ratios suggest that there are differences in the transplacental transfer of these compounds. Several studies showed, however, that there is a strong correlation between PFAA levels measured in the different biological matrices and at different times during pregnancy. For this reason, in the meta-analysis, we combined all these studies. We also conducted subgroup analyses according to pregnancy timing and matrix of collection. While for PFOA we did not find a marked heterogeneity between subgroups, for PFOS the heterogeneity was marked, with a stronger effect in late pregnancy and even stronger in umbilical cord blood. This is consistent with the reduction in PFOS serum levels during pregnancy; a difference of 1 ng/mL in umbilical cord blood would correspond to a higher difference if the sample were taken early during pregnancy. This would result in a higher difference in BrthW when using umbilical cord blood PFOS levels as a reference. Consequently, to account for these differences, we used a correction coefficient according to timing and matrix of collection to conduct the meta-regression of effect estimate associated with mean/median PFOA/PFOS blood levels in the study population.

Shape of the dose–response curve

One problem of this systematic review is that the meta-analysis was conducted separately for studies that presented a regression coefficient of BrthW on unadjusted blood PFOA/PFOS levels, and studies that applied a logarithmic transformation for PFOA/PFOS levels. This was unavoidable as the two models have different interpretations and cannot be combined. In any case, for PFOA, there was an indication that BrthW tended to decline for increasing exposure both in studies using untransformed and log-transformed levels. For PFOS, the summary estimate for eight studies analyzing untransformed PFOS was close to zero and not significant, although with high heterogeneity ($I^2 = 74\%$). Conversely, the meta-analytic estimate of nine studies presenting log-transformed PFOS levels was that BrthW decreases by 46 g for an increase in PFOS blood levels of 2.7 times. This estimate was statistically significant and heterogeneity between studies was low ($I^2 = 25\%$). The difference in these results may be due to the true form of the relation between PFOS levels and BrthW. If this were the case, the relation should be steeper at lower PFOS levels, since the logarithmic transformation tends to accentuate differences at lower values. However, it is also possible that the difference in results is due to the differences between the studies included in the two meta-analyses or to a lower influence of outliers when using the logarithmic transformation.

Transformations of data before analysis are used for various reasons. A common one is that several statistical procedures assume that the variables are normally distributed, and the violation of the assumption of normality can affect the validity of results. Thus, in the presence of right-skewed distributions, the logarithmic transformation is often used to improve the normality of the data.

Our analysis was performed on published data, and measures of goodness of fit for the individual studies were not available, thus we cannot determine how well the two types of analyses (untransformed and log-transformed) represented the data. However, both models are not entirely appropriate in the presence of a threshold effect, as suggested by animal studies in this case. In fact, in a sample of pregnant women from the same population followed by Fei et al. (2007) and by Bach et al. (2016), no relationship was found in the latter study, where blood levels were lower. A re-analysis of the available raw data using more flexible dose–response models could provide more information on the shape of the dose-response relationship.

Combination of human and animal evidence, and placing in a causal relationship grid

Epidemiology

PFOA

For PFOA, there was a significant inverse relationship when untransformed values were considered, although with significant moderate heterogeneity; for log-transformed values, there was a significant inverse relationship, but with low heterogeneity. There were 16 epidemiologic studies from different areas of the world, mostly with low risk of bias, although the different methods did not allow to consider more than

12 studies together. These studies encompassed a wide range of human blood concentrations (disregarding the sample time). However, in humans, the shape of the dose–response curve has not been sufficiently investigated, particularly for what concerns a possible threshold effect. Also, the clinical relevance of the observed effect needs to be better understood. Overall, therefore, we evaluated the epidemiological evidence for an inverse association between PFOA maternal blood levels and BrthW as moderately likely, at least for the highest blood levels.

PFOS

For PFOS, there was a non-significant inverse relationship when untransformed values were considered, with significant heterogeneity, while for log-transformed values, there was a significant inverse relationship, with low heterogeneity. There were 13 available epidemiologic studies from different areas of the world, and mostly with low risk of bias, although the different methods did not allow to consider more than eight studies together. The most recent largest study (Bach et al. 2016) did not find any association. The same considerations as for PFOA are valid, i.e. studies encompassed a wide range of concentrations but the shape of the dose–response curve, the possibility of a threshold effect and the clinical relevance of the observed effect, need further investigation. Overall, therefore, we evaluated the epidemiological evidence for an inverse association between PFOS maternal blood levels and BrthW as insufficient tending to moderately likely. As compared with PFOA, the uncertainty of the evaluation is further increased for PFOS by the fact that there does not appear to be a dose-response relationship when untransformed blood levels are analyzed.

