HEALTH-BASED MAXIMUM CONTAMINANT LEVEL SUPPORT DOCUMENT: PERFLUORONONANOIC ACID (PFNA)

New Jersey Drinking Water Quality Institute
Health Effects Subcommittee
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Acronyms

BMD — Benchmark Dose Modeling
BMDL — Lower 95% Confidence Limit
CAR — Constitutive androstane receptor
DWQI — Drinking Water Quality Institute
ECHA — European Chemical Agency Risk Assessment Committee
FTOH – Fluorotelomer alcohol
GD — Gestational Day
GM — Geometric mean
IRIS — Integrated Risk Information System
KO — Knockout (PPAR-alpha null)
LD_{50} — Lethal Dose
LOAEL — Lowest Observed Adverse Effect Level
MCL — Maximum Contaminant Level
NHANES — National Health and Nutrition Examination Survey
NJDEP — New Jersey Department of Environmental Protection
NJDOH — New Jersey Department of Health
NOAEL — No Observed Adverse Effect Level
PFC — Perfluorinated Chemical
PFHxS — Perfluorohexane sulfonate
PFOA — Perfluorooctanoic acid
PFOS — Perfluorooctane sulfonate
PFNA — Perfluorononanoic acid
PFUnDA — Perfluoroundecanoic acid
PND — Postnatal Day
POD — Point of Departure
PPAR --- Peroxisome proliferator activated receptor
PVDF — Polyvinylidene fluoride
PXR — Pregnane X receptor
RfD — Reference Dose
RSC — Relative Sources Contribution
SDWA — Safe Drinking Water Act
UCMR3 — Unregulated Contaminant Monitoring Rule 3
UFs — Uncertainty factors
USEPA —United States Environmental Protection Agency
WT — Wild Type

ABSTRACT

A Health-based Maximum Contaminant Level (Health-based MCL) for perfluorononanoic acid (PFNA, C9) was developed to protect for chronic (lifetime) drinking water exposure. The Health-Based MCL is based on a study of developmental effects in which pregnant mice were exposed to PFNA for 16 days. The Health-based MCL is further supported by data on effects in the offspring in the same study, and on increased liver weight and other effects in additional rodent studies from the same and other laboratories. Benchmark dose modeling was performed on PFNA levels in blood serum that caused increased maternal liver weight in the pregnant mice. Appropriate uncertainty factors were applied to protect sensitive human subpopulations, account for differences between human and experimental animals, protect for chronic exposure, and account for the incomplete toxicology database for PFNA. Based on available toxicokinetic data from animal and humans, a ratio of 200:1 was used to estimate the increase in PFNA in human blood serum from ongoing exposure to a given concentration of PFNA in drinking water. A chemical specific Relative Source Contribution factor of 50% was developed based on the most recent NHANES data for the 95th percentile PFNA serum levels in the U.S. general population. Using this information, a Health-based MCL protective for chronic drinking water exposure of **13 ng/L (0.013 µg/L)** was derived.

INTRODUCTION

Development of Health-based MCLs by New Jersey Drinking Water Quality Institute

The New Jersey Drinking Water Quality Institute (DWQI), established by the 1984 amendments to the New Jersey Safe Drinking Water Act (SDWA) at N.J.S.A. 58:12A- 20, is charged with developing standards (Maximum Contaminant Levels; MCLs) for hazardous contaminants in drinking water and for recommending those standards to the New Jersey Department of Environmental Protection (NJDEP). The Health Effects Subcommittee (formerly "Lists and Levels Subcommittee") of the DWQI is responsible for recommending health-based drinking water levels (Health-based MCLs) as part of the development of MCLs (NJDWQI, 1987; 1994; 2009).

Health-based MCLs are based on the goals specified in the 1984 Amendments to the NJ SDWA. For carcinogens, it is generally assumed that any level of exposure results in some level of cancer risk, and a one in one million (10^{-6}) risk level from lifetime exposure is specified in the statute. Health-based MCLs for carcinogens are thus set at levels that are not expected to result in cancer in more than one in one million persons ingesting the contaminant for a lifetime. For non-carcinogenic effects, it is generally assumed that exposure below a threshold level will not result in adverse effects. As specified in the statue, Health-based MCLs are set at levels which are not expected to result in in "any adverse physiological effects from ingestion" for a lifetime.

Other factors such as analytical quantitation limits and availability of treatment removal technology are also considered in the final MCL recommendation. For carcinogens, the 1984 Amendments to the NJ SDWA require that MCLs are set as close to the one in one million lifetime risk goal as possible "within the limits of medical, scientific and technological feasibility." For non-carcinogens, MCLs are set as close to the goal of no adverse effects as possible "within the limits of practicability and feasibility."

On March 21, 2014, the Commissioner Bob Martin of the NJDEP requested that DWQI recommend an MCL for perfluorononanoic acid (PFNA, C9).

To support the development of an MCL recommendation by the DWQI, the Health Effects Subcommittee has developed a Health-based Maximum Contaminant Level for PFNA. As specified in the Amendments to the NJ SDWA, this Health-based MCL is intended to be protective for chronic (lifetime) drinking water exposure.

Document development process

The Subcommittee conducted a literature search of the PubMed and Toxline databases for potentially relevant information. Additional references were identified through backward searching. In total, approximately 455 references were identified (Appendix 1). All of these references were screened by title, abstract and/or full text. Based on this screening, 169 references relevant to human health effects, human biomonitoring, animal toxicology, pharmacokinetics, and *in vitro* studies were designated for "further consideration" while 292 other references not relevant to these areas were excluded. Some references that were excluded as irrelevant to these topics were used to inform supporting sections of this assessment, such as the "Background Information" and "Environmental Sources, Fate, and Occurrence" sections.

In May 2014, the DWQI posted a request for public input regarding data or technical information about the toxicology, epidemiology, toxicokinetics, or other health effects topics related to PFNA that should be considered in developing an MCL. The Health Effects Subcommittee received and considered comments relevant to these topics that were submitted by the public

NJDEP has recently developed a draft Interim Specific Ground Water criterion for PFNA. Like Health-based MCLs, NJ Interim Specific Ground Water Criteria are intended to be protective for chronic (lifetime) drinking water exposure. The Health Effects Subcommittee reviewed the Draft Technical Support Document for the Interim Specific Ground Water Criterion for PFNA (NJDEP, 2014). Some sections of the Health-based MCL Support Document were developed *de novo* by the Health Effects Subcommittee, while other sections are based on updates of relevant information from the Draft Technical Support Document. In 2014, NJDEP solicited public comments on the Draft Technical Support Document for the Interim Specific Ground Water Criterion for PFNA. The Health Effects Subcommittee reviewed these comments and NJDEP's draft responses.

In April 2015, the DWQI posted the Draft Health-based MCL Support Document for public comment. All comments relevant to the Support Document were reviewed by the Health Effects Subcommittee. Revisions, where appropriate, are incorporated into this updated version.

BACKGROUND INFORMATION

Perfluorinated chemicals (PFCs) are a class of anthropogenic chemicals with structures consisting of a totally fluorinated carbon chain of varying length and a charged functional group, such as carboxylic or sulfonic acid (Lindstrom et al., 2011). The eight carbon compounds, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) have been the most intensively studied PFCs in the past. Recent research has focused on a wider range of PFCs including PFNA, the nine carbon perfluorinated carboxylic acid, which is the subject of this document. Because PFOA and PFNA are closely related and share similar properties, some of the discussions to follow include comparisons with information on PFOA as a part of the basis for evaluation of PFNA.

Physical and Chemical Properties (ATSDR, 2009; ALS Environmental, 2014)

Chemical Name:	Perfluorononanoic acid
Synonyms:	PFNA, C9
CAS Number:	375-95-1
Chemical Formula:	$C_9HF_{17}O_2$
Chemical Structure:	$CF_3(CF_2)_7COOH$
Molecular Weight:	464.08
Physical State:	white crystalline powder
Melting Point:	65-68 ^oC
Boiling Point:	218 ^oC at 740 mm Hg
Vapor Pressure:	No data
Water Solubility	9.5 g/L at 25 ^oC
Log octanol/water partition coefficient:	Not applicable
Taste Threshold (water):	No data
Odor Threshold (water):	No data
Odor Threshold (air):	No data

PFNA is a fully fluorinated carboxylic acid. Because carbon-fluorine bonds are among the strongest found in organic chemistry, PFNA and other PFCs are extremely stable and resistant to chemical reactions (Post et al., 2013).

PFNA and other PFCs contain a long perfluorocarbon tail that is both hydrophobic and oleophobic (repels both water and oil) and a charged functional group that is hydrophilic. Because they form a separate layer when mixed with hydrocarbons and water, measurement of the octanol:water partition coefficient is not practical (Prevedouros et al., 2006).

PFNA is manufactured as its ammonium salt, ammonium perfluorononanoate (APFN), which dissociates in water. At the pH range found in drinking water (6.5-8.5), PFNA is present almost totally in the non-volatile anionic form, the perfluorononanoate anion (Goss, 2008; Rayne and Forest, 2010).

Production and Use

The production, industrial uses, and environmental fate of PFNA were reviewed by Prevedouros et al. (2006). Production of PFNA began in 1975; it was made primarily in Japan. It is produced as the linear isomer of its ammonium salt (ammonium perfluorononanoate, APFN). It was estimated that 10% of the APFN that was produced was released to air and water at the production facility, resulting in global emissions of 70,000 to 200,000 kg PFNA between 1975 and 2004.

PFNA is the primary component of Surflon S-111 (CAS # 72968-3-88), a commercial mixture of linear perfluorinated carboxylic acids. Prevedouros et al. (2006) report the composition of Surflon S-111 by weight as PFNA, 74%; perfluoroundecanoic acid (PFUnDA, C11), 20%; perfluorotridecanoic acid (C13), 5%; PFOA (C8), 0.78%; perfluorodecanoic acid (C10), 0.37%; and perfluorododecanoic acid (C12), 0.1%.

The primary historic use of PFNA was as a processing aid in the emulsion process used to make

fluoropolymers, mainly polyvinylidene fluoride (PVDF), similar to the use of PFOA as a processing aid in the production of polytetrafluoroethylene (PTFE). PFNA is used to solubilize the monomer, vinylidene fluoride, used to make PVDF (Prevedouros et al., 2006). Prevedouros et al. (2006) lists the 2002 production capacities of major producers of PVDF by the emulsion process which uses PFNA/Surflon S-111. The two highest capacity facilities using the emulsion process in 2002 were located in Calvert City, KY (8.4×10^6 kg/yr) and Thorofare (West Deptford), NJ (7.7×10^6 kg/yr), with lower capacity sites in France and Japan.

PVDF is resistant to high temperatures and is chemically non-reactive. Uses of PVDF include: in tanks, valves, pipes, and other components which come into contact with reactive chemicals; as insulation for wire and printed circuit boards; as a coating in pressure and thermal optic sensors; as a binder for electrodes on lithium ion batteries; in artificial membranes used for biomedical applications, for monofilament fishing lines; and in architectural coatings (TOEFCO, 2014). PFNA is not an intended component of PVDF and is present only at trace levels (100-200 ppm) in the PVDF fluoropolymer used in commercial and industrial products that is produced with PFNA (Prevedouros et al., 2006).

It is estimated that 60% of the PFNA used in PVDF manufacturing worldwide was released to the environment, resulting in global emissions of 400,000 to 1,400,000 kg from 1975-2004 (Prevedouros et al., 2006). Data provided to NJDEP about PFC use at the PVDF manufacturing facility located in Thorofare (West Deptford), NJ indicate that 86.6% of the 125,069 kg of the Surflon S-111 PFC mixture (primarily PFNA) used between 1991-2010 was released to the environment (air and water) (Roux Associates Inc., 2013). The environmental fate of PFNA is discussed below.

The manufacture and use of PFOA, PFNA, and other long-chain perfluorinated carboxylates is currently being phased out by eight major manufacturers through a voluntary stewardship agreement with USEPA, with the intent to reduce global facility emissions and product content of these chemicals by 95% by 2010, and with the ultimate goal of eliminating emissions and product content by 2015 (USEPA, 2010, 2012a). The manufacturer of PVDF at the facility located in Thorofare is a participant in the voluntary stewardship agreement. However, other manufacturers of long-chain PFCs that are not participants in the voluntary stewardship agreement continue to manufacture these compounds, in the U.S. and particularly overseas (USEPA, 2009; Lindstrom et al., 2011). It is not known if PFNA is produced by manufacturers that are not part of the voluntary stewardship agreement with USEPA.

Data provided to NJDEP show that Surflon S-111, the PFC mixture consisting primarily of PFNA, was not used in 2011-2012 at the PVDF manufacturing facility located in Thorofare, NJ (Roux Associates Inc., 2013). In 2010, only 171 kg were used, compared to 6,341-8,467 kg/year in each of the previous 10 years.

Evaluations by other government agencies

No health-based guidance values or standards have been developed for PFNA by U.S. federal agencies including USEPA, U.S. states, or other nations.

The European Chemical Agency (ECHA) Risk Assessment Committee finalized its harmonized classification and labeling opinion (CLH) for PFNA in September 2014 (ECHA, 2014). The ECHA classifications are related to hazard identification and

qualitative weight of evidence for various endpoints and do not include dose-response, quantitative risk assessment, or criteria development. ECHA concluded that PFNA is a presumed human reproductive toxicant for damage to the unborn child; a suspected human reproductive toxicant for fertility effects; a suspected human carcinogen; causes specific target organ toxicity to liver, thymus, and spleen after prolonged or repeated exposure; and causes harm to the breast-fed child through effects on or via lactation. These conclusions are based on data on PFNA itself, as well as supporting information from PFOA, to which it is closely related. The background document for the ECHA classification of PFNA was prepared by the Swedish Environmental Agency (Swedish Environmental Agency, 2013).

ENVIRONMENTAL SOURCES, FATE, AND OCCURRENCE

Because of the extreme stability of their carbon−fluorine bonds, PFCs are extremely persistent in the environment. PFCs are highly water-soluble in comparison with other well-studied persistent and bioaccumulative organic pollutants which have much lower water solubilities, such as polychlorinated dioxins and PCBs (Post et al., 2013). Although the production and use of PFNA is being phased out by major U.S. manufacturers, environmental contamination and human exposure to PFNA are anticipated to continue for the foreseeable future due to its persistence, formation from precursor compounds (discussed below), and the potential for continued production by other manufacturers in the U.S. and/or overseas (USEPA, 2009; Lindstrom et al., 2011).

PFCs including PFNA are found in environmental media, including wildlife, in worldwide locations including remote polar regions. In addition to release from industrial facilities where it is made or used, an additional possible source of PFNA in the environment is its formation under some conditions from precursor compounds such as fluorotelomer alcohols (FTOH), used industrially and in consumer products (Butt et al., 2010; Buck et al., 2011).

The fluorotelomer alcohol 8:2 FTOH [$CF_3(CF_2)_7CH_2CH_2OH$] is converted to some extent to both PFNA and PFOA through non-biological chemical reactions in the atmosphere (Ellis et al., 2004) and through metabolic reactions in soil bacteria, under some conditions, and in fish (Butt et al., 2014).

Polyfluoroalkyl phosphoric acid diesters such as diPAPs 8:2 (larger molecules found in grease proof food contact papers, wastewater treatment plant sludge, and paper fibers from paper mills; D'eon et al., 2009) release FTOH that can degrade to PFCs. Fluoroacrylate polymers, used in commercial products, may also degrade in soil to release FTOH (Russell et al., 2008; Washington et al., 2009). Since PFNA and other PFCs do not degrade appreciably, environmental PFC levels could be increased by even a small rate of conversion of the precursors to the terminal PFC product.

Two major pathways have been proposed for long-range transport of PFCs such as PFNA to remote locations worldwide (Lau et al., 2007; Butt et al., 2010). The relative contribution of each of these pathways is not known. The first pathway involves the atmospheric transport of volatile precursors, such as FTOH, followed by oxidation to PFCs (e.g. PFOA and PFNA) which are then deposited onto the land or the water. The second pathway involves long-range aqueous transport of perfluorinated carboxylates such as PFOA and PFNA in their anionic forms to remote locations by currents on the ocean's surface.

Drinking Water

As discussed above, large amounts of PFNA were discharged to air, soil, and surface water at facilities where it was used as a processing aid in the production of the fluoropolymer PVDF (Prevedouros et al., 2006; Roux Associates Inc., 2013). Like other ground water contaminants, PFCs that are released to the environment can reach drinking water wells via the well-established pathways of migration of a ground water plume that has been contaminated either directly from surface spills and/or by contaminated surface water mixing with ground water drawn in by pumping wells. Air emission has also been established as a pathway for ground water contamination by the related compound, PFOA. In an industrial facility where PFOA was used as a processing aid in fluoropolymer production, ground water used for drinking water was contaminated up to 20 miles or more from the emission source (Shin et al., 2011). A pathway for this contamination was deposition from air onto soil, followed by migration through the soil to ground water (Davis et al., 2007). PFNA emitted to air from PVDF production facilities may reach ground water through the same pathway. This pathway, discussed further below, is being investigated as a possible source of PFNA in drinking water wells in the vicinity of a New Jersey PVDF production facility that emitted PFNA to air and water for about 25 years (Integral, 2013).

In addition to industrial releases, sources of PFCs found in ground water or surface water include: discharge from wastewater treatment plants that treat domestic and/or industrial waste; street- and storm water runoff; release of aqueous firefighting foams; and land application of biosolids or contaminated industrial waste (Post et al., 2013). Another source of PFCs in the environment is the biodegradation in soil, sludge, and wastewater of precursor compounds such as fluorotelomer alcohols (FTOH), as discussed above.

PFCs, including PFNA, have been found in raw and finished public drinking water from both ground and surface water sources in the U.S. and worldwide (Post et al., 2013; USEPA, 2015a). Available information indicates that PFCs, including PFNA, are not removed from drinking water by conventional treatment processes, but may be removed by granular activated carbon, reverse osmosis, and possibly ion exchange treatment systems designed for this purpose (Rahman et al., 2013).

PFNA has been found less frequently and at lower concentrations than PFOA and PFOS in drinking water studies from the U.S. and around the world. Comparison of occurrence frequencies for PFNA among drinking water studies is complicated by the fact that the reporting levels in these studies vary widely. In a literature review of drinking water occurrence studies worldwide (Post et al., 2013), the highest reported concentration of PFNA outside of Gloucester County, NJ was 58 ng/L in Catalonia, Spain (Ericson et al., 2009).

Post et al. (2013) reported on a study of the occurrence of PFCs in raw water from 31 NJ public water supplies (29 sampled by NJDEP in 2009, and two sampled by a water company in 2010-2013 using the same laboratory and method). In this study, PFNA was found in three NJ ground water sources at concentrations (72-96 ng/L) higher than the highest raw or finished drinking water level (58 ng/L) reported elsewhere in the studies located in the literature. At these three NJ sites, PFNA was the sole or predominant PFC detected, whereas PFNA was a minor component of a mixture of PFCs when it was reported in drinking water at locations elsewhere in the world.

The highest PFNA concentration (96 ng/L) reported in the 2009 NJDEP drinking water study was at a public water supply well (Paulsboro Water Department) in southern NJ about 2 miles from the

West Deptford, NJ facility that used and discharged PFNA from 1985 until 2010 (Post et al., 2013). In follow-up sampling of this well in 2013, PFNA was found at 140 ng/L in raw water and 150 ng/L in finished water (Post et al., 2013). This well is currently not in use, and installation of treatment to remove PFCs from this well is planned. PFNA levels in another recently constructed well of this public water supply were lower (< 20 ng/L) in September 2013 testing. PFNA data are not available from two other wells of this water system which were used only on a limited basis until May 2012 and are not currently in use. PFNA was also detected at up to 72 ng/L in wells of a second public water supply (NJ American Logan-Birch Creek) located about 10 miles from the industrial facility (Post et al., 2013). The presence of PFNA (80 ng/L) reported at a third site by Post et al. (2013), located in northern NJ, was not confirmed in follow-up sampling in 2013.

In further public water supply sampling reported to NJDEP through March 2014, PFNA was also found in a public water supply well in West Deptford, within the same township as the industrial facility, at up to 48 ng/L in 2013, and in wells of 5 other Gloucester County public water supplies at up to 50 ng/L.

Under the USEPA Unregulated Contaminant Monitoring Rule 3 (UCMR3; USEPA, 2012b), nationwide monitoring of finished water for 30 unregulated contaminants, including PFNA and 5 other PFCs, is being conducted in 2013−2015 by all U.S. public water supplies serving more than 10,000 people and 800 representative PWS serving less than 10,000 people. Comparison of the UCMR3 PFC occurrence data with other PFC occurrence studies is complicated by the fact that the Reporting Level for UCMR3 monitoring of PFNA (≥ 20 ng/L) is much higher than the Reporting Levels in the NJDEP studies and other monitoring data reported to NJDEP and in the drinking water occurrence studies reported in the literature (generally ≤ 5 ng/L, reviewed by Post et al., 2013). In initial UCMR3 data from 3483 public water supplies outside of New Jersey reported to USEPA through January 2015, PFNA (20 ng/L or above) was found in only six public water systems outside of New Jersey (USEPA, 2015a; Table 1). As of January 22, 2015, PFNA was found in UCMR3 monitoring in three public water supplies sites in Gloucester County, NJ (Woodbury City Water Department, up to 56 ng/L; Monroe Township MUA, up to 28 ng/L; West Deptford Township Water Department, 30 ng/L) including one public water supply (Monroe Township MUA) which had not previously reported detections of PFNA to NJDEP. In all but two of the non-NJ public water supplies reporting PFNA in UCMR3, other PFCs were also present, while PFNA was the only PFC reported at the three Gloucester County, NJ, sites.

Table 1. New Jersey versus national PFNA detections (≥20 ng/L) in public water supplies in initial UCMR3 results

New Jersey *(as of 1/22/15)*		***National (other than NJ)*** *(as of January 2015)*	
Number of PWS	*% of PWS*	*Number of PWS*	*% of PWS*
3/122	2.5%	6/3483	0.2%

In private well testing results reported to NJDEP as of July 18, 2014, PFNA (at ≥ 2.5 ng/L) was detected in wells at 26 of 94 (28%) of residences tested in the vicinity of the West Deptford industrial facility. Fifteen of the wells had PFNA levels above 20 ng/L, and the highest concentration found was 1,500 ng/L. Point of entry treatment systems (POETS) have been installed on those wells with PFNA levels of ≥ 20 ng/L that are currently used for potable purposes.

Ambient Surface Water

In 2007−09, PFNA was found in the Delaware River water at up to 976 ng/L starting near and downstream of the discharge location of the above-mentioned industrial facility; this is higher than the surface water concentrations elsewhere in the U.S. and worldwide in studies located in the literature. Elevated levels of PFUnDA (C11), a component of the Surflon S-111 mixture used at the facility, were also found in the Delaware River at these same locations (DRBC, 2012).

Wildlife

PFCs with eight or more fluorinated carbons (PFNA and longer chain carboxylates, PFOS and longer chain sulfonates) are bioaccumulative in fish, while shorter chain-length PFCs are not (Conder et al., 2008). PFNA and other PFCs are found in biota, including marine mammals and other species, worldwide including in remote Arctic and Antarctic regions. The presence of PFCs in these species is believed to result from exposure both to these compounds and to precursors that are metabolized to PFCs (Houde et al., 2011).

In a study of PFC levels in blood taken in 2003 from bottlenose dolphins in Bermuda, the East and West coasts of Florida, Charleston, SC, and Delaware Bay, NJ, the mean PFNA level in Delaware Bay dolphins (326 ng/g) was much higher than at the other sites (13-63 ng/g) (Houde et al., 2005). These higher levels in Delaware Bay may have resulted from discharges of PFNA from local industrial sources.

In 2004-07, PFNA and PFUnDA levels were elevated in fillets from white perch and channel catfish from the same Delaware River locations where elevated levels were found in surface water in 2007−2009 (DRBC, 2009). In more recent data from 2010 and 2012 at these Delaware River locations, PFNA was not detected ($\geq$ 0.25 ng/g, 2010; $\geq$ 0.5 ng/g, 2012) (DRBC, personal communication). Liver and serum were not analyzed in these studies.

HUMAN BIOMONITORING

Human Serum

PFNA is one of four PFCs [PFOA, PFOS, PFNA, perfluorohexane sulfonate (PFHxS)] that are detected in the serum of greater than 99% of a representative sample of the U.S. population in National Health and Nutrition Examination Survey (NHANES) conducted by the U.S. Centers for Disease Control and Prevention (CDC; Kato et al., 2011; CDC, 2015); PFCs are also ubiquitous in the serum of populations worldwide (reviewed in Lau, 2012; Post et al., 2012). These four PFCs are biologically persistent, with human half-lives of several years, as discussed in detail in the Toxicokinetics section below.

In the U.S population as a whole, serum levels of PFNA are generally lower than for the other three ubiquitous PFCs. In the most recent NHANES data from 2011-12 (CDC, 2015), geometric mean serum levels were PFNA, 0.88 ng/ml; PFOA, 2.08 ng/ml; PFOS, 6.31 ng/ml; and PFHxS, 1.28 ng/ml. Based on the infrequent occurrence of PFNA reported in U.S. public drinking water supplies in UCMR3 and other studies (discussed above), it is unlikely that the mean and median PFNA serum levels found in the U.S. general population in NHANES are influenced by drinking water exposures. To further verify this conclusion, local health officers from several counties reporting PFNA in UCMR3 through July 2014 were contacted by the Health Effects Subcommittee. Several of these counties reported that they had no information indicating that

their location participated in NHANES in 2011-12, while one county was not contacted because it did not have a health department.

Table 2. Geometric mean and 95% confidence interval and selected percentiles of PFOS, PFOA, PFHxS, and PFNA serum concentrations (ng/mL) for the U.S. population 12 years of age and older: Data from NHANES 2011-2012 [a]

	Geometric Mean (95% Confidence Interval)		Selected Percentiles			
			50th	75th	90th	95th
PFHxS	1.28	1.15-1.43	1.27	2.26	3.81	5.43
PFOS	6.31	5.83-6.82	6.51	10.48	15.62	21.68
PFOA	2.08	1.95-2.22	2.08	3.02	4.35	5.67
PFNA	0.88	0.80-0.97	0.86	1.30	1.95	2.54

[a] CDC (2015)

In another series of studies of PFC serum levels in U.S. blood donors, the geometric mean from the most recent data (2006) was 0.97 ng/ml (Olsen et al., 2011). Median PFNA serum levels in the epidemiology studies of the general population from around the world that are reviewed in the Human Studies section below ranged from 0.3 ng/ml to 2.36 ng/ml. As discussed below, the lower median values are from studies of European populations, and the two highest median values (2.3 and 2.36 ng/ml) are from Taiwanese studies. A number of other studies of general population human serum levels of PFNA from locations worldwide, which did not assess associations with health endpoints, are not reviewed herein.

In data from 2001-02 NHANES (Kato et al., 2009), PFNA and other PFCs in pooled serum samples from male and female children, age 3-5 and 6-11 years, of non-Hispanic white, non-Hispanic black, and Mexican-American ethnicity were generally similar in both age categories and both genders, with some differences among racial and ethnic groups.

Human Breast Milk

PFNA and other PFCs have been found in human breast milk in the general population of the U.S. and other nations. Fujii et al. (2012) sampled breast milk from 90 women (30 each from Japan, Korea, and China) and compiled these results, as well as data from other studies conducted worldwide that had been reported in the literature. Detection frequencies and concentration ranges for PFNA in these studies varied widely, with some studies finding no samples with PFNA above a detection limit of 8.8 ng/L while other studies reported maximum levels of >100 ng/L. In the only study conducted in the U.S. (Tao et al., 2008a), PFNA was found at >5.2 ng/L in 13 of 45 (29%) of breast milk samples collected in Massachusetts in 2004, with a mean of 7.26 ng/L, a median of 6.97 ng/L, and a maximum of 18.4 ng/L.

Human Seminal Fluid

PFNA and other PFCs were found in human seminal fluid in a study of Sri Lankans. The mean and median concentrations were 0.007 and 0.005 ng/ml, respectively, and concentrations were significantly correlated with serum PFNA concentrations (Guruge et al., 2005).

SOURCES OF HUMAN EXPOSURE

Sources of human exposure to PFCs include drinking water, food, food packaging, carpets, upholstery, and clothing treated for water and stain resistance, house dust, protective sprays and waxes, and indoor and outdoor air. Since PFNA bioaccumulates in fish, consumption of contaminated fish in locations where PFNA has been discharged into surface waters is a potential exposure route. The primary use of PFNA is as a processing aid in the production of PVDF, a material which is not used as widely in consumer products as the materials made with some other PFCs. Humans may also be exposed to PFCs including PFNA that are formed from fluorotelomer alcohols in environmental media (discussed above) and by metabolism of fluorotelomer alcohols in the human body (Henderson and Smith, 2007; Nilsson et al., 2010; reviewed by Butt et al., 2014). Fluorotelomer alcohols and their precursors, such as polyfluoroalkylphosphoric acid diesters (diPAPs), have been used in consumer products such as greaseproof food packaging paper.

In contrast to other persistent and bioaccumulative organic compounds that are not water-soluble, ingestion of contaminated drinking water can be an important source of human exposure to PFCs. Elevated serum levels of PFOA, PFOS, and PFHxS have been found in communities with contaminated private wells and/or public water supplies. However, no studies of serum levels in communities exposed to PFNA in drinking water have been conducted. Because of their long half-lives in the body, ongoing exposure to even relatively low drinking water concentrations of biologically persistent PFCs substantially increases total human exposure. For example, ongoing drinking water exposure to PFOA increases PFOA serum levels with a serum:drinking water ratio of 100:1 or greater (Emmett et al., 2006; Post et al., 2012; discussed in detail below). Consistent with their higher daily water consumption rate (ml/kg/day), serum levels are generally higher in young children than in adults exposed to the same PFC concentration in drinking water (Emmett et al., 2006; Mondal et al., 2012).

Because PFNA exists in drinking water in its non-volatile anionic form, inhalation exposure is not expected from non-ingestion uses of drinking water such as showering, bathing, laundry, and dishwashing. In contrast, these are important exposure routes for volatile drinking water contaminants. Similarly, dermal absorption of PFNA during showering and bathing is insignificant compared to exposure through ingestion (NJDOH, 2014). The evaluation was based on skin permeability data for PFOA (Franko et al., 2012), a compound which is expected to have a slightly higher potential for dermal absorption than PFNA.

Commercially available infant formula products does not appear to be a major source of exposure to PFNA or other PFCs in the U.S. Tao et al. (2008b) evaluated PFCs in 21 samples of 5 brands of infant formula representing >99% of the U.S. market. Products tested included milk-, organic-, and soy-based formula, packed in cans, glass, or plastic, in liquid, powdered, and concentrated liquid forms. PFNA was not detected (<2.2 ng/L) in any sample. Other PFCs (for which detection levels varied) were also not detected (PFOA, PFBS, PFHpA) or were infrequently found (PFOS – one detection at 11.3 ng/L; PFHxS-two detections at up to 3.59 ng/L). In this study, PFCs were also analyzed in 12 samples of 11 brands of dairy milk purchased in Albany, NY in 2008, with only one detection of PFHxS at 3.83 ng/L.

TOXICOKINETICS

Absorption

PFCs, including PFOA which differs from PFNA by only one fluorinated carbon, are generally well absorbed orally (Lau, 2012; Post et al., 2012). While oral absorption of PFNA has not been quantitatively evaluated, oral absorption occurs rapidly as indicated by its presence in serum in rodents soon after oral administration (Tatum-Gibbs et al., 2011).

Ammonium perfluorononanoate, the ammonium salt of PFNA, was absorbed by inhalation when generated as a dust, as demonstrated by its acute toxicity in rats exposed by this route (Kinney et al., 1989).

No information on the extent of dermal absorption of PFNA was located. PFOA penetrated rat and human skin in an *in vitro* system (Fasano et al., 2005), and caused liver toxicity in rats (Kennedy, 1985) and immune effects in mice (Fairley et al., 2007), after dermal exposure. The dermal permeability coefficient of PFOA (14,000 ng/L [14 µg/L] in water, pH 5.01) was estimated as 8.8×10^{-5} cm/hr (Fasano et al., 2005). The permeability coefficient of PFNA is expected to be close to, but slightly less than that of PFOA (F. Frasch, personal communication).

Distribution and Metabolism

Like other PFCs, PFNA is chemically non-reactive and is not metabolized (Lau et al., 2012).

PFNA is primarily distributed to serum, kidney, and liver. After repeated administration to mice and rats, liver concentrations are higher than serum concentrations, while concentrations in the kidney are lower than in the serum (Tatum-Gibbs et al., 2011).

PFCs in general have an affinity for binding to proteins. Available information indicates that PFNA, like other PFCs, is almost totally bound to albumin and other proteins in the serum (Lau, 2012). PFNA was found to bind (>98%) to plasma proteins *in vitro* (Ohmori et al., 2003).

Excretion

PFCs, including PFNA, are excreted in urine and feces, with the rate of excretion generally decreasing with increasing carbon chain length (Lau, 2012).

Rodents

The toxicokinetics of PFNA and PFOA have been studied in mice and rats, and half-lives in these species are shown in Table 1. PFNA is excreted several-fold more slowly than PFOA in both genders of both of these rodent species.

Both PFOA and PFNA are slowly excreted in both male and female mice and in male rats, with much more rapid excretion for both PFCs in female rats. In these species and genders, estimates of PFNA half-lives were 2 to 30-fold longer than for PFOA. Rates of fecal elimination are slow, and are similar in male and female rats (Kudo et al., 2001). The differences in excretion rates between genders are believed to result from gender differences in renal organic anion transporters (OATs) that control urinary excretion rates. These proteins are responsible for the active transport (secretion or reabsorption) of many organic anions into and out of the kidney and other organs (Han et al., 2012; Weaver et al., 2010). In rats administered 20 mg/kg/day by intraperitoneal injection for 5 days, castration reduced the levels of PFNA in the liver, while PFNA levels in the livers were not decreased in castrated rats that were treated with testosterone (Kudo et al., 2000). These results

suggest that the rapid excretion of PFNA in male rats is dependent on testosterone.

In pharmacokinetic studies of linear (*n*-) and branched (*iso*-) PFNA after a single-dose (male rats only; Benskin et al., 2009) and with subchronic dosing (males and females dosed for 12 weeks; De Silva et al., 2009), the linear form was excreted somewhat more slowly than the branched form. Half-lives were similar to those in the studies shown in Table 3. The half-lives in male rats were 41-48 days for linear PFNA and 21-32 days for branched PFNA, while the half-lives in females were 2.1 days for linear and 0.82 days for branched PFNA. In the male rats in these studies, the half-lives of PFNA were 3-5 times longer than for PFOA, based on comparison of groups treated with the same isomer for the same time period.

PFNA has been measured in urine and feces in several of the rat pharmacokinetic studies (Kudo et al., 2001; Benskin et al., 2009; De Silva et al., 2009; Mertens et al., 2010). Because urinary excretion of PFNA is very slow in male rats (discussed above), fecal excretion becomes proportionally more significant as compared to female rats in which urinary excretion is rapid. In male rats, a large percentage (65-68%; Benskin et al., 2009) is excreted in the feces (Kudo et al., 2001; Benskin et al., 2009).

Table 3. Half-lives of PFNA and PFOA in Male and Female Mice and Rats (days)

	PFNA		***PFOA***		***PFNA: PFOA $t_{1/2}$ Ratio***	
	Male	*Female*	*Male*	*Female*	*Male*	*Female*
Rat	30.6[a]/29.6[b]	1.4[a]/2.4[b]	4-6[c]	0.08-0.17[c]	5.0-7.5	8.2-30
Mouse	34.3[a]/68.4[b]	25.8[a]/68.9[b]	19[d]	17[d]	2.0-4.0	1.4-3.6

[a] Tatum-Gibbs et al. (2011).
[b] Ohmori et al. (2003).
[c] Johnson et al. (1979)
[d] Lau et al. (2005)

Humans

Data on the human half-life of PFNA are extremely limited. Human half-lives of several PFCs (PFOA, PFOS, PFHxS, PFBS, PFBA) have been estimated from data on declines in serum levels after occupational or drinking water exposures ended (summarized in Lau et al., 2012 and Post et al., 2012). For PFNA, no such data are available.

Zhang et al. (2013a) estimated the human half-lives of a series of PFCs, including PFOA and PFNA, based on renal clearance estimates. In women less than 50 years old, modeled excretion through menstrual blood loss was also considered. The study included 86 adults (age 21-88 years) from the Chinese general population. The median serum PFNA concentration in the subjects was 0.37 ng/ml, which was about two-fold lower than the median of 0.86 ng/ml in the 2011-12 NHANES (CDC, 2015). Renal clearance estimates for each PFC in each participant were based on paired urine and blood or serum measurements. The PFNA half-life estimates in males and older females for PFNA (n=50) ranging from 0.34 to 20 years, while for PFOA (n=66), the range was 0.059 to 14 years. In younger females, the range for PFNA (n=16) was 0.38 to 7.7 years and for PFOA (n=20) was 0.19 to 5.2 years.

Available data indicate that blood loss (e.g. through menstruation, blood donation, or

venesection) is an excretion route for PFCs (Harada and Koizumi, 2009; Taylor et al., 2014; Lorber et al., 2015; MDH, 2013). The estimates of PFNA half-life in women under 50 years of age are based on modeling of this pathway and are considered more uncertain than the estimates for men and older women. Although children were not included in this study, the increased excretion rate due to menstrual blood loss is not applicable to children. Similarly, the additional clearance through menstrual blood loss is not relevant to pregnant women. Other potential clearance pathways, such as fecal excretion, were not considered by Zhang et al. (2013a), but were believed by the researchers to be less significant than elimination through urine and menstrual blood.

Median and geometric mean values represent estimates of the 50th percentile value and are less affected by outliers than mean values. As shown in Table 4, the ratios of estimated half-lives for PFNA and PFOA in men and older women, based on medians and geometric means, are 2.06 and 2.67 years, respectively. For younger women for whom menstrual clearance was modeled, the estimated ratios are closer to 1. As noted above, the estimates for younger women are more uncertain than the estimates for men and older women.

In summary, while the half-lives estimated by urinary clearance are less definitive than those based on serum level declines, these results support the conclusion that PFNA is more persistent in humans than PFOA. A longer human half-life of PFNA as compared to PFOA is consistent with the toxicokinetic data from rodents.

Table 4. Estimated half-lives of PFNA and PFOA in Humans (years)

	PFNA		***PFOA***		***PFNA:PFOA $t_{1/2}$ Ratio***	
Based on decline in serum levels	No information		2.3-10.1[a]/3.8[b] years		----	----
Based on urinary excretion, with estimated menstrual clearance in females <50 years of age[c]	*all males and females >50 years:*	*females 21-50 years:*	*all males and females >50 years:*	*females 21-50 years:*	*all males and females >50 years:*	*females 21-50 years:*
Mean	4.3	2.5	2.6	2.1	1.65	1.19
Geometric Mean	3.2	1.7	1.2	1.5	2.67	1.13
Median	3.5	1.5	1.7	1.8	2.06	0.83

[a]Multiple studies reviewed in Post et al. (2012) – communities with drinking water exposures.
[b]Olsen et al. (2007) - retired workers.
[c]Zhang et al. (2013a) – Chinese general population.

Fetal exposure - Maternal and cord blood serum levels

Fetal exposures to PFNA are important because developmental effects are among the most sensitive toxicological endpoints for PFNA in animals (see Toxicology section below). PFNA, like other PFCs, is transferred from the mother to the fetus in animal studies (Das et al., 2015; Wolf et al., 2010). Like other PFCs, PFNA is found in human umbilical cord blood (reviewed below), placenta, and amniotic fluid (Stein et al., 2012; Zhang et al., 2013b), thereby demonstrating that maternal-fetal transfer also occurs in humans.

In human studies, PFNA levels in fetal cord blood serum generally correlate with maternal serum levels. In nine studies in which both maternal and cord blood PFNA levels were measured, the mean cord blood serum:maternal serum (or plasma) ratios ranged from about 0.3 to about 1, with a median value of about 0.5 (Monroy et al., 2008; Fromme et al., 2010; Beesoon et al., 2011; Kim et al., 2011a; Liu et al., 2011a; Needham et al., 2011; Gutzkow et al., 2011; Ode et al, 2013; Zhang et al., 2013b).

Infant Exposure – Distribution to Human Breast Milk

Infants drink much more fluid (breast milk or formula which may be prepared with drinking water) on a body weight basis than older children and adults, and the intake rate is highest in the youngest infants. For example, the mean drinking water intakes in infants who consume drinking water are 137 ml/kg/day from birth to 1 month of age, and 53 ml/kg/day from 6-12 months of age (USEPA, 2008). For breast fed infants, mean breast milk intakes in these age groups are 150 ml/kg/day from birth to 1 month of age and 83 ml/kg/day from 6-12 months of age (USEPA, 2008). In contrast, the mean daily drinking water intake is 13 ml/kg/day for children 11 or more years of age and adults (USEPA, 2008) and 26 ml/kg/day for lactating women (USEPA, 2011). Thus, infants who consume formula prepared with contaminated drinking water receive a higher dose of the contaminant than older children and adults. Breast-fed infants will also receive higher exposures than older children and adults for contaminants that are transferred to breast milk at concentrations even several-fold below the concentration in the drinking water source.

As discussed in Human Biomonitoring above, PFNA is found in human breast milk. The importance of breast milk as a route of exposure of PFNA and other PFCs is illustrated by the data of Fromme et al. (2010; Table 5). Maternal and cord blood serum PFNA concentrations were studied in 53 German mothers at birth and in their breast-fed infants. Although mean and median infant (cord blood) serum levels were less than in maternal serum at birth, serum PFNA increased at 6 months to levels higher than in maternal serum, presumably from exposure through breast milk. At age 19 months, a time point at which breast feeding had stopped or was decreased, serum levels had decreased to close to maternal levels, presumably due to decreased exposure on a body weight basis, combined with dilution due to rapid growth. Similar findings would be expected in infants who are fed with formula prepared with drinking water contaminated with PFNA rather than with breast milk, assuming that the PFC concentrations in the drinking water are the same as in the breast milk.

Table 5. PFNA (ng/ml) in serum from 53 mother:infant pairs[a]

	Mother			*Fetus/infant*		
	Pregnancy	At delivery	6 months after delivery	Cord blood	6 months after birth	19 months after birth
N (% > LOQ)	44 (86)	38 (83)	47 (83)	33 (30)	40 (90)	24 (83)
Mean	0.8	0.8	0.7	0.4	1.1	0.7
Median	0.6	0.6	0.5	<0.4	1.0	0.6
95th percentile	2.8	3.0	2.0	1.5	2.3	1.4

[a] Fromme et al., 2010

Relationship between PFC drinking water concentrations and serum levels

Because PFNA and other persistent PFCs are stored in the serum of humans with a half-

life of several years, human serum levels are a reliable and stable measure of internal dose. In communities with drinking water supplies contaminated by PFOA and other persistent PFCs (PFOS and PFHxS), mean and median serum PFC levels were elevated above means and medians in the general population (reviewed in Post et al., 2013).

For persistent compounds in general, the relationship between daily intake (as expressed either as a dose (ng/kg/day) or a concentration in drinking water (ng/L)) and body burden is proportional to the compound's half-life (USEPA, 2003). Since blood serum (along with liver and kidney) is a major site of distribution for PFCs, serum levels are an indicator of body burden for these compounds. Thus, the serum:drinking water ratio for persistent PFCs is expected to be proportional to their half-lives, provided that a constant fraction of total intake is distributed to serum.

PFOA

The relationship between drinking water concentration and serum concentration has been extensively evaluated for PFOA. It is well established that ongoing human exposure to PFOA in drinking water increases serum levels, on average, by ≥100 times the drinking water concentration. As discussed below, this ratio is supported by data from several studies of populations whose public water supplies or private wells were contaminated with a wide range of PFOA concentrations (60 ng/L to 13,300 ng/L), as well as three toxicokinetic modeling efforts (Emmett et al., 2006; Hoffman et al., 2011; Clewell, 2006, 2009; Post et al., 2009a, 2009b, 2012; Worley and Fisher, 2015).

Although upper percentile exposure factors are typically used in risk assessment, 100:1 represents a central tendency (or lower) estimate for the ratio in exposed populations. The ratio can be higher or lower among individuals due to differences in daily water consumption rates and physiological parameters related to excretion rate.

Emmett et al. (2006) reported a median ratio of 105:1 in residents of Little Hocking, Ohio age 6 years or older who were exposed to 3,550 ng/L (3.55 µg/L) in their drinking water, with a 25%-75% interquartile range of 62:1-162:1. For six individuals with private wells included in this study, the ratios ranged from 142:1 to 855:1 (Emmett et al., 2006). Post et al. (2009a) evaluated five other Ohio and West Virginia communities with lower drinking water concentrations (≥ 60 ng/L) in the same vicinity and found ratios of 100:1 to 330:1 in the four communities for which reliable estimates can be made based on a relatively narrow range of reported PFOA drinking water concentrations.

A ratio of ≥100:1 is also supported by data from several other studies. In 108 users of contaminated private wells with mean and maximum PFOA levels of 200 ng/L and 13,300 ng/L in the same Ohio/West Virginia region discussed above (Hoffman et al., 2011), the estimated ratio was 141:1 (95% CI: 135:1–148:1) based on regression modeling, and 114:1 based on a one-compartment toxicokinetic model. The 100:1 ratio is also consistent with observations in 98 Minnesota residents tested 34 months after exposure to contaminated drinking water ended if the expected post-exposure decline in serum levels is considered (MDH, 2009).

A lower serum:drinking water ratio of approximately 50:1 was observed in a German community whose drinking water source was contaminated with PFOA and other PFCs (Hölzer et al., 2008). Possible reasons for this difference are the use of bottled water by some participants who were aware of the contamination for up to 6 months before their blood was sampled,

uncertainty about the duration and time course of the water contamination, or differences in drinking water consumption patterns between German and U.S. residents.

Clewell (2006, 2009) developed a factor, 0.127 (ng/kg/day)/(ng/ml), that relates intake of PFOA (ng/kg/day) and human serum level (ng/ml). The factor was derived from a pharmacokinetic model and was validated with data from the exposed community in Little Hocking, Ohio. Using average daily water intake recommended by USEPA (2011) of 16 ml/kg/day, application of this factor predicts a serum:drinking water ratio of 126:1.

This observed serum:drinking water ratio of approximately 100:1 is also in agreement with a one-compartment model (Harada et al., 2005) which predicts that ingestion of 0.0017 μg/kg/day would result in serum levels of 13 μg/L (ng/ml) in males and 8 μg/L (ng/ml) in females, or a mean of 10.5 μg/L (ng/ml). Assuming a drinking water intake of 16 ml/kg/day (USEPA, 2011), a dose of 0.0016 μg/kg/day would result from a water concentration of 0.106 μg/L. The ratio between a serum concentration of 10.5 μg/L and this water concentration of 0.106 μg/L is 99:1, very close to the median ratio reported by Emmett et al. (2006).

Finally, a serum:drinking water of approximately 100:1 is predicted by a recently developed physiologically based pharmacokinetic (PBPK) model that relates PFOA drinking water concentration to serum level. This model considers urinary clearance rates and partition coefficients in humans for fat, liver, and kidney reported in the literature, as well as the most current drinking water exposure parameters (Worley and Fisher, 2015).

PFNA

The toxicokinetic studies discussed above indicate that rodent half-lives for PFNA are 2 to 30-fold longer than for PFOA (Table 3), and that the median and geometric mean half-life of PFNA in humans (except in women of childbearing age) for PFNA is at least twice that of PFOA (Table 4). (PFC half-life estimates in women of childbearing age in this study include a modeling component and are more uncertain.) These animal and human data support an estimated half-life of PFNA at least twice that of PFOA. Additionally, as discussed above, a 100:1 ratio for PFOA likely underestimates the median value for this parameter. Taken together, this information indicates that an estimated serum:drinking water ratio of 200:1 for PFNA is not overly stringent.

Based on an assumed daily drinking water intake of 16 ml/kg/day (USEPA, 2011), the corresponding increase in daily dose of PFNA (ng/kg/day) that results in a 1 ng/ml increase in PFNA in blood serum is 0.08 ng/kg/day/(ng/ml). This value is derived as follows:

$$\frac{\text{200 ng/ml PFNA in blood serum}}{\text{(1 ng/ml PFNA in drinking water) x (16 ml/kg/day drinking water ingested)}}$$

$$= \frac{\text{12.5 ng/ml PFNA in blood serum}}{\text{ng/kg/day PFNA ingested}}$$ which is equivalent to: (0.08 ng/kg/day)/(ng/ml)

For comparison, the estimated daily dose of PFOA estimated to result in a 1 ng/ml increase in serum level (0.127 ng/kg/day/(ng/ml); Clewell (2006, 2009) is associated with a serum:drinking water ratio of 126:1. This estimated daily dose is 1.49 times the daily PFNA dose (0.08 ng/kg/day) estimated to increase the serum level by 1 ng/ml.

Based on the above toxicokinetic considerations, ongoing exposure to drinking water with 150 ng/L

PFNA (the highest concentration reported in public drinking water in New Jersey or elsewhere) is estimated to increase PFNA serum levels, on average, by 30 ng/ml (µg/L; ppb) in serum. This represents about a 34-fold increase from the general population geometric mean serum value of 0.88 ng/ml (CDC, 2015).

HEALTH EFFECTS

Human Studies

Overview

In total, 44 human epidemiology studies evaluating associations of PFNA with health endpoints were reviewed and are discussed below. An individual table summarizing the design, study populations, outcomes and exposures, results, and limitations of each of these studies is found in Appendix 2. All of these studies, with the exception of one study of occupationally exposed workers evaluated the general population, and none of them were conducted in communities known to have drinking water contamination with PFNA.

The epidemiology studies evaluated associations of PFNA with serum lipids; metabolic parameters including diabetes; effects on the immune system, thyroid, and reproductive system; liver enzymes; birth outcomes; and several other endpoints. Associations of cancer with PFNA have been investigated in one study. The studies were conducted on populations in the U.S. as well as several Asian nations, several European nations, and Canada. Populations from different studies may not be comparable due to differences in age, pregnancy status, basis for enrollment, and PFNA exposure ranges (see below), as well as nationality. Summary tables for some health outcome categories are included below (Table 6A-G).

The range of serum levels in the general population studies that evaluated these effects is lower than those which may result from exposure to drinking water contaminated from PFOA. Although exposure to workers was higher, the occupational study is limited in its ability to detect associations for reasons discussed above. Therefore, the available data are not sufficient to evaluate the potential for PFNA to impact these parameters at higher exposures such as may occur from ingestion of contaminated drinking water.

PFCs other than PFNA, including PFOA, PFOS, and PFHxS, are ubiquitously present in the serum of the general population at concentrations generally higher than PFNA (Lau et al., 2012). A general issue in interpretation of epidemiology studies of PFNA is co-exposure to other PFCs, which in some instances were highly correlated with PFNA. Although some of the studies controlled for co-exposure to other PFCs when evaluating associations with PFNA, many studies did not include this adjustment, limiting their ability to determine the independent impact of PFNA.

General Population Studies

A strength of the general population studies is their use of PFNA serum levels as the basis for exposure assessment. Because of the long human half-life of PFNA, serum levels at a single time do not rapidly fluctuate with short term variations in exposure and reflect long-term exposures (see Toxicokinetics section above). Serum levels thus provide an accurate measure of internal exposure for each study participant, an advantage over studies based on external exposure metrics such as drinking water concentrations.

Of the general population studies, 28 were cross-sectional and one included a cross-sectional component. A general limitation of cross-sectional studies is that they evaluate information on both exposure and outcome at the same point in time, limiting their ability to establish temporality.

Occupational Studies

Occupational studies are often considered useful for evaluating effects of environmental contaminants because exposure levels are generally higher than in the general population or in communities exposed through site-specific environmental contamination. However, the sole occupational study of PFNA has several important limitations (Mundt et al., 2007). Importantly, Mundt et al. (2007) used work history rather than serum PFNA data for exposure classification. Potentially relevant to the evaluation of this study, PFOA serum levels in the least exposed groups of workers in some occupational studies of PFOA production workers were well above the general population range (reviewed in Post et al., 2012); this may also have been the case for PFNA exposures in the group classified as having no exposure by Mundt et al. (2007). Associations of PFOA, as well as PFOS, with some clinical parameters, including cholesterol and liver enzymes, exhibit a steep dose-response curve in the lower exposure range found in the general population with a plateau at higher exposures such as those found occupationally. For dose-response curves of this type, even the least exposed workers may have exposure levels that fall on the plateau portion of the dose-response curve. Thus, workers at a facility who are assumed to be non-exposed may not necessarily represent an appropriate group for comparison with more highly exposed groups of workers within a facility (Post et al., 2012). Other limitations of this study are discussed as relevant to specific endpoints, below.