Toxicology

Overall, in almost all animal studies, PFOA and PFOS show a similar dose–response trend. Both birth and fetal weight decreases show a threshold, and a similar slope from the threshold up to the highest dose tested, and statistically significant decrease of fetal growth is observed at higher doses. Moreover, for both PFOA and PFOS, fetal weight seems to be less affected compared with BrthW.

In the majority of the studies on both PFOA and PFOS, developmental and pup toxicity effects were also observed (Tables 2 and 4), which is not entirely consistent (at least for PFOS) with the hypothesized MoA of PPARs involvement during embryo development, as described above (Abbott 2009). However, even if evidences in animals show that these nuclear receptors are activated by PFOS and PFOA, no other key events have been identified, and thus the relevance for development of their activation is not clear. Given the different consequences of PPARs activation in humans, and in the absence of other information, the role of this activation for effects on human development is unknown. When human serum concentrations of PFOA and PFOS are compared with extrapolated animal serum concentrations, a remarkable distance between internal doses is evident. In fact, PFOA and PFOS effective serum concentrations in rodents are of 2–3 orders of magnitude higher than in humans. This large difference between human and animal internal dose have already been reported by other authors (Apelberg et al. 2007; Fei et al. 2007; Monroy et al. 2008).

Application of framework for the integration of toxicology and epidemiology for causal inference and risk assessment

The weight of evidence of epidemiology and toxicology was considered in the framework for the integration of toxicology and epidemiology for causal inference and risk assessment, as proposed previously (Adami et al. 2011; Woodruff & Sutton 2014).

Consequently, an encompassing judgment statement was assigned to qualify the toxicology evidences. Usually, the weight of evidence approach relies on the evaluation of all available toxicological information (e.g. *in vivo, in vitro,* and mechanistic), but given the intrinsic characteristics and the complexity of the effect (BrthW), we considered more appropriate to evaluate only *in vivo* experimental studies conducted in rodents. Taking into consideration all reviewed animal data, the overall toxicological evidence for a dose-dependent effect of PFOA and PFOS on BrthW is judged plausible. Hence, combining toxicological and epidemiological evidence in a qualitative way, the causal relationship falls in the "likely" category (Figures 11 and 12). However, this is only a qualitative judgment which does not take into account information on Mode of Action (MoA), including quantitative analysis of the dose–response in animals compared with human exposure. For these compounds, an MoA has not yet been clearly identified and agreed, besides the hypothesized PPARs involvement in animals development, although there appears to be qualitative concordance of the apical effect (i.e. BrthW) between animals and humans.

Further, a refinement was done by comparing human and animal PFOA and PFOS serum concentrations associated with the effect. Given the strong discrepancy in terms of effective serum concentrations in rodents compared with the concentrations found in epidemiological studies, the uncertainty regarding the biological plausibility of a causal relationship between PFOA or PFOS exposure and lower BrthW in humans is increased. In fact, the 2–3 orders of magnitude difference in serum concentration between rodents and humans suggests that there might be some, not yet identified, confounding factors that lead to a spurious association. This reduction in the biological plausibility is indicated in Figures 11 and 12, where the "Epid-tox" causal relationship moves down along the biological plausibility axis (blue arrows).

One innovative aspect of this study is the attempt to extend the comparison of human and animal evidence from qualitative evaluation to quantitative aspects, including the shape of the dose–response relationship.

Another innovative aspect is the attempt to apply the Epid-Tox framework for combining toxicological and epidemiological evidence to establish causal inference (Adami et al. 2011). One appeal of the Epid-Tox framework is that it promotes transparency and rigor with the final aim of providing solid support to evidence-based decision making. This

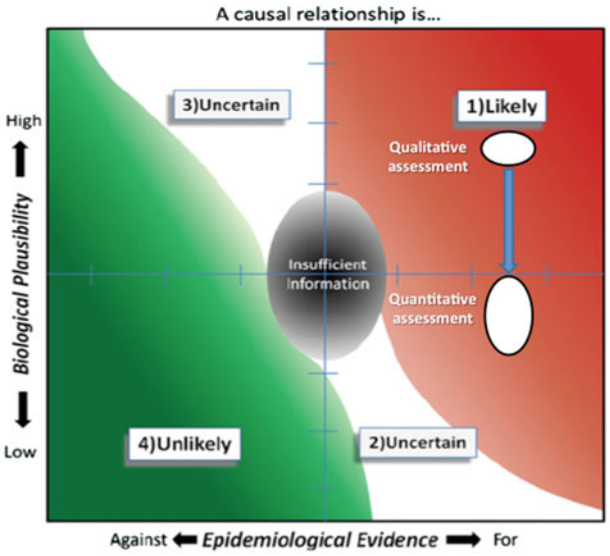

Figure 11. Graphical representation, according to Adami et al. (2011), of the integration of toxicological and epidemiological evidence for decreased BrthW after exposure to PFOA. The arrow indicates the direction of the quantitative refinement after taking into account the comparative quantitative information.

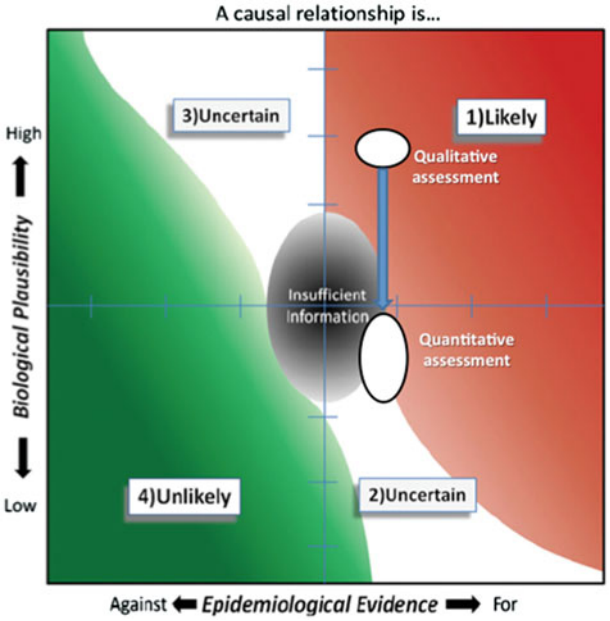

Figure 12. Graphical representation, according to Adami et al. (2011), of the integration of toxicological and epidemiological evidence for decreased BrthW after exposure to PFOS. The arrow indicates the direction of the quantitative refinement after taking into account the comparative quantitative information.

method has also been criticized, and the authors themselves acknowledge that it will likely need some modification. However, as they state "the refinement of any method occurs by working examples through it". Thus, our work provides a case study for testing the applicability of the Epid-Tox framework. In fact, our study shows that the placement in the causal grid needs further clarification. The aim is "to evaluate how strong is the evidence for or against a causal relationship in humans" (Adami et al. 2011). Thus, a good understanding of the mode of action is critical for performing interspecies comparisons. Unfortunately, the available information does not always provide this understanding. This can add uncertainty on the biological plausibility, as in this case.

These conclusions are evidence-based, in the field of both epidemiology and toxicology. Availability of new data and better information on comparative toxicokinetics may allow further refinement for a clearer characterization of the causal relationship, or lack thereof. This is based on the assumption that observation of BrthW changes in laboratory animals could be meaningfully extrapolated to human.

Acknowledgements

The authors wish to thank Lesley Rushton, Karlene Lavelle, Nicholas Synhaeve, and Alan Poole for their most helpful suggestions as reviewers of our CEFIC Long Range Research Initiative project. The authors are grateful for the help of their research to the chemist Andrea Colombo, Istituto di Ricerche Farmacologiche Mario Negri, for the support on the chemical assessment quality.

The authors gratefully acknowledge the comments provided by five reviewers elected by the Editor and anonymous to the authors. These comments were extremely helpful in revising the final manuscript.

Declaration of interest

The employment affiliation of the authors is as shown on the cover paper. However, the authors preparation of the paper was as independent professional not in their role as employees. The views expressed are not necessarily those of their employer. The authors have not been involved in any legal or regulatory proceedings with regard to the contents of the paper.

Funding

This work was funded under CEFIC Long Range Research Initiative Contract number EMSG58. CEFIC was not involved in the preparation or approval of the manuscript. The findings and conclusions in this manuscript are those of the authors and do not necessarily represent the views of CEFIC.

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