Studies in Exposed Communities

To our knowledge, there have been no studies of populations exposed to PFNA through contaminated drinking water or other environmental media. It is relevant to note that extensive information from communities with drinking water contaminated with the closely related compound PFOA is available from the C8 Health Project. This is a community health study of approximately 70,000 Ohio and West Virginia residents with at least one year of exposure to drinking water contaminated with PFOA at $\leq$50 ng/L to over 3000 ng/L (Frisbee et al., 2009; C8 Science Panel, 2014; reviewed in Post et al., 2012).This study is notable because of its large size, the wide range of exposure levels, and the large number of parameters evaluated. Associations of PFOA serum concentrations were reported for a number of health endpoints, some of which have not been evaluated for PFNA in humans, including two types of cancer (Barry et al., 2013; Vieira et al., 2013). For other health endpoints, no associations were found in the C8 Health Project population. C8 Science Panel reports and citations for peer- reviewed publications presenting the results of these studies are found at the C8 Science Panel website (http://www.c8sciencepanel.org/).

Table 6A. Summary of findings from epidemiologic studies of PFNA and serum lipids

Citation	Study Population	Study Details	TC	HDL	Non-HDL	LDL	TG
Fu et al., 2014	China, random selection of attendees to health check-up clinic	**Study Design:* Cross-sectional **Study Size*: n=133 **Study Population Age*: 0-88 years **Exposure (Median)*: 0.37 ng/mL	↑[a]	—[a]		↑[a]	—[a]
Lin et al., 2009	General U.S. Population (NHANES, 99-2000 & 03-2004)	**Study Design:* Cross-sectional **Study Size*: adolescents – n=474 / adults – n=969 **Study Population Age*: 12-20 years; > 20 years **Exposure (Mean):* 0.70 ng/mL; 0.81 ng/mL		adolescents ↑[b] / adults —[b]			adolescents —[b] / adults —[b]
Lin et al., 2011	Individuals with abnormal urinalysis results from population-based screening program in Taiwan	**Study Design:* Cross-sectional **Study Size*: n=287 **Study Population Age*: 12-30 years **Exposure (Median):* 1.68 ng/mL		—[a]			—[a]
Lin et al., 2013a[a]	Individuals with abnormal urinalysis results from population-based screening program in Taiwan	**Study Design:* Cross-sectional **Study Size*: 664 (246 w/ elevated blood pressure and 398 w/ normal blood pressure) **Study Population Age*: 12-30 years **Exposure (Geo Mean)*: range 0.38-25.4 ng/ml, males – 1.19 ng/ml, females – 1.00 ng/ml				—	—[a]
Mundt et al., 2007	Occupational, U.S. factory	**Study Design:* Cross-sectional and retrospective cohort **Study Size*: n=592 **Study Population Age*: not stated **Exposure (Median)*: not available	↑-	—		—	—
Nelson et al., 2010	General U.S. Population (NHANES, 03-2004)	**Study Design:* Cross-sectional **Study Size*: n=416 to n=860 **Study Population Age*: <80 years **Exposure (Median):* 1.0 ng/mL	↑-	—	↑	—	
Starling et al., 2014a	Norway, pregnant women, 03-2004	**Study Design:* Cross-sectional **Study Size*: n=891 **Study Population Age*: not stated **Exposure (Median)*: 0.39 ng/mL	—[b]	↑[b]		—[b]	—[a,b]

↑ = statistically significant increased association, ↓ = statistically significant decreased association, ↑- = inconsistent positively associated finding (findings from different models resulted in both statistically and non-statistically significant associations), ↓- = inconsistent negatively associated finding, — = not statistically significant, [statistical significant determined at α=0.05]
TC= total cholesterol, HDL= high density lipoprotein cholesterol, LDL=low density lipoprotein cholesterol, TG=triglycerides
[a] Outcome log-transformed for use in linear regression; [b] Exposure log-transformed for use in linear regression

Table 6B. Summary of findings from epidemiologic studies of PFNA and select metabolic effects

Citation	Study Population	Study Details	Glucose	Insulin	HOMA	Diabetes	BMI
Halldorsson et al., 2012	Denmark, mother-offspring pairs – pregnant women recruited 88-1989	*_Study Design:_ Prospective birth-cohort *_Study Size_: n=345 *_Study Population Age_: not stated *_Exposure (Median)_: maternal 0.3 ng/mL					↑-
Lin et al., 2009	General U.S. Population (NHANES, 99-2000 & 03-2004)	*_Study Design:_ Cross-sectional *_Study Size_: adolescents: n=474/ adults: n=969 *_Study Population Age_: 12-20 years; > 20 years *_Exposure (Mean):_ 0.70 ng/mL; 0.81 ng/mL	adolescents —[b] / adults —[b]	adolescents ↓[a, b] / adults —[a, b]	adolescents —[a, b] / adults —[a, b]		
Lin et al., 2011	Individuals with abnormal urinalysis results from population-based screening program in Taiwan	*_Study Design:_ Cross-sectional *_Study Size_: n=287 *_Study Population Age_: 12-30 years *_Exposure (Median):_ 1.68 ng/mL	—	—[a]	—[a]		
Lin et al., 2013a[a]	Individuals with abnormal urinalysis results from population-based screening program in Taiwan	*_Study Design:_ Cross-sectional *_Study Size_: 664 (246 w/ elevated blood pressure and 398 w/ normal blood pressure) *_Study Population Age_: 12-30 years *_Exposure (Geo Mean)_: range 0.38-25.4 ng/ml, males – 1.19 ng/ml, females – 1.00 ng/ml			—[a]		—
Lind et al., 2014	Sweden, 2001-2004	*_Study Design:_ Cross-sectional *_Study Size_: n=1,016 *_Study Population Age_: >=70 years *_Exposure (Median)_: 0.7 ng/mL		—	—	↑-	
Nelson et al., 2010	General U.S. Population (NHANES, 03-2004)	*_Study Design:_ Cross-sectional *_Study Size_: n=416 to n=860 *_Study Population Age_: <80 years *_Exposure (Median):_ 1.0 ng/mL					—

↑ = statistically significant increased association, ↓ = statistically significant decreased association, ↑- = inconsistent positively associated finding (findings from different models resulted in both statistically and non-statistically significant associations), ↓- = inconsistent negatively associated finding, — = not statistically significant, [statistical significant determined at α=0.05]
Insulin= defined as proinsulin/insulin ratio or serum insulin, HOMA=homeostasis model assessment of insulin resistance, BMI= body mass index
[a] Outcome log-transformed for use in linear regression; [b] Exposure log-transformed for use in linear regression

Table 6C. Summary of findings from epidemiologic studies of PFNA and immune system outcomes

Citation	Study Population	Study Details	Vaccine Response	Common Cold:GI	Asthma	IM	AD	Wheeze
Dong et al., 2013	Taiwan, 2009-2010	*_Study Design:_ Case-control *_Study Size_: Asthmatics n=231 and non-asthmatics n=225 *_Study Population Age_: 10-15 years *_Exposure (Median)_ : Cases (1.0 ng/mL) and Controls (0.8 ng/mL)			↑	↑		
Granum et al., 2013	Norway, 2007-2008, recruitment from mother-child cohort	*_Study Design:_ Prospective birth cohort *_Study Size_: n=56-99 *_Study Population Age_: Children 3 years *_Exposure (Median):_ 0.3 ng/mL	↓-	Common Cold ↑- (GI) ——	—		—	—
Humblet et al., 2014	General U.S. Population (NHANES, 99-2000 & 03-2008)	*_Study Design:_ Cross-sectional *_Study Size_: n=1,877 *_Study Population Age_: 12-19 years *_Exposure (Median)_: Medians ranges: 0.8-0.9 ng/mL			—			—
Wang et al., 2011	Taiwan, children of pregnant women	*_Study Design:_ Prospective birth cohort *_Study Size_: n=244 *_Study Population Age_: Birth through 2 years *_Exposure (Mean):_ 2.30 ng/mL				—[a, b]	—	

↑ = statistically significant increased association, ↓ = statistically significant decreased association, ↑- = inconsistent positively associated finding (findings from different models resulted in both statistically and non-statistically significant associations), ↓- = inconsistent negatively associated finding, — = not statistically significant, [statistical significant determined at α=0.05]

IM= immunological markers which may include AEC (absolute eosinophil count, IgE (immunoglobulin E), and/or ECP (eosinophilic cationic protein); GI=gastrointestinal illness; AD= atopic dermatitis/eczema

[a] Outcome log-transformed for use in linear regression; [b] Exposure log-transformed for use in linear regression

Table 6D. Summary of findings from epidemiologic studies of PFNA and thyroid hormones and related outcomes. Page 1 of 2

Citation	Study Population	Study Details	TSH	TT4	FT4	TT3	TG	TD	Hypo	Hyper
Bloom et al., 2010	Subgroup of NY State sportfish anglers	*_Study Design:_ Cross-sectional *_Study Size_: n=31-38 *_Study Population Age_: 31-45 years *_Exposure (Geo Mean)_: 0.79 ng/mL	—[a,b]		—[b]					
Jain, 2013	General U.S. Population (NHANES, 07-2008)	*_Study Design:_ Cross-sectional *_Study Size_: n=1,733 *_Study Population Age_: > 12 years *_Exposure (Median)_: not presented	—[a,b]	—[a,b]	—[a,b]	—[a,b] (FT3): —[a,b]	—[a,b]			
Ji et al., 2012	Korea, recruited from cohort study	*_Study Design:_ Cross-sectional *_Study Size_: n=633 *_Study Population Age_: > 12 years *_Exposure (Mean):_ 2.09 ng/mL	—[a,b]	—[a,b]						
Kim et al., 2011b	South Korea, pregnant women from three clinics and paired infants	*_Study Design:_ Prospective birth cohort *_Study Size_: (pregnant): n=44 / (mother-infant pairs) n=26 *_Study Population Age_: >25 years *_Exposure (Median)_: 0.44 ng/mL /0.45 ng/mL	pregnant — / pairs —	pregnant — / pairs —		pregnant — / pairs —				
Lin et al., 2013b	Individuals with abnormal urinalysis results from population-based screening program in Taiwan	*_Study Design:_ Cross-sectional *_Study Size_: n=551 (221 with elevated BP) *_Study Population Age_: 12-30 years *_Exposure (Geo Mean)_: 1.01 ng/mL	—[a]		↑				—	
Lopez-Espinosa et al., 2012	Community- Based (C8 Health Project)	*_Study Design:_ Cross-sectional *_Study Size_: n=10,725 *_Study Population Age_: 1-17 years *_Exposure (Median):_ 1-4 to 1.8 ng/mL	—[a, b]	↑[a,b]				—	—	—
Mundt et al., 2007	Occupational, U.S. factory	*_Study Design:_ Cross-sectional *_Study Size_: n=592 *_Study Population Age_: not stated *_Exposure (Median_: not available	—	—	—	—				

Table 6D. Summary of findings from epidemiologic studies of PFNA and thyroid hormones and related outcomes. Page 2 of 2

Wang et al., 2013	Norway, Pregnant women from case-control study	**Study Design:* Cross-sectional **Study Size*: n=903 (400 subfecund, 550 controls) **Study Population Age*: 18-44 years **Exposure (Median)*: 0.51 ng/mL	— [a]					—		
Wang et al., 2014	Central Taiwan, pregnant woman population and neonates at birth	**Study Design:* Cross-sectional / Prospective birth cohort **Study Size*: (pregnant): n=285 / (neonate) n=116 **Study Population Age*: Mean age 28.8 years **Exposure (Median)*: M-1.51 ng/mL	pregnant — / neonate —	pregnant ↓ / neonate ↓	pregnant ↓ / neonate —	pregnant — / neonate ↓				
Webster et al., 2014	Canada, pregnant women in CHirP study	**Study Design:* Prospective birth cohort **Study Size*: n=152 **Study Population Age*: >18 years **Exposure (Median)*: 0.60 ng/mL	↑-	—	—					
Wen et al., 2013	General U.S. Population (NHANES, 07-2010)	**Study Design:* Cross-sectional **Study Size*: n=1,180 **Study Population Age*: >20 year **Exposure (Geo Mean)*: 1.54 ng/mL	— [a,b]	— [b]	— [a,b]	— [a,b]	— [a,b]		— [a,b]	— [a,b]

↑ = statistically significant increased association, ↓ = statistically significant decreased association, ↑- = inconsistent positively associated finding (findings from different models resulted in both statistically and non-statistically significant associations), ↓- = inconsistent negatively associated finding, — = not statistically significant, [statistical significant determined at α=0.05]

TSH=thyroid stimulating hormone, TT4=total thyroxine, FT4=free thyroxine, TT3=total triiodothyronine, FT3= free triiodothyronine, TG=thyroglobulin, TD=thyroid disease, Hypo=hypothyroidism, Hyper=hyperthyroidism

[a] Outcome log-transformed for use in linear regression; [b] Exposure log-transformed for use in linear regression

Table 6E. Summary of findings from epidemiologic studies of PFNA and reproductive outcomes. Page 1 of 2

Citation	Study Population	Study Details	Age @ M	Meno	Hyst	Eclp	Endo	SM	SDD	SQP
Christensen et al., 2011	United Kingdom, girls recruited from mothers in a cohort	*_Study Design:_ Nested case-control *_Study Size_: Cases n=218, controls n=230 *_Study Population Age_: 8-13 years *_Exposure (Median):_ 0.6 ng/mL	__ [b]							
Leter et al., 2014	Greenland, Poland, Ukraine, Male partners of pregnant women	*_Study Design:_ Cross-sectional *_Study Size_: n=262 *_Study Population Age_: > 18 years *_Exposure (Mean)_: Greenland (2.2 ng/ml), Ukraine (1.1 ng/ml), Poland (1.4 ng/ml)						__ [b]		
Louis et al., 2015	16 counties in Michigan and Texas, Male partners of couples planning pregnancy	*_Study Design:_ Cross-sectional *_Study Size_: n=501 *_Study Population Age_: Mean 31.8 years *_Exposure (Median)_: Michigan (1.0), Texas (1.65 ng/mL)								__ [b]
Louis et al., 2012	Two U.S. cities, women in operative sample (OS) and population-based sample (P)	*_Study Design:_ Case-control *_Study Size_: OS: case n=190, controls n=283, P: cases n=14, controls n=113 *_Study Population Age_: >18-44 years *_Exposure (Geo Mean)_: 0.58-0.71 ng/mL					↑- [b]			
Specht et al., 2012	Greenland, Poland, Ukraine, fertile male partners of pregnant women	*_Study Design:_ Cross-sectional *_Study Size_: n=604 *_Study Population Age_: >18 years *_Exposure (Median)_: Greenland (1.4), Ukraine (1.0), Poland (1.2)							__ [b]	
Starling et al., 2014b	Norway, pregnant women recruit from cohort	*_Study Design:_ Nested case-control *_Study Size:_ cases=466, controls n=510 *_Study Population Age_: 16-44 years *_Exposure (Median)_: 0.54 ng/mL				__ [b]				

Table 6E. Summary of findings from epidemiologic studies of PFNA and reproductive outcomes. Page 2 of 2

Citation	Study Population	Study Details	Age @ M	Meno	Hyst	Eclp	Endo	SM	SDD	SQP
Taylor et al. 2014	General U.S. Population - women (NHANES, 99-2000 & 03-2010)	**Study Design:* Cross-sectional **Study Size*: n=2,732 **Study Population Age*: 20-65 years **Exposure (Median)*: pre-meno (0.90), meno (1.20), hyster (1.30 ng/mL)		↑	↑					
Toft et al., 2012	Greenland, Poland, Ukraine, fertile male partners of pregnant women	**Study Design:* Cross-sectional **Study Size*: n=588 **Study Population Age*: > 18 years **Exposure (Medians)*: Greenland (1.7), Ukraine (1.0), Poland (1.2 ng/mL)								—[b]

↑ = statistically significant increased association, ↓ = statistically significant decreased association, ↑- = inconsistent positively associated finding (findings from different models resulted in both statistically and non-statistically significant associations), ↓- = inconsistent negatively associated finding, — = not statistically significant, [statistical significant determined at α=0.05]

Age @ M=age at menarche, Meno=menopausal status, Hyst=hysterectomy, Eclp=preeclampsia, Endo=endometriosis , SM=sperm methylation. SDD= sperm DNA damage, SQP=semen quality parameters

[a] Outcome log-transformed for use in linear regression; [b] Exposure log-transformed for use in linear regression

Table 6F. Summary of findings from epidemiologic studies of PFNA and neurobehavioral outcomes

Citation	Study Population	Study Details	ADHD	IRI	MI	AB
Braun et al., 2014	Cincinnati OH, mother-child pairs	*_Study Design:_ prospective birth cohort *_Study Size_: n=175 *_Study Population Age_: pregnant women, children followed till age 5 *_Exposure (Mean):_ 0.90 ng/mL				—[b]
Gallo et al., 2013	Community- Based (C8 Health Project)	*_Study Design:_ Cross-sectional *_Study Size_: n-21,024 *_Study Population Age_: > 50 years *_Exposure_: (40^{th} percentile) = 1.0-1.2 ng/mL; (60^{th} percentile)=1.3-1.4 ng/mL			—[b]	
Gump et al., 2011	Oswego County, NY - Subset of children recruited from mailed invitation	*_Study Design:_ Cross-sectional *_Study Size_: n=83 *_Study Population Age_: 9-11 years *_Exposure (Median)_: 0.82 ng/mL		—[a, b]		
Hoffman et al., 2010	General U.S. Population Children, (NHANES, 99-2000 & 03-2004)	*_Study Design:_ Cross-sectional *_Study Size_: n=571 *_Study Population Age_: 12-15 years *_Exposure (Median):_ 0.6 ng/mL	—			
Ode et al., 2014	Sweden, children with ADHD and matched controls selected from study base	*_Study Design:_ Matched case-control *_Study Size_: cases n=206, controls n=206 *_Study Population Age_: children *_Exposure (Mean):_ not provided	—			
Power et al., 2013	General U.S. Population Children, (NHANES, 99-2000 & 03-2008)	*_Study Design:_ Cross-sectional *_Study Size_: n=1,766 *_Study Population Age_: 60-85 years *_Exposure (Geo Mean):_ 1.01 ng/mL			—[b]	

↑ = statistically significant increased association, ↓ = statistically significant decreased association, ↑- = inconsistent positively associated finding (findings from different models resulted in both statistically and non-statistically significant associations), ↓- = inconsistent negatively associated finding, — = not statistically significant, [statistical significant determined at α=0.05]
ADHD=attention deficient/Hyperactivity Disorder, IRI=impaired response inhibition, MI=memory impairment, AB=autistic behaviors
[a] Outcome log-transformed for use in linear regression; [b] Exposure log-transformed for use in linear regression

Table 6G. Summary of findings from epidemiologic studies of PFNA and liver enzymes, bilirubin, and uric acid

Citation	Study Population	Study Details	ALT	GGT	AST	ALP	TB	UA
Gleason et al., 2015	General U.S. Population Children, (NHANES, 2007-2010)	*_Study Design:_ Cross-sectional *_Study Size_: *_Study Population Age_: ≥ 12 years *_Exposure (Median):_ 1.4 ng/ml	↑ [a,b]	↑- [a,b]	— [a,b]	— [a,b]	— [a,b]	↑ [b]
Lin et al., 2010	General U.S. Population (NHANES, 99-00 & 03-04)	*_Study Design:_ Cross-sectional *_Study Size_: n=2,216 *_Study Population Age_: ≥ 18 years *_Exposure (Mean)_: 0.8ng/ml	— [b]	— [a,b]			↑ [a,b]	
Lin et al., 2013a	Individuals with abnormal urinalysis results from population-based screening program in Taiwan	*_Study Design:_ Cross-sectional *_Study Size_: n=664 (246 with elevated BP) *_Study Population Age_: 12-30 years *_Exposure_: (≤ 60th percentile) = 1.58 ng/mL; (> 90th percentile)=6.78 ng/mL						—
Mundt et al., 2007	Occupational, U.S. factory	*_Study Design:_ Cross-sectional *_Study Size_: n=592 *_Study Population Age_: not stated *_Exposure (Median)_: not available	↑	—	—	—	—	—

↑ = statistically significant increased association, ↓ = statistically significant decreased association, ↑- = inconsistent positively associated finding (findings from different models resulted in both statistically and non-statistically significant associations), ↓- = inconsistent negatively associated finding, — = not statistically significant, [statistical significant determined at α=0.05]
ALT=alanine aminotransferase, GGT=gamma-glutamyl transferase, AST=aspartate aminotransferase, ALP=alkaline phosphatase, TB=total bilirubin or unspecified bilirubin, UA=uric acid
[a] Outcome log-transformed for use in linear regression; [b] Exposure log-transformed for use in linear regression

Serum Lipids

Associations of PFNA with serum lipids were evaluated in six cross-sectional studies from the general population (Fu et al., 2014; Lin et al., 2009; Lin et al., 2011; Lin et al., 2013a; Nelson et al., 2010; Starling et al., 2014a) and one occupational study which that included both cross sectional and retrospective cohort design components (Mundt et al., 2007). General populations studied included the general U.S. population (Lin et al., 2009; Nelson et al., 2010), a random selection of health clinic attendees in China (Fu et al., 2014), individuals with abnormal urinalysis results in Taiwan (Lin et al., 2013a), and pregnant women in Norway (Starling et al., 2014). In these general population studies, median or geometric means for PFNA serum levels ranged from 0.37 ng/L to 1.68 ng/L; PFNA serum level data was not provided in the occupational study (Mundt et al., 2007). Parameters evaluated in one or more of these studies were total cholesterol, high density lipoprotein cholesterol (HDL), non-HDL, low density lipoprotein cholesterol (LDL), and triglycerides. Study details are provided in the tables for individual studies (Appendix 2) and the summary table for serum lipids above (Table 6A).

Three of four cross-sectional studies evaluating total cholesterol found evidence of a positive association with PFNA (Fu et al., 2014; Nelson et al., 2010; Mundt et al., 2007) while the fourth study, in pregnant women, found no significant association (Starling et al., 2014). In the two cross-sectional studies that reported positive associations and provided PFNA serum data, there was an increasing trend of serum PFNA with total cholesterol (Fu et al., 2014; Nelson et al., 2010). The occupational study additionally included a component employing a retrospective cohort design that evaluated men only but found no association with total cholesterol (Mundt et al., 2007).

The results of six studies evaluating high density lipoprotein cholesterol (HDL) and PFNA were inconsistent; one study found a positive association among adolescents but not among adults (Lin et al., 2009), one found a positive association in pregnant women (Starling et al., 2014a) and four others found no association (Nelson et al., 2010; and Mundt et al., 2007). HDL was the only lipid-related endpoint that was analyzed in both studies of the general U.S. population, and both studies consistently found no association of PFNA and HDL among adults (Lin et al., 2009; Nelson et al., 2010). The results of six studies evaluating triglycerides and PFNA all found no evidence of an association (Fu et al., 2014; Lin et al., 2009; Lin et al., 2011; Lin et al., 2013a; Mundt et al., 2007; and Starling et al., 2014a).

Four of five studies evaluating low density lipoprotein cholesterol (LDL) and PFNA did not find associations (Lin et al., 2013; Nelson et al., 2010; Starling et al., 2014a; and Mundt et al., 2007), while one study found a positive association (Fu et al., 2014).

Selection bias may be an issue in Fu et al. (2014) since the study included only individuals attending a health clinic check-up such that individuals concerned with existing health issues may be more likely to be included. This may also be true in Lin et al. (2013a), which included individuals with an abnormal urinalysis from a population-based screening program in which the final study population was made up of 246 (37%) individuals with elevated blood pressure. Information bias is unlikely to have an impact in the general population studies which relied on serum concentrations and clinical biomarkers. In contrast, Mundt et al. (2007) relied on medical

record abstraction of clinical parameters and work histories for exposure classification. Other limitations of Mundt et al. (2007) include small sample size that may limit power to detect associations, possibility of healthy worker effect, and the possibility that exposure in the least exposed groups may be well above the population exposure range in occupationally exposed individuals.

The epidemiologic data provide evidence of consistency, specificity, and exposure-response for PFNA and increased total cholesterol, although data on temporal relationship and strength of an association are limited. The possibility that PFNA causes increased cholesterol is further supported by evidence from epidemiology studies of PFOA, a closely related compound with similar toxicological effects. The epidemiology database for PFOA includes multiple studies of different designs in the general population, communities with drinking water exposure, and workers with occupational exposure, and suggests that a causal relationship may exist between PFOA and increased cholesterol (reviewed by Post et al., 2012; ECHA, 2014b).

The available epidemiological evidence does not support an association with PFNA and other serum lipid outcomes including HDL, LDL, and triglycerides.

Mode of action studies relevant to effects of PFNA on cholesterol and other lipid endpoints are discussed in the Mode of Action section (below).

Metabolic Parameters

Four cross-sectional studies (Lin et al., 2009; Lin et al., 2011; Lind et al., 2014; and Nelson et al., 2010) evaluated the association of PFNA with one or more metabolic parameters: glucose, insulin, the homeostasis model assessment of insulin resistance (HOMA), clinically diagnosed diabetes, and BMI. The study populations evaluated in these studies may not be comparable since these four studies differed in the ages, nationalities, and enrollment basis for their study populations; the ranges of serum PFNA levels; and the endpoints evaluated. Serum PFNA levels were similar in two studies of the general U.S. population (Lin et al., 2009; Nelson et al., 2010) and elderly individuals in the general Swedish population (Lind et al., 2013), and were relatively higher in a Taiwanese study participants with abnormal urinalysis results (Lin et al., 2013). Serum levels of maternal participants in a Danish prospective birth-cohort of mother-offspring pairs were several-fold lower than in the other studies (Halldorsson et al., 2012). Study details are provided in the tables for individual studies (Appendix 2) and summary table for metabolic endpoints (Table 6B).

Statistically significant results in the four cross sectional studies included a negative association with insulin and PFNA among 12- 20 year olds (but not among those greater than 20 years of age) in the U.S. general population (Lin et al., 2009), and a positive association with diabetes and PFNA among the elderly in Sweden when the outcome was treated as a quadratic term, but not when treated linearly (Lind et al., 2014). The association of insulin with PFNA had a linear coefficient of small magnitude (Lin et al., 2009), and the association with diabetes had a small effect estimate with a wide confidence range (OR=1.25, 95% CI 1.08-1.44; Lind et al., 2014). As summarized in Table 6B, no associations were found for insulin in two other cross-sectional

studies, or for glucose in any of the three cross-sectional studies in which it was evaluated; diabetes was not assessed in any of the other studies.

Three studies evaluated the relationship of PFNA and BMI. A cross-sectional study of the U.S. general population found no evidence of an association with this endpoint (Nelson et al., 2010) and a Danish prospective birth cohort study, based on maternal PFNA serum levels for subjects evaluated in young adulthood, found an inconsistent positive association (Halldorsson et al., 2012), and a study of young adults with abnormal urinalysis results found no association (Lin et al., 2013a). In Halldorsson et al. (2012), PFNA serum levels were several-fold lower than in the other studies of metabolic effects and fell within a narrow range, and PFNA was correlated with PFOA, which was found at much higher levels in serum. When PFOA was controlled for in an analysis of BMI and PFNA, the association became insignificant (Halldorsson et al., 2012). Five studies evaluated the relationship of PFNA and HOMA and found no association; all were cross-sectional (Lin et al., 2009; Lin et al., 2011; Lin et al., 2013a; Lind et al., 2014; and Nelson et al., 2010).

Selection bias may be an issue in Lin et al. (2011) which included individuals with an abnormal urinalysis from a population-based screening program. Also losses due to follow-up in Halldorsson et al. (2012), a prospective birth cohort, may present bias. Information bias in unlikely to have an impact in these studies which relied mostly on serum concentrations and clinical biomarkers.

The small number of studies that evaluated concurrent exposure to PFNA and metabolic effects provide only limited evidence of associations of PFNA with these endpoints. The sole study that evaluated prenatal exposure suggested a potential association with PFNA and metabolic effects, but limitations in this study impact the ability to determine the independent impact of PFNA. The results of this study are not sufficient to develop conclusions about the potential for early life exposure to PFNA to cause these effects in adulthood.

Parameters related to glucose metabolism were affected by PFNA in mice (Fang, 2012a). These studies are discussed in the Animal Toxicology and Mode of Action sections below.

Immune System Outcomes

Information on the effects of PFNA on immune system outcomes in human observational studies comes from a case-control study (Dong et al., 2013), two prospective birth cohorts (Granum et al., 2013 and Wang et al., 2011) and a cross-sectional study (Humblet et al., 2014). Study details are provided in the tables of individual studies (Appendix 2) and summary table of immune system outcomes above (Table 6C).

Epidemiologic information on the effects of PFNA on human immune system outcomes is limited. Three studies evaluated PFNA and asthma among adolescents; a case-control study in Taiwan which found a positive significant association (Dong et al., 2013), a small (n=55 to 99) prospective birth cohort which found no association (Granum et al., 2013), and a larger cross-sectional study of the general U.S. population which found no association (Humblet et al., 2014). Two studies assessed wheeze and found no association (Granum et al., 2013 and Humblet et al.,

2014). The Taiwanese case-control study additionally assessed immunological markers and found a positive association, while a prospective birth cohort of children of pregnant women in Taiwan found no association with either immunological markers or atopic dermatitis (Dong et al., 2013 and Wang et al., 2011, respectively). Granum et al.(2013) also found no association with atopic eczema. The results of these studies do not provide evidence of associations with PFNA and the outcomes evaluated. Although some studies found significant associations, findings from additional studies were inconsistent.

A prospective birth cohort study in Norway found evidence of decreased response to rubella vaccine but not three other vaccines, inconsistent evidence of positive association with the common cold depending on which age period was evaluated, and no association with gastrointestinal illness (Granum et al., 2013). In this study, the association with decreased vaccine response was stronger for PFNA than for three other PFCs (PFOA, PFOS, and PFHxS); serum levels of PFOS and PFOA were 4 to 14 fold higher than PFNA. Other studies that have found associations with other PFCs and decreased vaccine response in children (Grandjean et al., 2012) and adults (Looker et al., 2013) did not evaluate PFNA. Information from the single study that evaluated PFNA is not sufficient to conclude whether or there is an association of PFNA and vaccine response.

Selection bias may influence estimates in Dong et al. (2013) case-control study. Losses due to follow-up may also influence findings in prospective birth cohorts (Granum et al., 2013; and Wang et al., 2011). Information bias may be present due to parental response to questionnaire-based outcome assessments.

PFNA and other biologically persistent PFCs, including PFOA and PFOS, cause immunotoxicity in animal toxicology studies (Lau et al., 2012). The studies which evaluated PFNA are discussed in the Animal Toxicology and Mode of Action sections below.

Thyroid hormones and other related outcomes

Studies of thyroid hormones and related outcomes including hypo- and hyperthyroidism and thyroid disease include seven general population cross-sectional studies (Bloom et al., 2010; Jain, 2013; Ji et al., 2012; Lin et al., 2013b; Lopez-Espinosa et al., 2012; Wang et al., 2013; and Wen et al., 2013), two prospective birth cohort studies (Kim et al., 2011b and Webster et al., 2014), one study which presents findings from a cross-sectional and prospective birth cohort study designs (Wang et al., 2014), and one occupational cross-sectional study (Mundt et al., 2007). These four studies differed in the age, nationality, and enrollment basis for their study populations; their ranges of serum PFNA levels; and the endpoints evaluated. Study details are provided in the tables of individual studies (Appendix 2) and the summary table for thyroid effects and other related outcomes (Table 6D).

All 11 studies evaluated associations with an increase of thyroid stimulating hormone (TSH). Only one study found limited evidence of a positive association with TSH (Webster et al., 2014) while the 10 other studies found no evidence of an association. Of the eight studies that evaluated total thyroxine (TT4), six found no association (Jain, 2013; Ji et al., 2012; Kim et al., 2011b; Mundt et al., 2007; and Webster et al., 2014), one found a negative association (Wang et

al., 2014a), and one found a positive association (Lopez-Espinosa et al., 2012). Five studies found no association with free thyroxine (FT4) (Bloom et al., 2010; Jain, 2013; Mundt et al., 2007; and Webster et al., 2014), one study found a positive association (Lin et al., 2013b), and in a report which included two separate studies, no association was found among neonates in a prospective cohort study and a negative association was found in pregnant women in a cross-sectional analysis (Wang et al., 2014). Hypothyroidism was evaluated in three of the 11 studies, and additional endpoints (free triiodothyronine, thyroglobulin, hyperthyroidism, and unspecified thyroid disease) were each evaluated in only one study; no associations were found for any of these endpoints.

Selection bias may be an issue in Lin et al. (2013b) which included individuals with an abnormal urinalysis from a population-based screening program. Information bias is unlikely to have an impact in these studies which relied mostly on serum concentrations of exposure and outcomes. Although, serum thyroid measures are collected at a single time point in many studies, the measures are maintained over time. Also reliance on recall for studies assessing thyroid disease, hypo-, and hyperthyroidism may bias results (Lopez-Espinosa et al., 2012). Small sample sizes in some studies may have limited their power to detect associations (Bloom et al., 2010; Kim et al., 2011b; Mundt et al., 2007; Webster et al., 2014).

In summary, the results of the available epidemiologic studies generally do not provide evidence of associations with PFNA and thyroid hormones. The limited evidence from epidemiological studies of PFNA and thyroid disease does not support associations with these endpoints.

An *in vitro* study that evaluated the effects of PFNA on thyroid-related endpoints (Long et al., 2014) is discussed in the Mode of Action section, below.

Reproductive Outcomes

Although a variety of reproductive outcomes have been evaluated in epidemiology studies of PFNA, data for each of these outcomes is very limited. Only one epidemiology study evaluated each of the following endpoints: age at menarche (Christensen et al., 2011), menopausal status (Taylor et al., 2014), hysterectomy (Taylor et al., 2014), preeclampsia (Starling et al., 2014b), endometriosis (Louis et al., 2012), sperm methylation (Leter et al., 2014), and sperm DNA damage (Specht et al., 2012). Semen quality parameters were the only outcome assessed in two studies (Louis et al., 2015; Toft et al., 2012). Study details are provided in the tables summarizing individual studies (Appendix 2) and summary table for reproductive outcomes (Table 6E).

There was a positive association for menopause and hysterectomy in a cross-sectional study of the U.S. population (Taylor et al., 2014) and minimal and inconsistent evidence of an association with endometriosis in a case-control study in two U.S. cities (Louis et al., 2012). As discussed in the Excretion subsection of the Toxicokinetics section (above), blood loss is an excretion route for PFNA and other PFCs. Because higher PFCs were associated with both natural menopause and hysterectomy, the authors conclude that the increased serum levels of PFNA with earlier menopause may be a result of reverse causality since menstruation is no longer an excretion pathway for PFNA.

Information bias is unlikely to have an impact in these studies which relied mostly on serum concentrations of exposure and outcomes.

As above, the range of serum levels in the general population studies assessing these reproductive endpoints is lower than exposure ranges which may result from exposure to drinking water contaminated from PFNA, limiting ability to assess potential associations with reproductive outcomes occurring at higher exposures ranges of PFNA.

In summary, the database for PFNA and each individual reproductive effect is very limited. Except for a minimal and inconsistent association with endometriosis in one study and an association with menopause likely due to reverse causality in another study, no associations with PFNA were found for the reproductive endpoints that were evaluated.

Studies that evaluated reproductive effects of PFNA in animals are discussed in the Animal Toxicology section (below).

Neurobehavioral Outcomes

Studies providing epidemiologic evidence of associations of PFNA with neurobehavioral outcomes include a cross-sectional study and a matched case-control study of attention deficit/hyperactivity disorder (ADHD) (Hoffman et al., 2010; Ode et al., 2014), a cross-sectional study of impaired response inhibition (Gump et al., 2011), two cross-sectional studies of memory impairment (Gallo et al., 2013; Power et al., 2013), and a prospective birth cohort study of autistic behaviors (Braun et al., 2014). Study details are provided in the tables for individual studies (Appendix 2) and summary table for neurobehavioral outcomes above (Table 6F).

Neither a matched case-control study in Sweden (Ode at el., 2014) nor a cross-sectional study in the U.S. general population (Hoffman et al., 2010) found evidence of an association with PFNA and ADHD. A cross-sectional study assessed impaired response inhibition and found a negative association with total PFC serum concentration and certain other PFCs but findings for the effect of PFNA alone were inconsistent (Gump et al., 2011). There is no further evidence of an association with memory impairment in two cross-sectional studies (Gallo et al., 2013 and Power et al., 2013), or autistic behaviors in a prospective birth cohort (Braun et al., 2014).

Information bias is possible in some studies due to self-reporting errors or parental report on outcome classification (Gallo et al., 2013; Hoffman et al., 2010; Power et al., 2013). Small sample sizes in some studies may limit the power of some studies to detect an association (Braun et al., 2014; Gump et al. 2011; Ode et al., 2014).

The limited epidemiologic evidence provides minimal evidence of associations of PFNA and neurobehavioral outcomes.

The potential for PFNA to cause neurobehavioral effects in animals has not been thoroughly evaluated, particularly as related to effects resulting from prenatal or early life exposures (see Discussion of Uncertainties, below).

Birth Outcomes

Evidence describing the associations of PFNA with birth outcomes is available from three prospective birth cohort studies. A nested prospective birth cohort (Monroy et al., 2008) evaluated birth weight, and gestational length. A prospective birth cohort study (Chen et al., 2012) assessed gestational age, birth weight, birth length, head circumference, ponderal index, preterm birth, low birth weight, and small for gestational age and another prospective birth cohort study (Kim et al., 2011b) evaluated birth weight. Serum PFNA concentrations varied over a 5-fold range among the study populations in the three studies, with geometric mean in cord blood of 2.36 ng/mL in Chen et al. (2012), and medians in cord blood of 0.94 ng/mL in Monroy et al. (2008) and 0.45 ng/ml in Kim et al. (2011b). Additional study details are provided in the tables summarizing individual studies (Appendix 2).

The three studies which evaluated PFNA and birth outcomes did not find associations with birth weight. It is relevant to note that these three studies also did not find associations of PFOA and decreased birth weight, inconsistent with the general body of data on this issue, suggesting that their ability to detect associations with PFCs may have been limited. Two recent systematic reviews evaluated the numerous studies of associations of serum PFOA and fetal growth, as indicated by birth weight and other parameters (Johnson et al., 2014; Bach et al. 2015). Both reviews found that PFOA was associated with decreased average birth weight in most studies, and Johnson et al. (2014) concluded that the overall body of data from human studies is sufficient to conclude that decreased fetal growth is associated with PFOA exposure. The only study that evaluated birth length and decreased ponderal index found small but significant increases (Chen et al., 2012). Small sample sizes in these studies limited the ability to form conclusions on the impacts of PFNA and other PFCs.

PFNA causes adverse effects on developmental endpoints, including neonatal mortality and postnatal growth and development, in animals. These studies and data relevant to potential modes of action for these effects are discussed in the Animal Toxicology and Mode of Action sections, below.

Liver Enzymes and Bilirubin

Three cross-sectional epidemiologic studies evaluated the association of PFNA and liver enzymes and/or bilirubin. Lin et al. (2010) assessed the associations of PFNA in the general U.S. population with the liver enzymes, ALT and GGT, and total bilirubin and found a positive association only with total bilirubin. Gleason et al. (2015) assessed the same parameters as Lin et al. (2010) as well as the enzymes, AST and ALP, also in the U.S. general population, and found a positive association with PFNA and ALT and limited evidence of an association with GGT. Mundt et al. (2007) evaluated AST, ALT, bilirubin, GGT, and ALP and found evidence of a positive association with ALT among occupationally exposed study participants. Serum PFNA concentrations in the two studies of the U.S. general population differed (Lin et al., 2010: median=0.7 ng/ml; Gleason et al., 2015: median=1.4 ng/ml), while Mundt et al. (2007) did not provide information on serum concentrations. Additional study details are provided in the tables summarizing individual studies (Appendix 2) and summary table of findings (Table 6G).

These results provide limited evidence of an association with ALT and PFNA. Associations of the closely related compound, PFOA, and ALT were also found in the U.S. general population, communities with drinking water exposure, and occupationally exposed workers (reviewed in Post et al., 2012). The limited epidemiological information that is available did not find associations for PFNA and AST, bilirubin (total), GGT, or ALP.

Information bias is unlikely to have an impact in the general population studies which relied on serum concentrations and clinical biomarkers. In contrast, Mundt et al. (2007) relied on medical record abstraction of clinical parameters and work histories for exposure classification. Other limitations of Mundt et al. (2007) include small sample size that may limit power to detect associations, possibility of healthy worker effect, and the possibility that exposure in the least exposed groups may be well above the population exposure range in occupationally exposed individuals.

The hepatic toxicity of PFNA and other PFCs in animals is well established (Lau et al., 2012). Hepatic toxicity of PFNA in animals and potential modes of action for these effects are discussed in the Animal Toxicity and Mode of Action sections, below.

Other outcomes

Cross-sectional studies evaluating associations of PFNA with several additional outcomes have been reported. Study details are provided in the tables for individual studies (Appendix 2).

Uric acid

Three studies evaluated PFNA and uric acid. There was evidence of a positive association of PFNA with a small increase in uric acid in a cross-sectional study of the U.S. general population (Gleason et al., 2015), but no such association was found in an occupational study (Mundt et al., 2007) or in a cross-sectional study of a Taiwanese study group with previously abnormal urinalysis results (Lin et al., 2013a). Elevated levels of uric acid are associated with a variety of health outcomes, and increased uric acid has been associated with PFOA (Gleason et al., 2015).

Estimated glomerular filtration rate (eGFR)

Watkins et al. (2013) found an association of PFNA and estimated glomerular filtration rate eGFR), a measure of kidney function, in a cross-sectional study of children from a community with elevated PFOA exposures from contaminated drinking water. This association likely results, at least in part, from reverse causality since PFNA is excreted by the kidney, so its elimination is slower when kidney function is impaired.

Carotid artery intima-media thickness

A cross-sectional study in a Taiwanese population with abnormal urinalysis results did not find an association with PFNA and carotid artery intima-media thickness (CIMT) a marker of subclinical atherosclerosis (Lin et al., 2013a). Selection bias may be present in this study which included individuals with an abnormal urinalysis from a population-based screening program in and which the final study population was made up of 246 (37%) individuals with elevated blood pressure.

LINE-1 DNA methylation LINE-1 DNA methylation

A cross-sectional study of U.S. adults from a community with elevated PFOA exposures from contaminated drinking water found no association of PFNA with % LINE-1 DNA methylation in leukocytes (Watkins et al., 2014). This endpoint has been linked to genomic instability, risk of cancer, cerebrovascular outcomes, and serum lipids.

Cancer

Epidemiologic evidence of PFNA and cancer is available from only one case-control study of prostate cancer (Hardell et al., 2014). This study found no association of PFNA and prostate cancer. Details are provided in the table for this study in Appendix 2.

As discussed in the Animal Toxicology and Discussion of Uncertainty sections (below), the carcinogenic potential of PFNA has not been evaluated in animals.

Summary of epidemiological information

Of the endpoints evaluated in the studies reviewed above, the evidence for association with PFNA is strongest for serum cholesterol and ALT. These associations with PFNA are consistent with similar associations for these parameters in most, but not all studies, of PFOA and PFOS - two PFCs that have been more widely studied than PFNA. Causality cannot be proven for the associations that were reported since they primarily come from cross-sectional studies. Therefore, human data were not used as the basis for the quantitative risk assessment. Because human epidemiology data were not used as the primary basis for risk assessment, a formal weight of evidence evaluation of causality for the human studies was not conducted.

For many of the other endpoints that were evaluated, minimal or no evidence was found for associations with PFNA. For many of these endpoints, the epidemiological data are limited to one or very few studies and are not sufficient to make general conclusions about potential effects of PFNA. For other endpoints such as thyroid hormones, a larger number of studies are available. Some of the endpoints for which no associations were found for PFNA have been associated with other PFCs, including PFOA and PFOS (reviewed by Lau et al., 2012; Post et al., 2012; Gleason et al., 2015). Results of animal studies and mode of action studies for these PFCs suggest that they cause similar toxicological effects as PFNA. In evaluating the differing results for PFNA and other PFCs, a potentially important consideration is that serum levels of PFNA are lower than for PFOA and PFOS in the general population studies. Additionally, as discussed above, the range of serum PFNA levels in the general population studies is lower than those which would result from exposure to drinking water contaminated from PFOA. There is no information on the potential for PFNA to impact these health endpoints in communities with higher exposures from contaminated drinking water.

The health effects of prenatal and early life exposures to environmental contaminants in adulthood are a current focus of environmental health research (Heindel and Vandenberg, 2015). Only one epidemiology study in which PFNA serum levels were low and fell within a small range, investigated effects of prenatal exposure to PFNA on metabolic effects in early adulthood. Additional research is needed to evaluate the potential of developmental exposures to PFNA to impact health later in life.

Animal Toxicology

Overview

Peer-reviewed toxicology studies of oral or inhalation exposure to PFNA include ten short term (14 day) oral studies, four oral reproductive/developmental studies, and one oral subchronic study, all conducted in rodents, and an acute inhalation study in rats (Kinney et al., 1989). Each of these studies, with the exception of two that assess only mode of action endpoints (Fang, 2012b, c), is summarized in a separate table in Appendix 3. Summary tables of information on those endpoints of toxicity for which there is sufficient information are included below (Tables 7A-F).

The 14 day studies include seven in male rats (Fang et al., 2009; 2010; 2012 a, b, c; Feng et al. , 2009, 2010), two in male mice (Fang et al., 2008; Wang et al., 2015) and one in both sexes of mice (Kennedy et al., 1987). These short term studies focused on specific endpoints of toxicity (e.g. liver, immune system, or testicular), and many of them include mechanistic components intended to elucidate the mode(s) of action for the observed effects. The rat subchronic study (Mertens et al., 2010) used Surflon S-111, a mixture of PFCs consisting primarily of PFNA (see below).

The reproductive/developmental studies include a two-generation study in rats dosed with Surflon S-111 (Stump et al., 2008), and studies of gestational exposure to PFNA in CD-1 mice (Das et al., 2015), wild type and PPAR-alpha knockout mice (Wolf et al., 2010), and rats (Rogers et al., 2014).

Other PFNA toxicology studies include five rodent studies using dosing via intraperitoneal injection (Goecke-Flora and Reo, 1996; Kudo et al., 2000, 2001, 2003, 2006; Rockwell et al., 2013), and a 7 day mouse oral gavage study which is available as an abstract (Das et al., 2013). Additionally, results of an unpublished study of Surflon S-111 that included a higher dose than the published studies (Wolterbeek, 2004) are discussed by Stump et al. (2008) and Mertens et al. (2010). These studies add to the body of knowledge of effects of PFNA and are discussed in the text. However, they were not considered for use as the basis for the risk assessment because the route of administration is not relevant to environmental exposure or because they were not published in peer reviewed form. For this reason, they are not included in individual study tables (Appendix 3) or the summary tables (Table 7).

Importance of serum PFNA data in interpretation of toxicology studies

Because the half-life of long-chain PFCs such as PFNA is much longer in humans (several years) than in rats and mice (days to weeks), a given administered dose (mg/kg/day) results in a much greater body burden (as indicated by serum level) in humans than in these animal species. Therefore, comparisons between effect levels in animal studies and human exposures are made on the basis of serum levels rather than administered dose.

Most of the PFNA toxicology studies do not provide serum PFNA data. PFNA serum levels were measured at the same time point as when endpoints of toxicity were assessed in two mouse studies, the developmental study of Das et al. (2015) and the 14 day study of Wang et al. (2015). In Das et al. (2015) the serum data are presented graphically, and the numerical

data used to generate the graphs, including statistical parameters, were provided to the Health Effects Subcommittee. Wang et al. (2015) provides numerical serum PFNA data but does not include statistical parameters for these data.

The only other toxicology studies in which serum PFNA data were reported are the developmental toxicity study in wild type (WT) and PPAR-alpha knockout (KO) mice (Wolf et al., 2010) and the studies of the Surflon S-111 PFC mixture consisting primarily of PFNA (Stump et al., 2008; Mertens et al., 2010).

In Wolf et al. (2010), serum PFNA levels were measured 23 days after dosing ended. At this time point, a considerable portion of the PFNA had been excreted, with additional loss of PFNA through transfer to breast milk in the lactating dams. Since these serum levels were not taken at the end of the dosing period, they are not indicative of the maximum exposure levels which may have caused toxicity.

Mertens et al. (2010) present serum PFC levels over time in each dosed group in graphs but do not provide the numerical data that are needed for dose-response modeling. Additionally, the data for the lower serum levels cannot be accurately estimated due to the scale of the graphs. Stump et al. (2008) provides only area under the curve graphs for serum levels of the Surflon S-111 mixture, and no serum data is presented for individual PFCs. Numerical serum PFNA data from these studies has been requested from the sponsors of these studies but has not been provided to date.

Issues related to interpretation of Surflon S-111 studies

The oral subchronic rat study (Mertens et al., 2010) and two-generation oral rat study (Stump et al., 2008), used Surflon S-111 (CAS # 72968-38-8), a commercial mixture of linear perfluorinated carboxylic acids containing primarily PFNA. These two studies are the only available studies with dosing for more than 18 days.

Estimation of PFNA doses

The specific composition of the Surflon S-111 used in these studies is not reported; this information has been requested but not provided from the study sponsors to date. The composition of Surflon S-111 by weight was reported by Prevedouros et al. (2006) as PFNA, 74%; perfluoroundecanoic acid (C11), 20%; perfluorotridecanoic acid (C13), 5%; PFOA (C8), 0.78%; perfluorodecanoic acid (C10), 0.37%; and perfluorododecanoic acid (C12), 0.1%. This composition is assumed in the evaluation of the two studies.

In these two studies, the daily doses of PFNA in the Surflon S-111 were 0.025, 0.125, or 0.6 mg/kg/day. Based on the assumed percentages of PFCs in Surflon S-111 given above, the doses of PFNA are estimated as 0.019, 0.09, and 0.44 mg/kg/day. For perfluoroundecanoic acid (C11), the next most abundant PFC in the mixture, the doses are estimated as 0.005, 0.025, and 0.12 mg/kg/day, and the PFOA doses are estimated to be about 1% of the PFNA doses (about 0.0002, 0.0009, and 0.004 mg/kg/day).

Evaluation of the contribution of PFNA to toxicity of Surflon S-111 PFC mixture

An important issue in interpretation of the two Surflon S-111 studies is whether the toxic effects resulted primarily from PFNA, the major component of Surflon S-111, or from the other PFC(s) present at lower concentrations in the Surflon S-111 mixture. Information on the relationship between toxicity and the relative serum levels of PFNA and the other PFCs in the Surflon S-111 mixture in these studies is key to the evaluation of this issue.

As discussed above, serum levels of PFOA, PFNA, C11, and C13 in males and females in each dose group over time were presented graphically by Mertens et al. (2010). However, it is not possible to accurately estimate the serum values at lower dose levels from the graphs due to their scale. The numerical serum data have been requested from the study sponsors but have not been provided to date.

Although the serum PFC data presented cannot be completely and precisely interpreted, several important general conclusions can be made from the serum PFC graphs and the toxicity results. These data suggest that the effects of Surflon S-111 in Mertens et al. (2010) are, at least primarily, due to PFNA, rather than C11 or the other PFCs present in even lower concentrations. Consistent with other pharmacokinetic studies in rats (discussed above), serum PFNA levels in male rats were much higher than in female rats given the same administered dose. In contrast, serum levels of C11 were similar, and generally somewhat higher, in females than in males at the same administered dose.

Effects common to both genders (including changes in clinical chemistry parameters, increased liver weight, and increased hepatic beta-oxidation) occurred at lower administered doses in males than females, and some effects (including liver histopathology) occurred in males but not in females.

The LOAELs and NOAELs for Surflon S-111 in Mertens et al. (2010) are: LOAELs: 0.125 mg/kg/day (males) and 0.6 mg/kg/day (females); NOAELs: 0.025 mg/kg/day (males) and 0.125 mg/kg/day (females) (Table 7 and Appendix 3). At these LOAELs, PFNA serum levels were similar in males and females, while C11 serum levels were about 10-fold higher in females than in males (at the end of the 90 day study). The PFNA serum levels at the NOAELS are also similar in males and females, based on very rough estimates from the graphs provided. If C11 were a major contributor to the toxicity of Surflon S-111, effects would be expected in 0.125 mg/kg/day females, and a greater response would be expected in 0.6 mg/kg/day females than in 0.125 mg/kg/day males. These data suggest that effects of Surflon S-111 are primarily due to PFNA, not C11, assuming that males and females are equally susceptible to the toxicity of these PFCs.

In agreement with these conclusions, Mundt et al. (2007) also attribute the greater toxicity of Surflon S-111 in male rats than female rats in the unpublished reports (WIL Research Laboratories, 2006) of the subchronic study (Mertens et al., 2010) to the higher serum PFNA levels in males as compared to female rats.
In the two-generation study (Stump et al., 2008), data on serum levels of PFNA and the other PFCs in the Surflon S-111 mixture are not presented. This information has been requested from the study sponsors but has not been provided to date. As was seen in

Mertens et al. (2010), effects common to both genders (increased liver and kidney weight, hepatocellular hypertrophy, renal tubule cell hypertrophy) occurred at lower administered doses in males than in females, and other effects (decreased body weight, hepatocellular necrosis) occurred only in males.

Furthermore, a recent 7 week oral reproductive/developmental study of C11 in rats (Takahashi et al., 2014) supports the conclusion that C11 is not primarily responsible for the toxicity of the Surflon S-111 mixture. Takahashi et al. (2014) identified 0.1 mg/kg/day as the NOAEL and 0.3 mg/kg/day as the LOAEL for repeated dose toxicity of C11 in males and females, based on the occurrence of centrilobular hypertrophy of hepatocytes. For reproductive/developmental toxicity, the NOAEL and LOAEL for C11 were identified as 0.3 mg/kg/day and 1 mg/kg/day, respectively, based on decreased body weight at birth and decreased body weight gain at PND 4. As discussed above, the doses of C11 are estimated as 0.005, 0.025, and 0.12 mg/kg/day in the 0.025 mg/kg/day, 0.125 mg/kg/day and 0.6 mg/kg/day Surflon S-111 groups (respectively) in Mertens et al. (2010) and Stump et al. (2008).

Although serum levels of C11 were not measured by Takahashi et al. (2014), it is notable that the dose of C11 (0.025 mg/kg/day) at the LOAEL in males in Mertens et al. (2010) is about 10-fold lower than the LOAEL (0.3 mg/kg/day) for systemic toxicity identified by Takahashi et al. (2014). Similarly, the highest C11 dose in Stump et al. (2008), 0.12 mg/kg/day, was well below the NOAEL for reproductive/developmental toxicity of 0.3 mg/kg/day identified by Takahashi et al. (2014). These observations are even more significant because the duration of exposure in Stump et al. (2008) and Mertens et al. (2010) was several fold longer than in Takahashi et al. (2014).

Finally, C11 and C13 were less potent than PFNA as *in vitro* activators of PPAR-alpha, a nuclear receptor believed to be involved in many effects of PFCs (discussed in Mode of Action section, below).

Based on the information reviewed above, it is concluded that PFNA is likely the primary contributor to the toxicity of Surflon S-111 reported by Stump et al. (2008) and Mertens et al. (2010).

Table 7A. Summary of decreased body weight after oral administration of PFNA

Citation	*Species/strain*	*Administered Dose (mg/kg/day)*	*Duration*	*Endpoint*	*NOAEL* (mg/kg/day)*	*LOAEL* (mg/kg/day)*
Kennedy et al. (1987)	Crl:CD-1 mouse	0, 3, 10, 30, 300 ppm in diet. Estimated as 0, 0.45, 1.5, and 4.5 mg/kg/day	14 days	↑ relative liver weight	10 ppm (estimated at 1.5 mg/kg/day) *(Data not provided; text states weight loss and generalized weakness occurred at this dose).*	30 ppm (estimated as 4.5 mg/kg/day) *(100% mortality at higher doses)*
Wang et al. (2015)	Male Balb/C mouse	0, 0.1, 1, 5	14 days	↓ body weight	1	5
Fang et al. (2008)	Male Balb/C mouse	0, 1, 3, 5	14 days	↓ body weight	1	3
Fang et al. (2009)	Male Sprague-Dawley rat	0, 1, 3, 5	14 days	↓ body weight	1	3
Mertens et al. (2010)	Sprague-Dawley Rat	Surflon: 0, 0.025, 0.125, 0.6 PFNA: 0, 0.019, 0.09, 0.44. Gavage	90 days, followed by 60 day recovery period.	↓ body weight *(Not attributable to ↓ food consumption)*	*Males:*Surflon: 0.125 PFNA: 0.09	Surflon: 0.6 PFNA : 0.44
					*Females:*Surflon: 0.6 PFNA : 0.44	-------
					Males, 60 day recovery: Surflon: 0.125 PFNA: 0.09	Surflon: 0.6 PFNA : 0.44
Stump et al. (2008)	Sprague-Dawley Rat	Surflon: 0, 0.025, 0.125, 0.6 PFNA: 0, 0.019, 0.09, 0.44. Gavage	18-21 weeks	↓ body weight (F0 and F1 males) (*Not attributable to ↓ food consumption)*	Surflon: 0.125 PFNA: 0.09	Surflon: 0.6 PFNA : 0.44
					Females: Surflon: 0.6 PFNA : 0.44	-------
Das et al. (2015)	Pregnant CD-1 mouse	0, 1, 3, 5, 10	GD 1-17	Maternal weight gain	5	10 *(substantial weight loss starting on GD 8; sacrificed on GD 13)*

* NOAELs are defined as the highest dose that did not produce a statistically significant (e.g., $p<0.05$) effect, and LOAELs are defined as the lowest doses with statistically significant (e.g., $p<0.05$) effects. For some endpoints, there were dose-related trends that included non-statistically significant changes at lower doses.

Table 7B. Summary of key endpoints for hepatic toxicity and carbohydrate/lipid metabolism after oral exposure to PFNA

Citation	*Species/ strain*	*Administered Dose (mg/kg/day)*	*Duration*	*Endpoint*	*NOAEL* (mg/kg/day)*	*LOAEL* (mg/kg/day)*
Kennedy et al. (1987)	Crl:CD-1 mouse	0, 3, 10, 30, 300 ppm (diet). Est 0, 0.45, 1.5, 4.5 mg/kg/day	14 days	↑ relative liver weight	----	3 ppm (estimated as 0.45 mg/kg/day)
Stump et al. (2008)	Sprague - Dawley Rats (F0 and F1)	Surflon: 0, 0.025, 0.125, 0.6 PFNA: 0, 0.019, 0.09, 0.44. Gavage	18-21 wks.	↑ liver weight (absolute and relative)	*Males:* Surflon: 0.025 PFNA: 0.019	Surflon: 0.125 PFNA: 0.09
					Females: Surflon: 0.125 PFNA: 0.09	Surflon: 0.6 PFNA : 0.44
				Hepatocellular hypertrophy (LOAEL is lower than for ↑ liver wt.)	*Males:* -----	Surflon: 0.025 PFNA: 0.019.
					Females: -----	Surflon: 0.6 PFNA: 0.44 *(Lower doses not evaluated)*
				Hepatocellular necrosis	*Males:* -----	Surflon: 0.025 PFNA: 0.019
Mertens et al. (2010)	Sprague - Dawley Rats	Surflon: 0, 0.025, 0.125, 0.6 PFNA: 0, 0.019, 0.09, 0.44. Gavage	90 day main study, 60 day recovery period.	↑ liver weight (absolute and relative) ↑ beta-oxidation in liver (marker of peroxisome proliferation).	*Males:* Surflon: 0.025 PFNA: 0.019	Surflon: 0.125 PFNA: 0.09
					Females: Surflon: 0.125 PFNA: 0.09	Surflon: 0.6 PFNA: 0.44
					Males, 8 weeks after dosing ended: Surflon: 0.125 PFNA: 0.09	Surflon: 0.6 PFNA: 0.44
					Females, 8 weeks post-dosing. Surflon: 0.6 PFNA: 0.44	----------
				Hepatocellular hypertrophy/ eosinophilic foci	*Males:* Surflon: 0.025 PFNA: 0.019	Surflon: 0.125 PFNA: 0.09
					Females: Surflon: 0.6 PFNA: 0.44	----------
					Males, 8 weeks after dosing ended: Surflon: 0.125 PFNA: 0.09 *(Females not evaluated)*	Surflon: 0.6 PFNA: 0.44
				Necrosis	*Males:* Surflon: 0.125 PFNA: 0.09	Surflon: 0.6 PFNA: 0.44
					Females: Surflon: 0.6 PFNA: 0.44	----------
					Males, 8 weeks after dosing ended: Surflon: 0.125 PFNA: 0.09 *(Females not evaluated)*	Surflon: 0.6 PFNA: 0.44
				Serum cholesterol	Surflon: 0.6 PFNA: 0.44	----------

* NOAELs are defined as the highest dose that did not produce a statistically significant (e.g., $p<0.05$) effect, and LOAELs are defined as the lowest doses with statistically significant (e.g., $p<0.05$) effects. For some endpoints, there were dose-related trends that included non-statistically significant changes at lower doses.

Table 7B *(continued)*. Summary of key endpoints for hepatic toxicity and carbohydrate/lipid metabolism after oral exposure to PFNA						
Citation	***Species/ strain***	***Administered Dose (mg/kg/day)***	***Duration***	***Endpoint***	***NOAEL* (mg/kg/day)***	***LOAEL* (mg/kg/day)***
Das et al. (2015)	Pregnant and non-pregnant female CD-1 mice	0, 1, 3, 5	GD 1-16 or GD 1-17.	↑ liver weight (absolute and relative) (End of dosing (GD 17) and PND 28 (4 weeks after dosing ended)) *Histopathology not assessed.* *Fetus and pup liver weight data presented in Table 5F.*	----	1
Wolf et al. (2010)	Female wild-type (WT) 129S1/SvlmJ mice and PPARα knockout (KO) mice on a 129S1/SvlmJ background; mated to males of same strain.	0, 0.83, 1.1, 1.5, 2	GD 1-18	↑ relative liver weight, 23 days after dosing ended (PND 21). *Histopathology not assessed.* *Pup liver weight data presented in Table 5F.*	*Non-pregnant WT:* ----	0.83
					Non-pregnant KO: 0.83	1.1
					Pregnant WT: ----	0.83
					Pregnant KO: 2	----
Fang et al. (2012a).	Male Sprague-Dawley rats	0, 0.2, 1, 5	14 days Histopathology not assessed	↑ liver glycogen	1	5
				↑ serum glucose	0.2	1
				↑ serum LDL	1	5
				↓ serum HDL	---	0.2
				↓ serum HDL/LDL ratio	0.2	1
Wang et al. (2015)	Male Balb/c mice	0, 0.2, 1, 5	14 days	↑ relative liver weight	----	0.2
				↑ liver lipids	----	0.2
				↓ serum lipids	1	5
				↑ serum liver enzymes	1	5
* NOAELs are defined as the highest dose that did not produce a statistically significant (e.g., $p<0.05$) effect, and LOAELs are defined as the lowest doses with statistically significant (e.g., $p<0.05$) effects. For some endpoints, there were dose-related trends that included non-statistically significant changes at lower doses.						

Table 7C. Summary of key renal effects after oral administration of PFNA

Citation	*Species/ strain*	*Administered Dose (mg/kg/day)*	*Duration*	*Endpoint*	*NOAEL* (mg/kg/day)*	*LOAEL* (mg/kg/day)*
Mertens et al. (2010)	Sprague-Dawley Rat	Surflon: 0, 0.025, 0.125, 0.6 PFNA: 0, 0.019, 0.09, 0.44. Gavage	13 weeks	↑ kidney weight Histopathological changes in the kidney	Surflon: 0.6 PFNA: 0.44	 --------
Stump et al. (2008)	Sprague-Dawley Rat	Surflon: 0, 0.025, 0.125, 0.6 PFNA: 0, 0.019, 0.09, 0.44. Gavage	18-21 weeks	↑ kidney weight (absolute and relative)	*F0 and F1 males:* Surflon: 0.025 PFNA: 0.019	 Surflon: 0.125 PFNA: 0.09
					F0 females: Surflon: 0.125 PFNA: 0.09	 Surflon: 0.6 PFNA: 0.44
					F1 females: Surflon: 0.6 PFNA: 0.44	 -------
				Renal cell hypertrophy	*F0 males:* Surflon: 0.025 PFNA: 0.019	 Surflon: 0.125 PFNA: 0.09
					F1 males: Surflon: 0.125 PFNA: 0.09	 Surflon: 0.6 PFNA: 0.44
					F0 females: Surflon: 0.125 PFNA: 0.09	 Surflon: 0.6 PFNA: 0.44
					F1 females: Surflon: 0.6 PFNA: 0.44	 ----------

* NOAELs are defined as the highest dose that did not produce a statistically significant (e.g., $p<0.05$) effect, and LOAELs are defined as the lowest doses with statistically significant (e.g., $p<0.05$) effects. For some endpoints, there were dose-related trends that included non-statistically significant changes at lower doses.

Table 7D. Summary of key endpoints for immune system toxicity of PFNA after oral exposure

Citation	*Species/ strain*	*Administered Dose (mg/kg/day)*	*Duration*	*Endpoint*	*NOAEL* (mg/kg/day)*	*LOAEL* (mg/kg/day)*
Fang et al. (2008)	Male Balb/c mice	0, 1, 3, 5 Gavage	14 days	↓ thymus weight (relative and absolute)	1	3
				% immature versus mature T cells in thymus	3	5
				Impairment of cell cycle progression in thymus	1	3
				↑ apoptosis in thymus	3	5
				↓ absolute spleen weight	1	3
				↓ relative spleen weight	3	5
				↓ specific types of innate immune cells in spleen	----	1
				Impairment of cell cycle progression in spleen	----	1
				↑ apoptosis in spleen	3	5
				↓ cytokine (IL-4) secretion in spleen	----	1
				↑ serum cortisol	1	3
				↑ ACTH	3	5
Fang et al. (2009)	Male Sprague-Dawley rats	0, 1, 3, 5 Gavage	14 days	Thymus weight (absolute and relative)	---	1 (↑) 5 (↓)
				Thymus histopathology	*Stated to be dose-related; doses not specified.*	
				Serum cytokine levels (↑ or ↓)	1	3
				↑ serum cortisol	3	5
Fang et al. (2010)	Male Sprague-Dawley rats	0, 1, 3, 5 Gavage	14 days	↓ absolute spleen weight	----	1
				↓ relative spleen weight	3	5
				↑ apoptosis in spleen	1	3
Mertens et al (2010); Stump et al. (2008)	Sprague-Dawley Rats (male and female)	Surflon: 0, 0.025, 0.125, 0.6 PFNA : 0, 0.019, 0.09, 0.44 Gavage	13 weeks; 18-21 weeks	Spleen and thymus weight	Surflon: 0.6 PFNA: 0.44	------- *Text reports that there were no effects; data not shown.*

* NOAELs are defined as the highest dose that did not produce a statistically significant (e.g., $p<0.05$) effect, and LOAELs are defined as the lowest doses with statistically significant (e.g., $p<0.05$) effects. For some endpoints, there were dose-related trends that included non-statistically significant changes at lower doses.

Table 7E. Summary of key endpoints for male reproductive system toxicity of PFNA after oral exposure

Citation	*Species/ strain*	*Administered Dose (mg/kg/day)*	*Duration*	*Endpoint*	*NOAEL* (mg/kg/day)*	*LOAEL* (mg/kg/day)*
Feng et al. (2009)	Male Sprague-Dawley rats	0, 1, 3, 5 Gavage	14 days	Testicular histopathology	3	5
				↑ apoptosis in testes (TUNEL assay)	----	3 *(Text states sharp ↑at 3 mg/kg/day: NOAEL not stated)*
				↑ Serum testosterone	-----	1 No effect at 3 mg/kg/day. Greatly ↓ at 5 mg/kg/day.
				↑ Serum estrogen	3	5
				% apoptotic testicular cells	1	3
				↑ testicular caspase-8 (part of death receptor pathway)	1	3
Feng et al. (2010)	Male Sprague-Dawley rats	0, 1, 3, 5 Gavage	14 days	Histopathology in seminiferous tubule.	*(Not evaluated at 1)*	3
				↑ testicular Wilms tumor protein	----	1
				↓ testicular transferrin (delivers iron needed for sperm production)	----	1
				↑ testicular Mullerian inhibiting substance	3	5
				↓ testicular inhibin B (marker of testicular toxicity)	----	1
Stump et al. (2008)	Male Sprague-Dawley rats	Surflon: 0, 0.025, 0.125, 0.6 PFNA (est.): 0, 0.019, 0.09, 0.44 Gavage	18-21 weeks	↓ sperm motility (F1), epididymis weight (F0, F1), and epididymis sperm concentration (F0)	Surflon: 0.125 PFNA: 0.09	Surflon: 0.6 PFNA: 0.44

* NOAELs are defined as the highest dose that did not produce a statistically significant (e.g., $p<0.05$) effect, and LOAELs are defined as the lowest doses with statistically significant (e.g., $p<0.05$) effects. For some endpoints, there were dose-related trends that included non-statistically significant changes at lower doses.

Table 7F. Summary of key endpoints for reproductive/developmental effects of PFNA after oral exposure

Citation	*Species/ strain*	*Administered Dose (mg/kg/day)*	*Duration*	*Endpoint*	*NOAEL* (mg/kg/day)*	*LOAEL* (mg/kg/day)*
Stump et al. (2008) See Table 7E for spermatogenic endpoints.	Male Sprague-Dawley rats	Surflon: 0, 0.025, 0.125, 0.6 PFNA: 0, 0.019, 0.09, 0.44. Gavage	*F0 and F1*: Starting at 6 weeks for at least 70 days prior to mating, throughout mating, gestation, and lactation.	↓ Fertility index in F0 males and females	-----	Surflon: 0.025 PFNA (estimated): 0.019 (No effect at higher doses)
				↑ relative liver weight in pups (PND 21)	*F1:* Surflon: 0.025 PFNA: 0.019	Surflon: 0.125 PFNA: 0.09
					F2: Surflon: 0.125 PFNA: 0.09	Surflon: 0.6 PFNA: 0.44
				Maternal body weight gain; Pregnancy rate; Number of live pups at birth; Post-natal mortality; Pup body weight; Post-natal development	Surflon: 0.6 PFNA: 0.44	-------
Rogers et al. (2014)	Sprague-Dawley Rats	0, 5	GD 1-20	↓ Maternal body weight gain	---	5
				↓ pup weight at birth	---	5
				Pup weight on PND 21 until age 56 weeks.	5	-------
				↑ systolic blood pressure in pups on PND 10.	----	5 (No effect on PND 26 and PND 56)
				↓ nephron endowment in renal glomeruli in PND 22	*Males:* ----	5 *(Not associated with body wt. or kidney wt. changes)*
					Females: 5	------

Table 7F *(continued)*. Summary of key endpoints for reproductive/developmental effects of PFNA after oral exposure						
Citation	***Species/ STRAIN***	***Administered Dose (mg/kg/day)***	***Duration***	***Endpoint***	***NOAEL* (mg/kg/day)***	***LOAEL* (mg/kg/day)***
Das et al. (2015)	CD-1 mice	0, 1, 3, 5, 10	GD 1-16 (sacrificed at term) GD 1-17 (allowed to give birth)	Maternal weight loss; full litter resorptions	5	10
				↑ fetal liver weight (Consistent with NOAEL/LOAEL for adult ↑ liver weight)	---	1
				Post-natal mortality (Sharp ↑ starting on PND 2 through PND 10.)	3	5 (Severe effects at this dose)
				Pup body weight (Persisted in males until 9 months of age)	1	3
				Post-natal development (Day of eye opening, vaginal opening, and preputial separation)	1	3
				↑ pup liver weight	*PND 1, 10, 24:* ----- (Consistent with NOAEL/LOAEL for adult ↑ liver weight in same study)	1
					PND 42: 1	3
					PND 70: 5	---
Wolf et al. (2010)	Female wild-type (WT) 129S1/SvlmJ mice and PPARα knockout (KO) mice on a 129S1/SvlmJ background; mated to males of same strain.	0, 0.83, 1.1, 1.5, 2	GD 1-18	↓ pregnancy rate	*WT:* 2	----
					KO: ---	0.83
				↓ number of live pups at birth	*WT:* 0.83	1.1
					KO: 2	---
				↑ post-natal mortality	*WT:* 0.83	1.1
					KO: 2	---
				↓ pup weight gain	*WT:* 1.5	2
					KO: 2	---
				Delayed eye opening	*WT:* 1.5	2
					KO: 2	---
				↑ pup liver weight (PND 21; 23 days after last dose)	*WT:* ----	0.83
					KO: 1.5 (Below NOAEL for ↑ maternal liver weight)	2
*NOAEL is defined as the highest dose that did not produce a statistically significant (e.g., $p<0.05$) effect. LOAEL is defined as the lowest dose with statistically significant (e.g., $p<0.05$) effects. For some endpoints, there were dose-related trends that included non-statistically significant changes at lower doses.						

Acute toxicity

No studies that determined the acute oral LD_{50} of pure PFNA were located. However, Mertens et al. (2010) state that the "approximate lethal dose" (unpublished data, calculated herein as 65 mg/kg) in rats for the Surflon S-111 mixture of PFCs consisting primarily of PFNA (see below) was 2.9-fold lower than the acute LD50 for PFOA of 198 mg/kg identified by Olson and Anderson (1983).

The inhalation LC_{50} in male rats (5 or 6 per group) exposed for 4 hours to six concentrations ranging from 67 to 4600 mg/m^3 of ammonium perfluorononanoate (the ammonium salt of PFNA) as a dust was 820 mg/m^3; the lowest dose that caused death was 590 mg/m^3. Animals were observed for 5-14 days after exposure and deaths occurred earlier with increasing dose (Kinney et al., 1989). As has been observed in animals acutely exposed to PFOA (reviewed in Lau et al., 2007; Post et al., 2012), severe body weight loss occurred in surviving rats of all but the lowest dose group.

Mortality

Mortality occurred in mice at PFNA doses of 10 mg/kg/day or higher in two 14 day mouse studies. In a 14 day dietary CD-1 mouse study (Kennedy, 1987), all animals in the 300 and 3000 ppm groups died. The doses at these dietary concentrations are estimated as 45 and 450 mg/kg/day (Appendix 3). The next lowest dose group, 30 ppm (estimated as 4.5 mg/kg/day) caused weight loss and generalized weakness. Consistent with these results, mortality occurred in 50% of male Balb/C mice dosed with 10 mg/kg/day PFNA for 14 days (Fang et al., 2008).

Body weight

Effects of PFNA on body weight in mice and rats are summarized in Table 7A. In the rat studies, the decreased body weight was not attributable to decreased food consumption, while food consumption was not evaluated in the mouse studies.

Weight loss occurred in mice exposed to PFNA at >3 mg/kg/day for 14 days (Kennedy et al., 1987; Wang et al., 2015; Fang et al., 2008; Fang et al., 2009). In an unpublished study of longer term exposure to Surflon S-111 in rats discussed in Stump et al. (2008), all males dosed with 2 mg/kg/day for 23 days were euthanized on day 23 due to severe clinical findings (no details provided) and severe body weight loss (Wolterbeek, 2004).

Decreased body weight gain also occurred in male (but not female) rats with longer exposures to 0.6 mg/kg/day Surflon S-111/0.44 mg/kg/day PFNA (Mertens et al., 2010; Stump et al., 2008). Body weight in rats exposed to this dose remained decreased 60 days after subchronic (90 day) exposure ended (Mertens et al., 2010).

Hepatic Toxicity

Toxicology data on hepatic effects of PFNA are summarized in Table 7B.

Liver enlargement

Liver enlargement is a well-established effect of PFCs including PFNA (Lau, 2012). PFNA has been evaluated for increased liver weight only in rodents (Table 7B), while PFOA is known to cause this effect in non-human primates as well as rodents (reviewed in Post et al., 2012). As discussed in detail in the Mode of Action section (below), the increased liver weight caused by

PFNA in rodents has both PPAR-alpha dependent and PPAR-alpha independent components (Wolf et al., 2010; Rosen et al., 2010). Hepatic beta-oxidation, a marker of PPAR-alpha activation, was significantly increased in male and female rats at the same PFNA doses at which liver weight was increased (Mertens et al., 2010).

The magnitude of increased liver weight was similar in male and female mice given the same dose (Kennedy, 1987). Since the half-lives of PFNA in male and female mice are similar (see above), these results suggest that male and female mice are equally susceptible to the liver weight increases induced by PFNA.

Statistically significant increases in liver weight occurred at doses as low as 0.2 mg/kg/day in mice in a 14 day study (Wang et al., 2015) and 0.09 mg/kg/day (0.125 mg/kg/day Surflon S-111) in rats after 13 week exposure (Mertens et al., 2010). Increased liver weight also occurred in fetuses after *in utero* exposure and in pups exposed prenatally and through breast milk (Das et al., 2015; Wolf et al., 2010, discussed in Reproductive/Developmental Toxicity, below).

Increased liver weight persists after dosing with PFNA has ended. In male rats dosed for 90 days, increased liver weight persisted at 0.44 mg/kg/day PFNA/0.6 mg/kg/day Surflon S-111 for at least 60 days (about two half-lives for PFNA; Mertens et al., 2010). In CD-1 mice exposed during pregnancy, dose-related increases in absolute and relative liver weight persisted at 3 and 5 mg/kg/day on PND 28, 4 weeks after the last dose (Das et al., 2015). Absolute and relative liver weights were also increased on PND 21, 23 days after the last dose, in both WT and PPAR-alpha KO mice gestationally exposed to lower doses of PFOA than those used in the CD-1 mouse study (Wolf et al., 2010). Relative liver weights were increased at all doses (0.83 to 2 mg/kg/day) in non-pregnant WT and PPAR-alpha KO females, with significance in all groups except the lowest dose (0.83 mg/kg/day) PPAR- alpha KO. In mice that had given birth, relative liver weight was significantly increased at all doses in WT, but was not increased in PPAPR-alpha KO at any dose. In interpreting these results, it is important to note that in mice that had given birth, PFNA serum levels in the KO mice were much lower than in the WT mice for reason(s) that were not determined. The NOAELs and LOAELs for increased liver weight are consistent in the WT and KO mice on a serum level basis, suggesting that the differences in response is likely due to kinetic differences unrelated to PPAR-alpha status.

Serum levels of liver enzymes

Increased serum liver enzymes AST and ALT, an indication of hepatic toxicity, were observed in male mice exposed to 5 mg/kg/day PFNA for 14 days (Wang et al., 2015). In contrast, these two liver enzymes and GGT were not affected in the 90 day rat study of Surflon S-111 which used lower doses of PFNA (up to 0.44 mg/kg/day; Mertens et al., 2010).

Histopathology

Quantitative liver histopathology data (frequency and severity) were reported only for the 90 day subchronic and two-generation studies of Surflon S-111 (Mertens et al., 2010; Stump et al., 2008). Microscopic evaluation of the liver was not performed in other shorter term studies which generally used higher doses, with the exception of the 14 day rat study conducted by Fang et al. (2012b). Fang et al. (2012b) did not perform a systematic quantitative evaluation for this endpoint and provide only a brief summary of their observations using different

terminology to describe the histopathological changes than that used in the Surflon S-111 rat studies.

In both the 13 week and two-generation (18-21 week) rat studies, there were dose-related increases in frequency and severity of histopathological changes in liver (Mertens et al., 2010; Stump et al., 2008). These effects occurred at lower doses in the 18-21 week study (0.019 mg/kg/day PFNA/0.025 mg/kg/day Surflon) than in the 13 week study (0.09 mg/kg/day PFNA/0.125 mg/kg/day Surflon), and a NOAEL was not identified in the longer 18-21 week study. These results suggest that these effects occur at lower doses when exposure duration is increased from 13 weeks to 18-21 weeks.

Histopathological changes in the liver, including necrosis, occurred in males in the two-generation rat study (Stump et al., 2008) at a dose (Surflon S-111, 0.025 mg/kg/day; PFNA, 0.019 mg/kg/day) below the doses that caused increased liver weight in the same study. Thus, histopathological changes, including necrosis, were a more sensitive endpoint than increased liver weight in this study. It is important to note that some of the histopathological changes in the liver reported by Fang et al. (2012b), Stump et al. (2008), and Mertens et al. (2010) are not typically associated with PPAR-alpha activation (peroxisome proliferation and increased smooth endoplasmic reticulum), suggesting that PFNA caused liver toxicity that was independent of PPAR-alpha in these studies.

Hepatocellular hypertrophy occurred at high frequency in both the 13 week study and the 18-21 week study (Mertens et al., 2010; Stump et al., 2008). In the 13 week study, the incidence in male rats was 60% at 0.09 mg/kg/day PFNA (0.125 mg/kg/day Surflon S-11), and 100% at 0.44 mg/kg/day PFNA (0.6 mg/kg/day Surflon S-111) both at the end of dosing and 60 days after exposure ended. In the longer duration (18-21 week) study, hepatocellular hypertrophy in F_0 and F_1 males occurred at lower doses and higher incidence than in the subchronic (13 week) study (77% at 0.019 mg/kg/day PFNA/0.025 g/kg/day Surflon S-111, and 100% at the two higher doses, 0.09 and 0.44 mg/kg/day PFNA (0.125 and 0.6 mg/kg/day Surflon S-111). This effect was not seen in females in the subchronic study, but occurred in F_0 females in the longer two–generation study. Hepatocellular hypertrophy was also evident in the photomicrographs of livers from rats treated with PFNA presented by Fang et al. (2012b), although the authors did not use this term to describe their findings (K. Cooper, personal communication).

Hepatocellular necrosis occurred only in high dose males in the 13 week study, but was found in all dose groups, with dose-related increases in frequency and severity, in both the F0 and F1 males after longer (18-21 week) exposure (Mertens et al., 2010; Stump et al., 2008). Other histological changes including inflammation, clear cell foci, and vacuolation, were also seen in all dosed groups of F_0 and F_1 males in the two-generation study, with severity and/or incidence increasing with dose.

Hepatic and serum glucose/glycogen and lipids

Summaries of key findings are shown in Table 7B.

Lau (2012) reported that PFOA causes hepatic lipid accumulation and decreased serum lipids in rodents, and similar effects have been observed in rodents treated with PFNA. In male mice

exposed to PFNA for 14 days, triglycerides and total cholesterol were increased in the liver at 0.2 and 1 mg/kg/day, but not at a higher dose, 5 mg/kg/day. In contrast, serum triglycerides and total cholesterol were decreased at 5 mg/kg/day but not at lower doses (Wang et al., 2015). Serum cholesterol was also decreased in all groups of male rats treated with $\geq$ 0.1 mg/kg/day Surflon S-111 for 14 days in an unpublished study (Wolterbeek et al., 2004) cited by Mertens et al. (2010). In a study presented in poster/abstract form, hepatic triglyceride levels and lipid accumulation were increased in both SV129 wild type (WT) and PPAR-alpha null (knockout, KO) mice given 10 mg/kg/day PFNA for 7 days (Das et al., 2013). In male rats, there was lipid accumulation in livers at 5 mg/kg/day, but not lower doses (Fang et al., 2012b). Potential modes of action for these effects is discussed in the Mode of Action section (below). In contrast, total serum cholesterol was not affected in the 90 day study of Surflon S-111 in rats exposed to lower doses of PFNA (up to 0.44 mg/kg/day); serum triglycerides were not measured (Mertens et al., 2010).

PFNA caused a dose-related increase in serum glucose, as well as a dose-related decrease in both serum HDL and a decrease in the serum HDL/LDL ratio in male rats dosed with 0.2-5 mg/kg/day PFNA for 14 days (Fang et al., 2012a). The authors note that a decreased serum HDL/LDL ratio often accompanies hyperglycemia. Liver glycogen content was increased in a dose-related manner, and the increase was significant at 5 mg/kg/day. In contrast, no effect on serum glucose was reported in the longer duration (subchronic) rat study at up to 0.44 mg/kg/day PFNA (Mertens et al., 2010).

Renal Effects:
Kidney weight and histopathology were evaluated in the 13 week and two-generation (18-21 week) studies of Surflon S-111 in rats (Mertens et al., 2010; Stump et al., 2008). In these studies, renal effects occurred after 18-21 weeks of exposure, but not after 13 weeks of exposure to the same doses. These results suggest that a longer exposure duration is needed to produce renal effects. Renal endpoints were also evaluated in newborn rats exposed during gestation (Rogers et al., 2014; see Reproductive/Developmental section) (Table 7C).

In the two-generation study with 18-21 week exposure (Stump et al., 2008), dose-related increases in kidney weight occurred in F_0 and F_1 males, and F_0 females. Dose-related increases in renal tubule cell hypertrophy were also found in F_0 and F_1 males in this study. This effect occurred in in 93-100% of high dose (0.6 mg/kg/day Surflon S-111/0.44 mg/kg/day PFNA) F_0 and F_1 males and at lower frequency in mid-dose (0.125 mg/kg/day Surflon S-111/0.09 mg/kg/day PFNA) F_0 males, but not in control or low-dose (0.025 mg/kg/day Surflon S-111/0.019 mg/kg/day PFNA) males, and severity increased with dose in the F_0 males. Other histopathological effects (renal inflammation, brown pigment, and capsular fibrosis) also occurred in a few treated F_0 males. Renal tubule cell hypertrophy was also observed in 27% of high dose F_0 females.

In contrast, there were no effects on kidney weight or renal histopathology in the subchronic (13 week) study. This study used the same doses as the two-generation (18-21 week) study (Mertens et al., 2010).

Immune system effects
Toxicity to the immune system occurred in three 14 day oral gavage studies using PFNA doses

of 1, 3, and 5 mg/kg/day (one in male mice, Fang et al., 2008; and two in male rats, Fang et al., 2009; Fang et al., 2010; Table 7D). Rockwell et al. (2013) also reported immune system toxicity in male and female mice 14 days after a single high intraperitoneal (i.p.) dose of PFNA (0.1 mM/kg, calculated as 46.4 mg/kg). The longer duration rat studies (Mertens et al., 2010; Stump et al., 2008) found no effects on thymus and spleen weight at up to 0.44 mg/kg/day PFNA, but did not evaluate other parameters of immune function (Table 7D).

PFNA caused dose-related decreases in spleen and/or thymus weights (absolute and relative) in all three 14 day studies. Apoptosis was increased in a dose-related manner in spleens of mice and rats, and in thymus of mice (not evaluated in rats; Fang et al., 2008, 2010). Serum levels of hormones that suppress the immune system (cortisol in mice and rats; ACTH in rats) were increased by PFNA, and serum levels of cytokines involved with immune system function were affected by PFNA in both species (Fang et al., 2008, 2009). The most sensitive endpoints for immune system toxicity in mice were impairment of cell cycle progression, decreases in specific types of innate immune cells, and decreased interleukin-4 secretion in the spleen (Fang et al., 2008). These three splenic effects occurred at 1 mg/kg/day, and a NOAEL was not identified. In the rat, the most sensitive endpoints were decreased absolute and relative thymus weight and decreased absolute spleen weight. The LOAEL for these effects was also 1 mg/kg/day with no NOAEL identified (Fang et al., 2009; Fang et al., 2010). Other components of these studies related to the modes of action for these effects are discussed in the Mode of Action section (below).

In male and female C57Bl/6 mice 14 days after a single i.p. dose of 46.4 mg/kg PFNA, the number of red blood cells and leukocytes in the spleen was reduced by 87.5-95% and thymocyte viablility was decreased significantly. Additionally, other parameters of immune function in the spleen and thymus were affected. This dose caused substantial body weight loss (31% in males, 38% in females), an approximate 3-fold increase in liver weight/body weight ratio, and a 60-70% decrease in spleen weight/body weight ratio (Rockwell et al., 2013).

Male reproductive system effects

Three studies in rats found effects of PFNA on the male reproductive system at doses $\leq$ 1 mg/kg/day. PFNA caused toxicity to the male reproductive system in two 14 day oral gavage studies using dose levels of 1, 3, and 5 mg/kg/day (Feng et al., 2009; Feng et al., 2010). Some endpoints related to the male reproductive system were also affected in the two-generation study of Surflon S-111 (Table 7E). Sensitive endpoints include histopathological changes in the seminiferous tubules, increased serum testosterone, changes in Sertoli cell secretions, reduced sperm motility, and decreased epididymis weight and sperm concentration.

PFNA caused dose-related disruption of spermatogenesis and increases in apoptosis in testicular cells of male rats (Feng et al., 2009). Hormones related to reproductive function were also affected. Serum estradiol increased at 5 mg/kg/day, while testosterone was increased at 1 mg/kg/day and decreased at 5 mg/kg/day. These hormones are also affected by other PFCs; testosterone was decreased in rats treated with perfluorodecanoic acid (C10; Bookstaff et al., 1990) and perfluorododecanoic acid (C12; Shi et al., 2009), and estradiol was increased in male rates exposed to PFOA for 1-12 months in a two year dietary study (Biegel et al., 2001).

PFNA also caused ultrastructural changes and effects on multiple functions of Sertoli cells, testicular cells that play a key role in spermatogenesis (Feng et al., 2010). In this study, levels of four proteins secreted by Sertoli cells in testes or serum were affected by 1 mg/kg/day PFNA, with no NOAEL identified. These include increases in Mullerian inhibiting substance and Wilms tumor protein (both involved with regulation of testosterone production and spermatogenesis), and decreases in transferrin (delivers iron needed for sperm production), and the glycoprotein inhibin B (modulates FSH secretion) which is considered to be a marker of testicular toxicity in rodents.

Effects on the male reproductive system were also reported in rats after 18-21 weeks of exposure to Surflon S-111 in the two-generation study (Stump et al., 2008). Sperm motility and progressive motility were significantly decreased in high dose (0.44 mg/kg/day PFNA/0.6 mg/kg/day Surflon S-111) F_1 males. Although it is stated that this effect is not test related because reproductive organ weights were not affected, the data tables show significantly decreased left epididymis weight in F_0 and F_1 males, and significantly decreased left epididymis sperm concentration in F_0 males, at 0.6 mg/kg/day Surflon S-111 (0.44 mg/kg/day PFNA).

Reproductive/developmental effects

Four studies of reproductive and developmental effects of PFNA have been conducted (Table 7F).

A study of CD-1 mice with gestational exposure to PFNA (1-10 mg/kg/day) evaluated reproductive outcomes, fetal abnormalities, and postnatal development (Das et al, 2015; previously reported as Lau et al., 2009). Since PPAR-alpha is known to mediate some of the developmental effects of the related compound PFOA, a second study in wild type (WT) and PPAR-alpha null ("knockout", KO) strains of mice was undertaken to elucidate the role of PPAR-alpha in developmental toxicity of PFNA (0.83 to 2 mg/kg/day; Wolf et al., 2010). The designs of these two mouse studies were similar to the analogous studies of PFOA at higher doses in CD-1 mice (1-40 mg/kg/day) and WT and KO mice (0.1-20 mg/kg/day) from the same laboratory (Lau et al., 2006; Abbott et al., 2007). A two-generation study of Surflon S-111 (0.019-0.44 mg/kg/day PFNA) assessed reproductive parameters and postnatal development in rats (Stump et al., 2008). Finally, a study using only one dose level of PFNA (5 mg/kg/day) in rats exposed on GD 1-20 focused on endpoints related to elevated blood pressure in offspring (blood pressure, nephron endowment, renal glucocorticoid receptor mRNA level; Rogers et al., 2014).

As discussed below, several developmental endpoints affected by PFNA in mice were not impacted by PFNA in rats (Stump et al., 2008; Wolf et al., 2010; Das et al., 2015). As is the case for PFOA, PFNA is excreted much more quickly in female rats than in female mice or male rats (see Toxicokinetics, above). Thus, the rat may not be the most appropriate model for evaluation of developmental effects of PFNA because the developing rat fetus receives a much lower dose than the developing mouse fetus at the same maternally administered dose.

PFNA in serum and liver in reproductive/developmental studies

As discussed above, comparisons between effect levels among animal studies of PFNA are most appropriately made on the basis of serum levels rather than administered dose. PFNA

levels in serum and liver are reported in the two mouse studies (Das et al., 2015; Wolf et al., 2010), but were not reported in the rat studies (Stump et al., 2008; Rogers et al., 2014). Das et al. (2015) measured PFNA levels in liver and serum in adult females and in fetal livers on GD 17 (one day after dosing ended), in adult females sacrificed on PND 28 (four weeks after dosing ended), and in pups on PND 1, 10, 24, 42, and 70. In Wolf et al. (2010), serum PFNA was measured in adult females and in two pups per litter only on PND 21 (23 days after the last dose).

While PFNA was not measured in breast milk in the mouse studies, the transfer of PFOA to milk in mice and the presence of PFCs including PFNA in human breast milk (discussed above) are well established (reviewed in White et al., 2011; Post et al., 2012). Exposure to PFNA in pups is therefore assumed to result from both *in utero* exposure to the PFNA administered to the mothers during pregnancy and from PFNA that was transferred to the breast milk after maternal dosing had ended.

The maternal serum data from GD 17 (one day after dosing ended) in CD-1 mice (Das et al., 2015) are valuable for quantitative risk assessment (see below) because they provide information on the maximum internal PFNA exposure to these mice at a time point when an endpoint for toxicity (maternal liver weight) was assessed. On GD 17, PFNA levels increased with dose in both serum and liver from pregnant and non-pregnant adult females, and in fetal livers. Serum levels were higher in non-pregnant than in pregnant adult female mice, with the lower levels in the pregnant mice presumably due to transfer to the fetal compartment.

On PND 28 (post-weaning, four weeks after dosing ended), serum levels were lower in dams that had nursed than in pregnant mice on GD 17 at 1 and 3 mg/kg/day, but were similar in pregnant GD17 versus post-weaning adult females at 5 mg/kg/day (Das et al., 2015). Although these PND 28 data do not reflect the maximum internal PFNA exposures which may have caused toxicity, they do provide information on the time course of internal exposure and the persistence of PFNA in these mice.

PFNA concentrations in liver generally paralleled serum levels in pregnant, non-pregnant, and post-weaning (PND 28) female adults and were several fold higher than serum PFNA levels in the same animals (Das et al., 2015). The PFNA levels in fetal livers on GD 17 were similar to the maternal serum levels at the same time point.

Serum levels in offspring soon after birth (PND 1) were close to or higher than maternal serum levels at a similar time point (GD 17). In offspring, PFNA levels in serum and liver decreased over time, with a slower decline in liver than in serum. At PND 1, PFNA levels in liver were about 3-fold higher than in serum. By PND 70, PFNA in serum had decreased to about 4-7% of PND 1 levels, while liver PFNA concentrations on PND 70 were about 12-18% of PND 1 levels.

PFNA persisted at low levels in liver and serum of treated offspring until 43 weeks (10 months), the last time point assessed. In male offspring at age 10 months, PFNA in serum was about 1% of PND 1 levels, while levels in liver were about 2-4% of PND 1 levels; liver and serum levels in female offspring were lower than in males at this time point. (Numerical

data obtained from C. Lau).

In the study of WT and PPAR-alpha KO mice (Wolf et al., 2010), serum PFNA was measured in adult females and in two pups per litter on PND 21 (23 days after the last dose). The serum levels measured at this time point in the adult females would be considerably lower than at the end of the dosing period. This decrease reflects excretion of PFNA in all adult females (pregnant and non-pregnant), as well as transfer to breast milk in those adult females which had delivered and nursed live pups. These serum levels thus do not reflect the maximum internal PFNA exposures which may have resulted in the observed toxicity and are not appropriate for use in quantitative risk assessment. However, these data are useful for comparison of the dose-response for toxicity in the two strains of mice (WT and PPAR-alpha KO) on the basis of internal, rather than external dose. Notably, in mice that had given birth, PFNA serum levels in the KO strain were much lower than in the WT strain given the same dose of PFNA, for reason(s) that were not determined.

Maternal weight gain and pregnancy outcome

In CD-1 mice, PFNA at 10 mg/kg/day caused substantial maternal weight loss and 100% full litter resorptions (Das et al., 2015). At lower doses ($\leq$5 mg/kg/day), maternal body weight gain was not affected, and there were no significant effects on reproductive parameters (full litter resorptions, the number of implants, number or percent live fetuses, prenatal liver loss, or fetal weight; Das et al, 2015).

The study of WT and PPAR-alpha KO mice suggests that PFNA caused both PPAR-alpha dependent and PPAR-alpha independent reproductive effects. PFNA ($\leq$ 2 mg/kg/day) did not affect maternal weight gain, number of uterine implants, or number of live plus dead pups per litter in either strain of mice. In WT mice, the number of live pups at birth was decreased at all doses (significant at some doses), and percent litter loss was increased at the highest PFNA dose, although this change was not significant (Wolf et al., 2010). Pregnancy rate was significantly ($p<0.001$) reduced in the PPAR-alpha KO mice, but was not affected in WT mice, suggesting that PFNA affects implantation in the absence of functioning PPAR-alpha (Wolf et al., 2010).

In the two-generation rat study of Surflon S-111, the fertility index was significantly decreased only in the low dose (0.025 mg/kg/day Surflon S-111/ 0.019 mg/kg/day PFNA) F_0 males and females. No effects were seen on other reproductive parameters in F_0 or F_1 rats. There were no effects on maternal weight gain or other parameters such as number of pups born and live litter size at any dose (Stump et al., 2008).

Maternal weight gain was significantly decreased in rats given a higher dose of PFNA, 5 mg/kg/day, on GD 1-20 (Rogers et al., 2014).

Fetal abnormalities

Exposure to PFNA did not increase fetal skeletal or visceral abnormalities in fetuses in CD-1 mice (Das et al., 2015). These endpoints were not evaluated in the other reproductive/developmental studies.

Neonatal and postnatal mortality

PFNA caused neonatal mortality in gestationally exposed mouse pups, and this effect appears to be dependent on PPAR-alpha.

In CD-1 mice, pup survival was severely affected at 5 mg/kg/day, while this dose did not affect maternal weight gain, cause overt maternal toxicity, or impact pregnancy outcome (Das et al., 2015). Fewer than 20% of the 5 mg/kg/day pups survived to PND 21, compared to greater than 80% of the controls. The neonatal mortality in the 5 mg/kg/day offspring was gradual, with a sharp increase in pup deaths throughout the first 10 days of life. These pups were weak and failed to thrive, although lack of maternal care was not observed. Furthermore, milk was present in the stomachs of the pups after death, indicating that they had been able to suckle and swallow (C. Lau, personal communication).

Das et al. (2015) contrast the neonatal mortality from 5 mg/kg/day PFNA on PND 2-10 to the findings in similar studies with PFOA and PFOS conducted in the same laboratory (Lau et al., 2003; Lau et al., 2006). These other PFCs caused neonatal mortality in mice in the first two days of life at higher doses (10-20 mg/kg/day), but mortality did not continue to occur at later time points.

Das et al. (2015) suggest that the neonatal deaths in the 5 mg/kg/day PFNA pups may be due to effects on intermediary metabolism (i.e. the utilization of nutrients to produce energy needed for growth through glycolysis and other metabolic pathways). This conclusion is supported by studies showing that PFNA is more potent than PFOA or PFOS as an activator of PPAR-alpha, a receptor that is involved with regulation of intermediary metabolism (discussed in the Mode of Action section below).

In the study of WT and PPAR-alpha KO mice (Wolf et al., 2010), the number of live pups at birth and pup survival from birth to weaning was reduced in a dose-related fashion at all PFNA doses in WT groups; these decreases were significant at the two highest doses. Most pup deaths occurred within the first few postnatal days, and WT pup survival at PND 21 was reduced to 36% at 1.5 mg/kg/day and 31% at 2 mg/kg/day. These parameters were not affected by PFNA in the PPAR-alpha KO mice.

There were no effects on postnatal survival in F_1 or F_2 pups in the two-generation rat study that used doses up to 0.44 mg/kg/day (Stump et al., 2008), and this endpoint was not assessed in the study of rats exposed to 5 mg/kg/day during pregnancy (Rogers et al., 2014).

Pup Body Weight

Body weights of CD-1 mouse pups on PND 1-24 were decreased by PFNA in a dose-related fashion at all doses, with statistical significance at 3 and 5 mg/kg/day (Das et al., 2015). At weaning, body weight decreases were substantial (27% and 50% lower than in controls at 3 and 5 mg/kg/day, respectively). These statistically significant body weight decrements persisted in both male and female offspring after weaning, and remained statistically significant in males until PND 287 (9 months of age) when most of the PFNA had been eliminated. It is the opinion of the study authors that the body weight decrements at 9 months of age are unlikely to be attributable to the low concentrations of PFNA remaining in the body at this time point (C. Lau, personal

communication).

These persistent delays in growth from PFNA are in contrast to the findings in a PFOA study of similar design in CD-1 mice in the same laboratory (Lau et al., 2006). In the PFOA study, body weights of pups from mothers dosed with 3 or 5 mg/kg/day during gestation were 25-30% lower than controls at weaning, but recovered and reached control levels by age 6.5 weeks in males and 13 weeks in females.

Pup weight at birth was not affected by PFNA ($\leq$ 2 mg/kg/day) in WT or KO mice (Wolf et al., 2010). Weight gain from birth until weaning was reduced in male and female WT pups at 2 mg/kg/day, but not at lower doses in WT or at any dose in PPAR-alpha KO pups.

In rats, there were no effects on body weight through weaning in F_1 or F_2 pups in the two-generation study at doses up to 0.44 mg/kg/day (Stump et al., 2008) or at PND 21 through 56 weeks of age in offspring exposed gestationally to a higher dose, 5 mg/kg/day (Rogers et al., 2014).

Developmental Delays

Markers of postnatal development were evaluated in three reproductive/developmental studies (Das et al., 2015; Wolf et al., 2010; Stump et al., 2008), but were not evaluated by Rogers et al. (2014).

In CD-1 mice, PFNA caused dose-dependent delays in day of eye opening, day of vaginal opening, and day of preputial separation, with statistically significant delays for all three endpoints in the 3 and 5 mg/kg/day PFNA groups (Das et al., 2015). The magnitude of these delays at 3 mg/kg/day PFNA were similar to those at the highest dose (20 mg/kg/day) in the analogous PFOA study (Lau et al., 2006), while 5 mg/kg/day PFNA caused much greater delays than 20 mg/kg/day PFOA (Table 8).

Table 8. Comparison of developmental delays in CD-1 mice from PFNA and PFOA

Endpoint	**PFNA (Das et al., 2015)**		**PFOA (Lau et al., 2006)**
	3 mg/kg/day	*5 mg/kg/day*	*20 mg/kg/day*
Eye opening	2 days	5 days	~3 days
Vaginal opening	3 days	7 days	~3 days
Preputial Separation	2 days	5 days	~1 day

Day of eye opening was also significantly delayed at 2 mg/kg/day PFNA in WT pups, but was not affected at lower doses in WT pups or at any dose in PPAR-alpha KO pups (Wolf et al., 2010). The other developmental endpoints evaluated by Das et al. (2015) were not assessed by Wolfe et al. (2010).

In the two-generation rat study (Stump et al., 2008), day of preputial separation and vaginal opening were not affected in F_1 pups at doses up to 0.44 mg/kg/day. These endpoints were not assessed in F_2 pups, and day of eye opening was not evaluated.

Effects on liver weight in adults, fetuses, and offspring

Adult females

Effects on liver weight in adult females in the three reproductive/developmental studies are summarized in the section on Hepatic Toxicity and Table 7B (above). They are discussed in more detail below because this endpoint is the basis for the quantitative risk assessment for PFNA (below). Liver weight was not assessed by Rogers et al. (2014).

Absolute and relative liver weights were statistically significantly increased in a dose-related fashion at all doses in pregnant and non-pregnant female CD-1 mice on GD 17 (Das et al, 2015). Maternal serum levels were also measured at this time point (discussed above). Dose-related statistically significant increases in absolute and relative liver weight persisted at the two higher doses (3 and 5 mg/kg/day) in dams on PND 28 (4 weeks after dosing ended).

Absolute and relative liver weights were increased by PFNA on PND 21 (23 days after the last dose) in both WT and PPAR-alpha KO mice (Wolf et al., 2010). Relative liver weights were increased at all doses in non-pregnant WT and PPAR-alpha KO mice, with significance in all groups except the lowest dose PPAR-alpha KO. In mice that had given birth, relative liver weight was significantly increased at all doses in WT, but was not increased in PPAPR-alpha KO at any dose. In interpreting these results, it is important to note that in mice that had given birth, PFNA serum levels in the KO mice were much lower than in the WT mice for reason(s) that were not determined. The NOAELs and LOAELs for increased liver weight based on serum levels are consistent in these WT and KO mice, suggesting that the differences in response is likely due to kinetic differences unrelated to PPAR-alpha status.

Absolute and relative liver weights were also increased in F_0 and F_1 females exposed to 0.6 mg/kg/day Surflon S-111 (0.44 mg/kg/day PFNA) for 18-21 weeks at post-weaning sacrifice (Stump et al., 2014). Lower dose groups were not evaluated.

Fetal liver weights

In CD-1 mice, absolute and relative fetal liver weights were significantly increased at all doses of PFNA, except that the increase in absolute liver weight was not significant at 5 mg/kg/day. The magnitude of the increases in liver weight was similar in all dose groups (Das et al., 2015). This endpoint was not evaluated in the other three reproductive/developmental studies.

Pup liver weights

Pup liver weight was evaluated at PND 1, 10, 24, 42, and 70 in CD-1 mice (Das et al., 2015), on PND 21 in WT and PPAR-alpha KO mice (Wolf et al., 2010), and on PND 21in F1 and F2 rats (Stump et al, 2008). This endpoint was not assessed by Rogers et al. (2014).

In CD-1 mouse pups, relative liver weights were increased in a dose-related fashion on PND 1 through PND 70. These increases were significant at all doses (1, 3, and 5 mg/kg/day) on PND 1 and 24, and at 3 and 5 mg/kg/day on PND 42 (Das et al., 2015).

In the study of WT and KO mice, relative liver weight on PND 21 was increased at all doses ($\geq$ 0.83 mg/kg/day) in WT mice but only at the highest dose (2 mg/kg/day) in PPAR-alpha KO mice (Wolf et al., 2010).

In rats in the two generation study, relative liver weights on PND 21 were significantly increased at $\geq$ 0.125 mg/kg/day Surflon S-111 (0.09 mg/kg/day PFNA) in F_1 pups and at 0.6 mg/kg/day Surflon S-111 (0.44 mg/kg/day PFNA) in F_2 pups.

Elevated blood pressure in offspring

This endpoint was evaluated only by Rogers et al. (2014). Systolic blood pressure was significantly increased on PND 10 in male and female rat pups after gestational exposure to 5 mg/kg/day PFNA. Blood pressure was not increased at later time points (PND 26 and 56). In male pups, nephron endowment (number of functioning nephrons present at birth) in renal glomeruli was significantly decreased when evaluated on PND 22; no effect was observed in female pups. These changes in nephron endowment were not associated with changes in body weight or kidney weight.

Summary of reproductive/developmental effects

In both mouse studies, PFNA caused dose-dependent maternal and developmental effects including postnatal mortality, decreased pup weight gain, and delays in reaching markers of development (Das et al., 2015; Wolf et al., 2010). It is notable that decreased growth from developmental exposure persisted well into adulthood (age 9 months), long after PFNA had been eliminated from the serum (Das et al., 2015). Both Das et al. (2015) and Wolf et al. (2010) conclude that PFNA is more potent than PFOA as a developmental toxicant, based on studies of PFOA in the same strains of mice used in the PFNA studies (Lau et al., 2006; Abbott et al., 2007). The greater toxicity of PFNA as compared to PFOA is likely related to both its greater intrinsic potency and longer persistence in the body.

As discussed in the Mode of Action section below, PPAR-alpha is considered to have an important role in human reproduction and development (Abbott, 2009). Developmental parameters (number of live pups per litter, pup survival, day of eye opening, pup weight gain) were adversely affected by PFNA only in the WT mice (Wolf et al., 2010). Based on higher serum levels in PPAR-alpha KO pups than WT pups, the authors conclude that the lack of effects in PPAR-alpha KO pups is not attributable to lower PFNA levels in their serum. The developmental effects of PFNA seen in this study thus appear to be dependent on PPAR-alpha. In contrast, pregnancy rate was significantly affected by PFNA in the PPAR-alpha KO but not the WT strain, suggesting that the presence of functioning PPAR-alpha prevents this effect.

Decreased maternal body weight gain, decreased neonatal weight, and delayed development of pups (reduced body weight gain, delayed age of markers development) were not observed in the rat two-generation study (Stump et al., 2008), although a higher dose of PFNA (5 mg/kg/day) caused decreased maternal weight gain and decreased pup weight at birth in rats (Rogers et al., 2014). Relevant to this point, the highest dose used in the rat two-generation study (0.44 mg/kg/day PFNA) was lower than the lowest doses in the mouse studies (0.83 and 1 mg/kg/day) and PFNA serum data are not available for this study. These results from developmental studies of PFNA in rats and mice are consistent with results in mice and rats for the related compound, PFOA (reviewed in Post et al., 2012). As is the case for PFOA, the rat may not be an appropriate model for assessment of developmental effects of PFNA because it is eliminated much more rapidly in female rats than in female mice (see Toxicokinetics, above). For this reason, the developing rat fetus receives a much lower dose than the developing mouse

fetus at the same maternally administered dose.

Additional considerations related to developmental toxicity

The potential for PFNA to cause the specific low-dose developmental effects seen in mice given 0.01 mg/kg/day of the closely related compound, PFOA, including delayed mammary gland development and histopathological effects on female reproductive system (reviewed in Post et al., 2012; Tucker et al., 2014), has not been evaluated. Other long chain PFCs, including PFOA, PFOS, and PFHxS, caused neurobehavioral effects in mice after developmental exposures (Johansson et al., 2008: Viberg et al., 2013), but PFNA has not been tested for these effects.

Additional systemic endpoints

In the subchronic and the two-generation studies of Surflon S-111 in rats (Mertens et al., 2010; Stump et al., 2008), effects were reported on several endpoints that were not included in other toxicology studies.

Hematology

In the subchronic study (Mertens et al., 2010), several hematological parameters were affected in the high dose (0.6 mg/kg/day Surflon S-111; 0.44 mg/kg/day PFNA) males at the end of the 13 week dosing and/or after the recovery period at week 21. These include increased prothrombin time and lymphocytes, and decreased red cells, hemoglobin, and hematocrit, and reticulocytes. No effects were seen in females.

Clinical Chemistry

In the high dose (0.6 mg/kg/day Surflon S-111/0.44 mg/kg/day PFNA) males in the subchronic study (Mertens et al., 2010), serum protein and globulin were decreased, while bilirubin, BUN, chloride (data not shown), and alkaline phosphatase were increased. In the mid dose (0.125 mg/kg/day Surflon S-111/0.09 mg/kg/day PFNA) males and high dose (0.6 mg/kg/day Surflon S-111/0.44 mg/kg/day PFNA) females, only increased alkaline phosphatase and decreased globulin were observed. The effects on these clinical chemistry parameters were more pronounced in males than females.

In an unpublished study of Surflon S-111 in male rats (Wolterbeek, 2004), serum phospholipids, cholesterol, and calcium were decreased in male rats in all dose groups (0.1, 0.5, and 2 mg/kg/day) after 14 days of exposure. Calcium and cholesterol were not affected in the 90 day study and phospholipids were not measured (Mertens et al., 2010).

Gastrointestinal Effects

Inflammation, ulceration, erosion, and hyperplasia occurred in the duodenum and stomach of some males in the 0.6 mg/kg/day Surflon S-111 (0.44 mg/kg/day PFNA) group. Minimal stomach erosion persisted in one male in the recovery group. In females, only the control and high dose (0.6 mg/kg/day Surflon S-111, 0.44 mg/kg/day PFNA) were evaluated, and these effects were not seen. Histopathological examination of the gastrointestinal organs was not performed in the rat two generation study of Surflon S-111 (Stump et al., 2010).

Chronic effects and carcinogenicity

Chronic studies, including studies designed to detect carcinogenicity have not been conducted

for PFNA. PFOA and PFOS, the two long-chain biologically persistent PFCs for which chronic studies have been conducted, caused tumors in rats (Sibinski, 1987; Biegel et al., 2001; Thomford, 2002).

Summary of toxicity in experimental animals

PFNA and/or Surflon S-111 (a mixture of PFCs consisting primarily of PFNA) caused multiple toxic effects in rats and mice. Detailed evaluation of the results of the Surflon S-111 studies support the conclusion that its toxicity is primarily caused by PFNA.

Effects of PFNA (and/or Surflon S-111) in rodents include body weight loss, increased liver weight and histopathological changes in the liver including necrosis, increased kidney weight and histopathological changes in the kidney, atrophy of spleen and thymus and changes in immune cell populations in these organs, histopathological changes and other effects in the testes, decreased pregnancy rate, maternal weight loss and full litter resorptions, neonatal and postnatal mortality, decreased weight gain persisting into adulthood in offspring, and delays in reaching markers of postnatal development.

Reproductive/developmental studies of both PFNA and the closely related compound, PFOA, have been conducted in CD-1 mice and in WT and PPAR-alpha KO mice. Comparison of the results of the studies of these two compounds indicates that PFNA is more toxic to reproduction and development than PFOA. Similarly, authors of the subchronic and two generation rat studies of Surflon S-111 concluded that Surflon S-111 is more toxic than PFOA, based on comparison with similar studies of PFOA in rats (Mertens et al., 2010; Stump et al., 2008). Notably, similar decreases in body weight in males occurred from 0.6 mg/kg/day Surflon S-111 (0.44 mg/kg/day PFNA) and 30 mg/kg/day PFOA in two generation studies (Stump et al., 2008). The greater toxicity of PFNA as compared to PFOA is likely due to both its longer persistence in the body and its higher intrinsic potency.

PFNA causes both PPAR-alpha dependent and PPAR-alpha independent toxicity, as is also true for PFOA. PFNA caused increased liver weight (adult, fetal, and pup) in both WT and PPAR-alpha KO mice. Some developmental/reproductive effects (number of live pups per litter, pup survival, day of eye opening, pup weight gain) occurred only in WT mice. Based on knowledge of the role of PPAR-alpha in human reproduction and development, these PPAR-alpha dependent effects are considered relevant to humans. In contrast, pregnancy rate was decreased by PFNA only in PPAR-alpha KO mice. Additionally, PFNA caused hepatic necrosis, an effect not typically associated with PPAR-alpha activation, at a dose which did not increase liver weight in rats exposed for 18-21 weeks.

Some endpoints of toxicity, including increased kidney weight and histopathological changes in the kidney, occurred after 18-21 weeks of exposure in the two-generation study, but not in the 13 week subchronic study which used the same doses of Surflon S-111. Furthermore, hepatic necrosis occurred only at the highest dose in the 13 week study but at all doses in the 18-21 week study. These results suggest that 13 weeks not a sufficient exposure duration to produce some of the effects that occur from longer exposures, and that toxicity occurs at lower doses from exposures longer than 13 weeks.

The half-life of PFNA differs among species and is much shorter in female rats than male rats. As expected from the rapid excretion of PFNA in female rats, higher administered doses were required to cause toxicity in female rats than in male rats. Similarly, reproductive and developmental effects occurred at higher administered doses in rats than in mice, a species in which females excrete PFNA slowly.

Because of the large differences in excretion rates in species and genders, quantitative risk assessment and extrapolation to human exposure levels are most appropriately based on internal dose (serum level) rather than administered dose. PFNA serum levels were evaluated in one 14 day study in male mice and two reproductive/developmental studies in mice. Additionally, serum levels that can be roughly estimated from graphs provided in the subchronic rat study are useful for comparison to other studies but are not appropriate for quantitative risk assessment.

Although the studies that do not provide data on PFNA serum levels are not appropriate as the basis for quantitative risk assessment, results of some of these studies suggest that PFNA causes toxicity at similar or lower internal doses lower than in the studies of similar duration which do provide serum levels. These results (discussed in detail above) include increased liver weight at 0.45 mg/kg/day in mice dosed for 21 days (Kennedy, 1987); increased serum glucose and other effects at 1 mg/kg/day, and related biochemical effects at 0.2 mg/kg/day, in mice dosed for 14 days (Fang et al., 2012a); immunotoxicity at 1 mg/kg/day in mice dosed for 14 days (Fang et al., 2008); and damage to testicular Sertoli cells and other related effects at 1 mg/kg/day in rats dosed for 14 days. Additionally Stump et al. (2008) and Mertens et al. (2010) discuss an unpublished study (Wolterbeek, 2004) in which serum cholesterol, calcium, and phospholipids were decreased in male mice dosed with 0.1 mg/kg/day Surflon S-111, a PFC mixture containing primarily PFNA, for 14 days.

There are several important data gaps in the toxicity information available for PFNA. Chronic studies, including studies designed to detect carcinogenicity, have not been conducted. The two other long chain PFCs (PFOA and PFOS) that have been tested chronically caused tumors in rats. Furthermore, additional non-carcinogenic toxicity from longer exposure durations may occur with chronic exposure. A two-generation study of PFNA in a species, such as the mouse, that is appropriate for evaluating reproductive/developmental effects has not been conducted. Additionally, the potential for PFNA to cause the specific low-dose developmental effects seen in mice at low doses of PFOA, including delayed mammary gland development, has not been evaluated. Finally, PFNA has not been tested for developmental neurotoxicity, such as has been found in studies of other long chain PFCs.

MODE OF ACTION

Although there is a considerable body of information on the mode(s) of action (MOA) for PFNA and other PFCs, the MOA of these compounds has not been fully characterized.

Perfluorinated carboxylic acids such as PFNA structurally resemble free fatty acids, and thus may act similarly to a free fatty acid in activating nuclear receptors such as PPAR-alpha, CAR (constitutive androstane receptor) and PXR (pregnane X receptor), binding to transporters and carrier proteins, and interacting with membranes (Butenhoff, 2009). However, PFNA and

other PFCs are non-reactive and thus are not substrates for biochemical reactions involving fatty acids.

Genotoxicity

In general, because PFNA and other PFCs are non-reactive and are not metabolized to reactive intermediates, they do not covalently bind to nucleic acids and proteins and are therefore unlikely to be genotoxic. PFOA and PFOS were not mutagenic in several *in vitro* assays in bacterial and mammalian cells, and did not induce micronuclei in mice *in vivo* (reviewed by USEPA, 2005; ATSDR, 2009). Only one study of the genotoxic potential of PFNA was located (Eriksen et al., 2010). In this study, five PFCs (PFNA, PFOA, PFHxA (C6), PFOS, and perfluorobutane sulfonic acid) were evaluated for their potential to generate reactive oxygen species and cause oxidative DNA damage in human HepG2 cells. Of the PFCs tested, only PFNA caused a modest but statistically significant increase in DNA strand breaks at concentrations of 100 µM and 400 µM, but this effect of PFNA was not related to generation of reactive oxygen species. Although it was stated that PFNA increased strand breaks only at cytotoxic concentrations, this point is unclear because cytotoxicity did not occur at 100 µM PFNA.

PPAR-alpha dependent and PPAR-alpha independent effects

PFNA activates PPAR-alpha, as well as other nuclear receptors such as constitutive androstane receptor (CAR) and pregnane X receptor (PXR), in rodents (Rosen et al., 2010; Das et al., 2015). These receptors are found in many tissues, and activation of these receptors affects the expression of genes involved in many pathways related to carcinogenicity, hepatotoxicity, metabolic functions, developmental toxicity, immunotoxicity, and other effects (Lau, 2012). Like other long chain PFCs, PFNA increases the activity of hepatic peroxisomal beta-oxidation biochemical, a marker for hepatic peroxisome proliferation in mice and rats (Goecke-Flora and Reo, 1996; Kudo et al., 2000; Kudo and Kawashima, 2003; Kudo et al., 2006; Mertens et al., 2010). PFNA induced the expression of genes in functional categories normally associated with PPAR-alpha in both WT and PPAR-alpha KO mice, suggesting that other PPAR isoforms (i.e. beta, delta, and/or gamma) present in the PPAR-alpha KO mice are activated by PFNA (Rosen et al., 2010). Genes associated with CAR were also activated by PFNA in WT and PPAR-alpha KO mice (Rosen et al., 2010).

PPAR-alpha is found in human liver, and fibrate drugs bring about decreases in cholesterol and lipids in humans through activation of hepatic PPAR-alpha. However, the human relevance of hepatic toxicity in rodents by environmental contaminants that activate PPAR-alpha has been subject to debate because of the lower levels and/or lower intrinsic activity of PPAR-alpha in human liver (reviewed in Post et al., 2012). The hepatic effects of PFNA in rodents have both PPAR-alpha dependent and PPAR-alpha independent components (Rosen et al., 2010; Wolf et al., 2010). Absolute and relative liver weights were increased by PFNA in both wild type (WT) and PPAR-alpha knockout (KO) mice (Wolf et al., 2010). In mice that had given birth, the magnitude of increased liver weight in WT and KO mice were similar when the two strains were compared on the basis of PFNA serum levels 23 days after dosing ended (Wolf et al., 2010). Additionally, PFNA caused hepatic necrosis, an effect not typically associated with PPAR-alpha activation, at a dose which did not increase liver weight in rats exposed for 18-21 weeks (Stump et al., 2008).

Furthermore, it is well established that the observed hepatic toxicity of the closely related compound, PFOA, also has both PPAR-alpha dependent and PPAR-alpha independent components (reviewed in Post et al., 2012). Two recent studies provide important additional data related to this issue. In CD-1 mice exposed *in utero* to PFOA, hepatocellular hypertrophy was observed at 3 and 13 weeks of age. These changes were accompanied by mitochondrial alterations in the liver cells, while there was no evidence of hepatic peroxisome proliferation, a marker of PPAR-alpha activation (Quist et al., 2015). A second study evaluated liver lesions at age 18 months in CD-1, WT, and PPAR-alpha KO mice with prenatal exposure to PFOA (Filgo et al., 2015). Liver carcinomas and/or adenomas did not occur in controls in any of the three mouse strains, but were found in PFOA-treated CD-1 and PPAR-alpha KO mice, but not WT mice. Non-neoplastic hepatic effects (hepatocellular hypertrophy, bile duct hyperplasia, and hematopoietic cell proliferation) were more frequent and/or more severe in PPAR-alpha KO than WT mice.

Wang et al. (2015) found that PFNA increased hepatic lipids only at lower doses (0.2 and 1 mg/kg/day, but not 5 mg/kg/day). In contrast, serum lipids were decreased only at the two highest doses. PFNA affected expression of genes and proteins leading to up-regulation of both PPAR-alpha and sterol regulatory element-binding proteins (SREBPs). PPAR-alpha promotes fatty acid oxidation and lipolysis, leading to decreased hepatic and serum lipids, while SREBPs have the opposite effect, promotion of lipid biosynthesis leading to increased hepatic and serum lipids. Wang et al. (2015) concluded that the different changes in serum and hepatic lipids at low versus high PFNA doses may result from the balance between these opposing effects. A potential explanation for the increased serum levels from PFNA and other PFCs in humans, but not rodents, is that these the balance between lipolysis and lipid biosynthesis may differ in these species due to interspecies differences in PPAR-alpha versus SREPBs.

Some developmental/reproductive effects of PFNA (number of live pups per litter, pup survival, day of eye opening, pup weight gain) occurred only in WT mice and appear to be PPAR-alpha dependent, while pregnancy rate was decreased by PFNA only in PPAR-alpha KO mice. The uncertainty about human relevance of hepatic effects of PPAR-alpha does not apply to PPAR-alpha's role in developmental toxicity and other non-hepatic effects, or to other PPAR isoforms such as PPAR-gamma. PPAR-alpha and other PPAR isoforms are expressed in many fetal and adult tissues in rodents and humans (Abbott et al., 2010). Based on their physiological roles, PPAR-alpha and other forms of PPAR are expected to have important roles in reproduction and development in these species (Abbott, 2009). In regard to human relevance of PPAR-alpha mediated effects of PFNA on development in mice, Wolf et al. (2010) state: "Relevance of the PPAR-alpha mechanism to humans has been criticized primarily based on the lower number of these receptors in the liver of human versus mouse. However, PPARα is implicated here in the developmental effects of PFNA as well, and the etiology of PPARα in other tissues of the embryo, fetus and neonate of the human and the mouse that are involved in gross development has not been fully determined. Therefore, the possibility of relevance of PPARα to a human response to PFNA cannot be dismissed." Additionally, the USEPA Science Advisory Board (2006) concluded that available data are insufficient to dismiss the relevance of the PPAR-alpha MOA in children.

Das et al. (2015) suggest that the neonatal deaths in the 5 mg/kg/day PFNA pups may be due to effects on intermediary metabolism (i.e. the utilization of nutrients to produce energy needed for growth through glycolysis and other metabolic pathways). Gene expression profiles in livers from fetuses and pups exposed to PFNA during gestation showed activation of expression of genes related to homeostatic control of lipid and glucose metabolism, including PPAR-alpha, CAR, and PXR (Das et al., 2015). A similar hypothesis has been suggested for the neonatal mortality and decreased pup growth caused by PFOA. Like PFNA, gestational exposure to PFOA affected the expression of genes associated with PPAR-alpha, other PPARs, CAR, and PXR, in both fetal and neonatal mice (Rosen et al., 2007; Abbott et al., 2010).

Both PPAR-alpha and PPAR-gamma were activated by PFNA in the thymus of mice and rats and in rat spleen (Fang et al., 2008, 2009, 2010). Based on these results, Lin et al. (2011) hypothesize that the increased levels of the hormone adiponectin associated with human PFNA exposure may be related to activation of PPAR-gamma. In the mouse thymus, PPAR-alpha and PPAR-gamma were activated at only the lowest dose of PFNA, while immunotoxicity was more severe at higher doses. The authors concluded that some immunotoxic effects are independent of both PPAR-alpha and PPAR-gamma and may be due to increased levels of ACTH and cortisol (Fang et al., 2008).

Estrogenic activity may also be involved in the mode of action of PFNA and other PFCs. Studies in rainbow trout, a species which has long been used as a model for human liver carcinogenesis because it is insensitive to peroxisome proliferation, suggest that PFNA and other PFCs have estrogenic activity and can promote liver tumor development through an estrogenic mechanism (Benninghoff et al., 2011, 2012).

Other MOA studies

The toxicity of PFNA in rodent spleen and thymus is associated with impairment of cell cycle progression and increased apoptosis in these immune system organs (Fang et al., 2008, 2009, 2010). The proportion of less mature versus mature cells in the thymus was affected by PFNA (Fang et al., 2008). In the spleen, the innate splenic immune cells appeared to be a specific target for PFNA. Potential modes of action for the immunotoxic effects of PFNA suggested by these studies include increased levels of the hormones ACTH and cortisol, altered levels of cytokines which regulate immune function, and induction of apoptosis via oxidative stress and activation of a mitochondria-related caspase-independent death signaling pathway.

The mode of action for testicular toxicity of PFNA in rats was investigated by Feng et al. (2009; 2010). Histological examination, staining for DNA fragmentation (TUNEL assay), and flow cytometry showed that apoptosis in testicular cells was increased in a dose-dependent manner by PFNA (Feng et al., 2009). Two independent pathways are involved with apoptosis in the testes. Data on gene expression and protein levels in the testes suggested that PFNA activates the death receptor pathway, but not the mitochondrial-dependent pathway, for apoptosis.

PFNA exposure affects multiple functions of Sertoli cells, testicular cells important for spermatogenesis (Feng et al., 2010). The ultrastructural disruptions observed *in vivo* may be related to upregulation of the intermediate filament protein vimentin. PFNA affected levels in testes or serum of four proteins secreted by Sertoli cells, and these effects are consistent with *in*

vitro studies of primary cultured Sertoli cells. Increases in MIS, WT1, and ABP may indirectly reduce free testosterone in testes, consistent with decreased testosterone in PFNA-treated rats observed by Feng et al. (2009). Decreased inhibin B is considered a marker for testicular toxicity and may indicate impaired secretory function of Sertoli cells and testicular sperm production. Decreases in transferrin and FSH-R caused by PFNA may impair spermatogenesis in the testes. Transferrin delivers iron needed for sperm production to the germ cells, and FSH-R (the receptor for FSH) regulates many Sertoli cell functions.

Fang et al. (2012b,c) suggest that effects on both Kupffer cells (hepatic macrophages) and hepatocytes contribute to the liver toxicity of PFNA. PFNA affected both expression of genes related to lipid metabolism in hepatocytes and release of cytokines related to inflammation from Kupffer cells (Fang et al., 2012c). Exposure to PFNA activates Kupffer cells, leading to release of the cytokines TNF alpha and IL1-beta. These cytokines suppress the expression of hepatic PPAR-alpha in hepatocytes, contribute to the accumulation of lipids in the liver, and increase the toxicity of PFNA to hepatocytes as assessed by serum liver enzyme levels. Consistent with these effects, inactivation of Kupffer cells by $GdCl_3$ affected the hepatic effects of PFNA. When Kupffer cells were inactivated, PFNA caused greater expression of PPAR-alpha, smaller increases in hepatic triglycerides, and smaller increases in serum levels of liver enzymes. *In vitro* studies were consistent with the in *vivo* studies.

Fang et al. (2012a) investigated potential modes of action for increases in serum glucose and hepatic glycogen caused by PFNA in mice. Hepatic levels of four proteins that are part of the insulin signaling pathway were significantly reduced at all doses of PFNA. A decrease in this pathway can induce insulin resistance leading to increased serum glucose. Additionally, hepatic levels of another protein, p-GSK3-beta, which is responsible for glycogen synthesis in the liver were increased at all doses, potentially explaining the observed increase in liver glycogen. Because the levels of p-GSK3-beta are regulated by the insulin signaling pathway, the increased levels of this protein could result from the inhibition of the insulin signaling pathway by PFNA. Oxidative stress may be involved in inhibiting the insulin signaling pathway.

Additional modes of action for PFNA and other PFCs have been suggested including oxidative stress, effects on other cell signaling pathways, inhibition of gap junctional intercellular communication, and epigenetic changes (reviewed by Lau, 2012).

MOA studies in zebrafish

Zebrafish are a useful vertebrate model for evaluating the underlying toxicological mode of action of environmental contaminants because of their rapid development and the similarity to higher vertebrates, including humans, of their genetic makeup, developmental processes, anatomy, physiology, and behavior. Studies of PFNA in zebrafish have evaluated effects on the liver, thyroid, development, and behavior. PFNA caused hepatic and thyroid toxicity at relatively low concentrations (0.05 – 0.1 mg/L) with no NOAEL identified, while developmental toxicity and behavioral effects occurred at higher PFNA concentrations.

Zhang et al. (2012a) studied liver toxicity and hepatic protein and gene expression in male zebrafish exposed to PFNA (0. 0.1, 0.5, and 1.0 mg/L) for 180 days. Histopathological changes in the liver became more severe as PFNA concentration increased and were similar to those

observed in rodents exposed to PFNA. Hepatic expression of 57 proteins related to many different function including metabolism of amino acids, tricarboxylic acid cycle intermediates, pyruvate, glucose, proteins, and nucleotides; structure and motility; stress and defense; signal transduction; and cell communication were affected at all PFNA concentrations. Results of gene expression studies indicated that the liver toxicity caused by PFNA could not be totally attributed to PPAR-mediated effects.

Hepatic effects differed in male versus female zebrafish exposed to PFNA (0, 0.01, 0.1, or 1 mg/L) for 180 days (Zhang et al., 2012b). PFNA levels were higher in males than in females exposed to the same concentration. In both sexes, body weight and length were decreased in a dose-dependent manner and hepatosomatic index (relative liver weight) was decreased significantly at lower doses, but not the high dose. In liver, total cholesterol was increased in both sexes, but triglycerides were increased in males and decreased in females. Furthermore, the pattern of expression of genes for 11 hepatic fatty acid binding protein in response to PFNA differed greatly between sexes. Expression 10 isoforms was increased in males and decreased in females exposed to PFNA, while one isoform showed the opposite pattern. PFNA also affected the expression of genes for proteins involved in regulation of fatty acid binding proteins (PPARs and Ccaat-enhancer binding proteins) differently in males and females.

Toxicity to the thyroid was reported in a trans-generational study of zebrafish exposed to PFNA (0, 0.05, 0.1, 0.5, and 1 mg/L) for 180 days (Liu et al., 2011b). In F_0 males exposed for 180 days starting at 23 days post fertilization, histopathological changes in the thyroid occurred at all PFNA concentrations and became more severe at higher concentrations. Plasma triiodothyronine (T3) was increased in those adult fish that were examined (F_0 males, and F_1 males and females) at concentrations as low as 0.05 mg/L. In livers of F_0 males, PFNA induced transthyretin, a protein involved with thyroid hormone transport, and decreased UDP-glucuronosyltransferases, an enzyme involved with thyroid metabolism. Gene expression related to thyroid hormone synthesis and metabolism was affected in F_1 larvae from parents exposed to PFNA, but that had developed in a PFNA-free environment, indicating that PFNA caused a transgenerational effect.

In a study of the effects of a series of PFCs on zebrafish development, fertilized eggs were exposed to PFNA (0, 6.25, 12.5, 25, 50, 100, and 200 mg/L). The developing embryos/larvae were examined before and after hatching until 96 hpf (hours post fertilization) (Zheng et al., 2011). At 72 hpf, the LC_{50} concentrations for lethality (opaque embryos) and the EC_{50} for delay of hatching (84 mg/L and 214 mg/L), respectively, were relatively high compared to concentrations of environmental concern. PFNA did not increase the occurrence of edema or malformations. Consistent with the results of this study, PFNA was not reported to cause malformations in rats or mice (Das et al., 2015; Stump et al., 2008). Based on LC_{50} values at 72 hpf, the relative potency of PFCs tested in this study was PFOS>PFNA>PFOA.

In a second study comparing developmental toxicity of a series of PFCs, fertilized zebrafish eggs were exposed to six concentrations of PFNA (0.03 – 10 mg/L) until 144 hpf (Ulhaq et al., 2013a). The LC_{50} was greater than 10 mg/L, and there was no effect on heart rate or hatching time within this concentration range. For sublethal effects, including spinal curvature, the EC_{50} was 16 mg/L. The relative toxicity of PFNA compare to other PFCs tested was PFOS>PFDA (C10)>PFNA>PFBS>PFBA, consistent with Zheng et al. (2012).

An additional study from this research group (Ulhaq et al., 2013b) evaluated effects on locomotor behavior in zebrafish larvae at PFC concentrations below those which caused lethality and/or overt toxicity. Exposure to PFNA at 10 mg/L caused a reduction in the activity of the zebrafish, while lower concentration ($\leq$ 3 mg/L) had no effect.

Relative potencies of PFNA and other PFCs

Based on comparison with similar studies of PFOA, PFNA (and Surflon S-111 consisting primarily of PFNA) is more potent than PFOA for developmental and systemic toxicity (Das et al., 2015; Mertens et al., 2010; Stump et al., 2008, Wolf et al., 2010). In support of this conclusion, PFNA (1 mg/kg/day) altered the expression of more genes than PFOA (3 mg/kg/day) in both WT and PPAR-alpha KO mice dosed for 7 days (Rosen et al, 2010). The greater toxicity of PFNA is likely due to both its longer persistence in the body (discussed above) and its greater intrinsic potency (discussed below).

In vitro studies of activation of mouse or human PPAR-alpha transfected into cultured cells by a series of PFCs provide information on the relative potency of these compounds for activation of PPAR-alpha. Based on the concentration eliciting 20% of maximal response, PFNA was the most potent activator of both mouse and human PPAR-alpha in a study of PFBA (C4), PFHxA (C6), PFOA (C8), PFNA (C9), and PFDA (C10) (Wolf et al., 2008). In a follow-up study that included additional PFCs (Wolf et al., 2012), longer chain PFCs (PFUnDA (C11) and PFDoA (C12)) were less potent activators of both the human and mouse PPAR-alpha receptors than PFNA, with particularly low activity for activation of the human receptor; these results indicate that potency for this effect in a series of perfluorocarboxylic acids does not necessarily increase with greater carbon chain length. It should be noted that activity in this assay is a measure of intrinsic potency and is independent of differences in half-life or other toxicokinetic parameters.

Gene activation profiles of a series of twelve PFCs including PFNA were also studied in primary hepatocytes from humans and mice (Rosen et al., 2013). PFNA was the most active PFC in human cells, based on the average molarity producing a 2-fold change in gene expression, and was also among the more active PFCs in mouse cells. However, the study's authors discuss results that indicate that primary hepatocytes are problematic as a model for *in vitro* effects, since many genes that are well known to be up-regulated by PFCs *in vivo* were not affected in these *in vitro* cell assays.

PFNA was also more potent than PFOA as a promoter of liver tumor development in rainbow trout after initiation with aflatoxin B_1 (Benninghoff et al., 2011). The liver tumor incidence after initiation with AFB was 13% with no promoter, 62% after promotion with PFOA (50 mg/kg/day), and 72% after promotion with PFNA (25 mg/kg/day). The percent of hepatic tumors which were adenomas (as opposed to carcinomas) was 30%, 15%, and 8% in the non-promoter, PFOA, and PFNA groups, respectively (Benninghoff et al., 2011). The number of tumors per fish and the mean tumor size were also higher for PFNA than PFOA. In another part of the study, changes in hepatic gene expression from PFCs were assessed. PFNA altered the expression of most of the same genes affected by PFOA and perfluorodecanoic acid (C10), but also altered the expression of a group of other genes not affected by the other two PFCs. In a subsequent study (Benninghoff et al., 2012), PFNA, as well as PFOA and PFDA, induced the

estrogen-dependent biomarker protein vitellogenin in young rainbow trout, and these PFCs also weakly activated the rainbow trout and human estrogen receptors *in vitro.*

Two additional studies evaluated the effects of PFNA and six other PFCs on *in vitro* effects on receptors for thyroid hormones, aryl hydrocarbons (AhR), androgens (AR), and estrogens (ER). The PFCs included in these studies were PFOA, PFNA, PFDA (C10), PFUnA (C11), PFDoA (C12), PFHxS, and PFOS. In the widely used T-screen assay, all of the PFCs except PFOA antagonized the effect of the thyroid hormone T3 on the growth of cultured rat pituitary GH3 cells. The potency of PFNA was similar to most of the other PFCs that caused this effect (Kjeldsen and Bonefeld-Jorgensen, 2013). Of the seven PFCs tested, only C10 and C12 activated AhR (Kjeldsen and Bonefeld-Jorgensen, 2013). PFNA did not induce transactivity of ER, while PFOA, PFOS, and PFHxS did cause this effect (Long et al., 2013). PFNA, as well as PFOA, PFOS, PFHxS, and C10, antagonized the effect of dihydrotestosterone on AR, and a mixture of these five PFCs caused a greater than additive effect on this endpoint (Long et al., 2013).

DEVELOPMENT OF HEALTH-BASED MCL

Weight of Evidence for Carcinogenicity

Health-based MCLs developed by the DWQI are intended to be protective for chronic (lifetime exposure) through drinking water. The 1984 Amendments to the NJ SDWA stipulate that Health-based MCLs be based on a one in one million lifetime cancer risk level for carcinogens and no adverse effects from lifetime ingestion for non-carcinogens.

There is no available information that can be used to evaluate the carcinogenic potential of PFNA because chronic carcinogenicity bioassays have not been conducted. Therefore, the Health-based MCL for PFNA is based on non-carcinogenic effects.

Key and Supporting Studies and Endpoints

Both the human epidemiology data and the animal toxicology were considered as part of the overall weight of evidence for the potential human health effects of PFNA. While some studies found associations of PFNA with health effects at exposures found in the general population, the extent of the available epidemiology data for PFNA is limited. Causality cannot be proven for the associations that were reported since they primarily come from cross-sectional studies. Therefore, human data were not used as the basis for the quantitative risk assessment. Because human epidemiology data were not used as the primary basis for risk assessment, a formal weight of evidence evaluation of causality for the human studies was not conducted.

The quantitative basis for the Health-based MCL is increased liver weight in pregnant mice in a developmental study conducted by USEPA in which mice were dosed with 0, 1, 3, or 5 mg/kg/day PFNA throughout gestation (Das et al., 2015). Increased liver weight is a well-established effect of PFNA and other PFCs in experimental animals. Das et al. (2015) was selected as the only study appropriate for use as the basis for quantitative risk assessment because it provides the numerical serum PFNA data, including statistical parameters, needed for benchmark dose (BMD) modeling. (These numerical data were provided by C. Lau.) PFNA serum levels at the time point when liver weight was measured are also available in a study of

male mice exposed to 0, 0.2, 1, or 5 mg/kg/day PFNA for 14 days (Wang et al., 2015). When compared on the basis of PFNA serum levels, the NOAELs and LOAELs for increased liver weight (Table 9) are consistent in male mice (Wang et al., 2015) and pregnant female mice (Das et al., 2015), and the dose-response curves for increased relative liver weight are similar in the two studies.

Table 9. PFNA serum level NOAELs and LOAELs for increased liver weight in mice

	NOAEL ***(ng/ml)***	**LOAEL** ***(ng/ml)***
Pregnant mice (Das et al., 2015)	-----	12,400
Male mice (Wang et al., 2015)	----	11,500

The only other toxicology studies for which serum PFNA data were reported numerically is the developmental toxicity study in WT and PPAR-alpha KO mice (Wolf et al., 2010). In Wolf et al. (2010), serum PFNA levels were measured 23 days after dosing ended. At this time point, a considerable portion of the PFNA would have been excreted, with additional loss of PFNA through transfer to breast milk in the lactating dams. Thus, these serum levels are not indicative of the exposure levels which resulted in toxicity and are not suitable for use as the basis for quantitative risk assessment.

In the studies of the Surflon S-111 PFC mixture consisting primarily of PFNA, Mertens et al. (2010) present serum PFC levels over time in each dosed group in graphs but do not provide the numerical data that are needed for dose-response modeling. Additionally, the data for the lower serum levels cannot be accurately estimated from the graphs, due to their scale. Stump et al. (2008) provides only area under the curve graphs for serum levels of the Surflon S-111 mixture, and no serum data is presented for individual PFCs. Numerical serum PFNA data from these studies have been requested from the sponsors of these studies but have not been provided to date.

Of the numerous effects observed in Das et al. (2015), increased maternal liver weight was selected as the critical endpoint for quantitative risk assessment because serum levels and liver weights were both measured at the same time point (GD 17), one day after the last dose. Liver weight increased in a dose related manner with a LOAEL of 1 mg/kg/day, and no NOAEL was identified.

Dose-response curves for endpoints of developmental toxicity assessed in the offspring in Das et al. (2015) are similar as for maternal liver weight. These endpoints were not used as the basis for quantitative dose response modeling of maternal serum levels because of greater uncertainty about the relationship of maternal serum levels on GD 17 to those effects that were assessed in offspring at later time-points.

Serum levels of PFNA measured in the offspring were not used in dose-response modeling of the delays in offspring developmental endpoints because they were measured after maternal dosing

ended and/or at different time points than when endpoints were assessed in the offspring. Thus, these serum levels in the offspring may not be indicative of the serum levels which caused the observed effects. It is notable that decreased body weights in male offspring persisted until at least 287 days of age, a time point at which almost all PFNA had been eliminated from the body.

PFNA caused similar effects at similar or lower doses in a study in male mice (Wang et al., 2015) and in a second developmental study in another strain of mice (Wolf et al., 2010). PFNA also caused several other types of toxicity in other studies at similar or lower doses and/or estimated serum levels than those ($\geq$1 mg/kg/day) causing increased maternal liver weight in Das et al. (2015). These studies are discussed in detail above, and include increased liver weight at 0.45 mg/kg/day in mice dosed for 21 days (Kennedy, 1987); increased serum glucose and other effects at 1 mg/kg/day, and related biochemical effects at 0.2 mg/kg/day, in mice dosed for 14 days (Fang et al., 2012a); immunotoxicity at 1 mg/kg/day in mice dosed for 14 days (Fang et al., 2008); and damage to testicular Sertoli cells and other related effects at 1 mg/kg/day in rats dosed for 14 days. Additionally Stump et al. (2008) and Mertens et al. (2010) discuss an unpublished study (Wolterbeek, 2004) in which serum cholesterol, calcium, and phospholipids were decreased in male mice dosed with 0.1 mg/kg/day Surflon S-111, a PFC mixture containing primarily PFNA, for 14 days. Histopathological effects in the liver, including necrosis, occurred in male mice dosed with Surflon S-111 at 0.025 mg/kg/day for 18 or 21 weeks, a dose at which hepatic enlargement did not occur (Stump et al. 2010). The results of these other studies, as well as the other effects in Das et al. (2015), provide further qualitative support to the dose and serum level chosen as the basis for quantitative risk assessment.

Determination of Point of Departure (POD)

Benchmark dose (BMD) modeling is a quantitative approach commonly used to estimate a generalizable NOAEL that does not directly depend on the specific doses/exposures in a given study. In this context, it is used to predict the dose (BMD) and its lower 95% confidence limit (BMDL) corresponding to a minimal response level (the BMR) that is consistent with the observed data. USEPA Benchmark Dose Modeling Software 2.6.0.86 (USEPA, 2015b) was used to perform BMD modeling of the data on liver weight on GD 17 in pregnant mice exposed to PFNA on GD 1-17 (Das et al., 2015).

The data used to generate the graphs presented in Das et al. (2015) were provided in numerical form from the investigators. Average serum levels in the pregnant mice on GD 17 in the 0, 1, 3, and 5 mg/kg/day groups were 13 ng/ml, 12,400 ng/ml, 18,300 ng/ml, and 57,100 ng/ml, respectively. Average liver weights in the 0, 1, 3, and 5 mg/kg/day groups on GD 17 were 2.24, 3.29, 4.36, and 5.26 g. The number of pregnant mice in the 0, 1, 3, and 5 mg/kg/day groups was 8, 8, 8, and 10, respectively.

BMD and the BMDL serum levels were determined for a 10% increase from the mean liver weight in the pregnant control mice using EPA BMD software (version 2.6.0.86).All models for continuous data included in the software were run. Results of the BMD modeling are shown in Table 10, and the complete output of the BMDS software for each model is presented in Appendix 4.

The Hill model and the Exponential model 5 gave almost identical AIC statistics, and these

were the lowest AIC values of the models run. Both of these models also show an excellent visual fit to the data. As there is no basis to choose between two models which have equally good statistics and fit, the average of the BMDLs from these two models was used as the Point of Departure. The BMDLs for the Exponential model 5 and the Hill model are 4.43 µg/ml and 5.43 µg/ml, respectively. The average of these values is 4.93 µg/ml which rounds to 4.9 µg/L (4900 ng/L).

Because the half-life of PFNA in female mouse serum is 34-69 days, the serum concentration of PFNA following the 17 days of dosing represents the maximum serum concentration during the dosing period, and the average exposure over this period was lower than the exposure at the end of the dosing period. Thus, attributing the liver weight effect to the serum concentration on day 17 is a non-conservative approach as the effect could have resulted from the lower average exposures experienced over the whole time period of dosing.

Table 10: Benchmark Dose Modeling for 10% Increase in Liver Weight in Pregnant Mice from Das et al. (2015)

Model	**AIC**	**P (χ^2)**	**Scaled Residual** (at dose closest to BMD)	**BMD (µg/ml)**	**BMDL (µg/ml)**
Exponential (BMDS model 2, 3)* (non-homogeneous variance) * Models 2 and 3 are identical,	+14.97074	< 0.0001 (This value does not meet the BMDS threshold of 0.1)	-0.3054	9.25	7.58
Exponential (BMDS model 4) (non-homogeneous variance)	-8.231961	0.002 (This value does not meet the BMDS threshold of 0.1)	-1.931	1.58	1.18
Exponential (BMDS model 5) (non-homogeneous variance)	-15.54591	NA (available degrees of freedom do not permit calculation of χ^2 model fit)	0.1075	6.77	4.43
Hill (non-homogeneous variance)	-15.545906	NA (available degrees of freedom do not permit calculation of χ^2 model fit)	0.107	7.76	5.43
Polynomial – 2nd deg. (non-homogeneous variance)	-9.889301	0.006 (This value does not meet the BMDS threshold of 0.1)	-1.88	1.76	1.40
Power Model with power unrestricted (non-homogeneous variance)	-4.937769	< 0.001 (This value does not meet the BMDS threshold of 0.1)	-2.09	0.26	0.02
Linear Model/Power Model with power ≥ 1.0) (non-homogeneous variance) **These two models are mathematically identical.	+10.746883	< 0.0001 (This value does not meet the BMDS threshold of 0.1)	-0.151	5.77	2.95
USEPA BMDS Software version 2.6.0.86 was used to model all available continuous response models.					

Derivation of health-based water concentration

Application of uncertainty factors

The choice of uncertainty factors is consistent with current USEPA IRIS guidance (USEPA, 2012c) and previous risk assessments developed by NJDEP and the DWQI.

Uncertainty factors (UFs) are applied to the POD serum level of 4.9 ug/ml (4900 ng/ml) derived above to obtain the target human serum level. The target human serum level (ng/ml serum) is analogous to a Reference Dose (RfD) but is expressed in terms of internal, rather than administered, dose.

The total uncertainty factor applied to the POD serum level is 1000, and includes the following uncertainty factors:

10 – Human variation, to account for variation in susceptibility across the human population and the possibility that the available data may not be representative of individuals who are most sensitive to the effect.

3 – Animal-to-human extrapolation, to account for toxicodynamic differences between humans and mice.

The typical uncertainty factor of 3 for toxicokinetic variability between species is not included because the risk assessment is based on comparison of internal dose (serum levels) rather than administered dose.

10 – Duration of exposure

The POD is based on a systemic effect (increased liver weight) resulting from exposure for only 17 days, while the value derived is intended to protect for chronic exposure. Other studies discussed above suggest that PFNA causes additional and/or more severe effects as exposure duration increases.

3 – Incomplete database

Gaps in the toxicology database for PFNA include the lack of developmental neurobehavioral studies, the absence of chronic/carcinogenicity studies, and lack of two-generation study in an appropriate species, as well as the lack of studies of specific developmental effects such as mammary gland development that are affected by low doses of the closely related compound, PFOA. Additionally, the results of Stump et al. (2008) suggest that PFNA causes liver necrosis at doses below those that cause increased liver weight, the endpoint used as the basis for the risk assessment.

The target human serum level is: $\frac{4{,}900 \text{ ng/ml}}{1000} = 4.9 \text{ ng/ml}.$

Relative Source Contribution factor

A Relative Source Contribution (RSC) factor that accounts for non-drinking water sources including food, soil, air, water, and consumer products is used in the development of health-based drinking water concentrations based on non-carcinogenic effects. This approach is used by the DWQI for Health-based MCLs, by USEPA for Maximum Contaminant Level Goals, and by other states in development of similar health-based drinking water values. An RSC is intended to prevent total exposure from all sources from exceeding RfD (USEPA, 2000). When sufficient chemical-specific information on non-drinking water exposures are not available, a default RSC of 0.2 is used, meaning that 20% of the RfD may come from drinking water (USEPA, 2000). When sufficient chemical-specific exposure data are available, a less stringent chemical-specific RSC may be derived, with floor-ceiling values of 20%-80% (USEPA, 2000).

In the most recent NHANES data from 2011-12, the geometric mean serum PFNA concentration was 0.88 ng/ml and the 95th percentile value was 2.54 ng/ml (CDC, 2015). PFNA serum levels in NHANES reflect background exposures to PFNA and PFNA precursors at levels prevalent throughout the U.S. from media such as food, water, air, dust, and consumer products. Based on the infrequent occurrence of PFNA reported in U.S. public drinking water supplies in UCMR3 and other studies (discussed above), it is unlikely that the mean and median PFNA serum levels in the U.S. general population reported by NHANES are influenced by drinking water exposures.

Non-drinking water exposures in N.J. may reflect multiple overlapping sources of release of PFNA including those background exposures that are influenced by air transport within N.J., and this may be particularly true in communities where drinking water has been impacted by past industrial use and discharge of PFNA. In contrast, mean national estimates of exposure, as indicated by the mean serum levels identified in NHANES, reflect exposures in large parts of the U.S. where there are few or no sources of PFNA manufacture or use.

Because PFNA is known to bioaccumulate in fish, the possibility of elevated exposures from recreationally caught fish is of particular concern in areas with past industrial release of PFNA. In 2004-2007, elevated levels of PFNA, as well as C11, were found in fillets from white perch and channel catfish from locations on the Delaware River in the vicinity of communities where drinking water is contaminated with PFNA (DRBC, 2009). However information provided by the Delaware River Basin shows that PFNA was not detected in fillets from these two species from the same Delaware River locations in more recent sampling in 2010 and 2012.

Because the most recent data do not suggest elevated exposures from recreationally caught fish in communities where PFNA is present in drinking water, the 95th percentile PFNA serum level of 2.54 ng/mL (Table 2), which rounds to 2.5 ng/ml, is assumed to represent a reasonable and protective estimate of total non-drinking water exposure. It was therefore considered appropriate to use this 95th percentile serum value to develop a chemical-specific RSC for PFNA. The RSC for PFNA is developed using the "subtraction" approach described by USEPA (2000), but on the basis of serum levels rather than administered dose.

The chemical specific RSC (%) for PFNA is developed by subtracting the NHANES (2011-12) 95th percentile PFNA serum level from the target human serum level for PFNA (above) of 4.9 ng/L, and dividing by the target human serum level.

$$\text{RSC} = \frac{\text{Target human serum level} - 95^{th}\ \%\ \text{NHANES serum level}}{\text{Target human serum level}} \times 100$$

$$\text{PFNA RSC} = \frac{4.9\ \text{ng/ml} - 2.5\ \text{ng/ml}}{4.9\ \text{ng/ml}} \times 100 = 49.0\%\ \text{(rounded to 50\%)}$$

Based on the above, the increase in human serum level that can result from drinking water exposure only is:

4.9 ng/ml x 0.5 = 2.45 ng/ml which rounds to 2.5 ng/ml (2500 ng/L).

Development of Health-based MCL

Development of the Health-based MCL for PFNA is based on the same general approach used to develop the New Jersey health-based drinking water guidance for PFOA. This approach is described in NJDEP (2007) and Post et al. (2009a) and is applicable to other persistent PFCs found in drinking water.

Because the half-life of long-chain PFCs such as PFNA is much longer in humans (several years) than in rats and mice, a given administered dose (mg/kg/day) results in a much greater internal dose (as indicated by serum level) in humans than in these animal species. Therefore, comparisons between effect levels in animal studies and human exposures were made on the basis of serum levels rather than administered dose.

As discussed above, ongoing exposure to PFOA in drinking water increases PFOA serum levels, on average, in a serum:drinking water ratio of at least 100:1 with several studies indicating mean ratios substantially above 100:1. The 100:1 ratio for PFOA used in development of the PFOA drinking water guidance is based on data from adults and is higher in infants and young children.

The half-life of PFNA is estimated as 2 to 30 times longer than that of PFOA in rats and mice, and limited human data indicate that its human half-life is at least twice as long as for PFOA (with the exception of the more uncertain data from women of childbearing age; Tables 3 and 4). These data on the relative half-lives of PFOA and PFNA indicate that use of a ratio of 200:1 is not overly stringent. Although upper percentile values for exposure parameters are typically used by USEPA and DWQI for drinking water risk assessment, the 200:1 ratio is intended to represent a central tendency estimate rather than an upper percentile value.

Based on the 200:1 ratio between PFNA serum levels and drinking water concentration derived above, an increase in PFNA serum level of 2500 ng/L is expected to result from ongoing exposure to 12.5 ng/L, which rounds to 13 ng/L, PFNA in drinking water. Therefore, the Health-based MCL for PFNA is calculated as **13 ng/L or 0.013 µg/L.**

DISCUSSION OF UNCERTAINTIES

- Ongoing exposure to PFNA at 13 ng/L (0.013 µg/L) in drinking water is estimated to contribute an additional 2.6 ng/ml, on average, to the PFNA concentration in blood serum already present in the general population based on a serum:drinking water ratio of 200:1. Thus, the average serum level in communities with drinking water at 13 ng/L (0.013 µg/L) is estimated at about 3.5 ng/ml, by adding the contribution to serum from drinking water (2.6 ng/ml) to the geometric mean (0.88 ng/ml, which rounds to 0.9 ng/L) serum level in the general U.S. population. This represents an increase of about 3-fold from the general population geometric mean. In infants and young children, the increase in serum levels from ongoing exposure to PFNA in drinking water would likely be greater than in adults, due to their greater water consumption on a body weight basis.

  Although the epidemiological data on PFNA are limited, several epidemiological studies have found associations of PFNA serum levels in the general population with potentially important health endpoints. Causality cannot be established for these effects because of the

cross-sectional design of the studies and because, in some studies, the associations cannot be definitively attributed to PFNA because of correlations with other PFCs. However, these data contribute to the overall body of evidence about the potential hazard of PFNA. Thus, there is uncertainty about the extent of protection provided by a Health-based MCL that will result in serum PFNA levels above the general population range.

- No scientific studies have been conducted in communities with elevated exposures to PFNA from drinking water or other environmental media. The sole study of workers with occupational exposure is of limited utility, in part because PFNA serum levels were not reported.

- Several important health endpoints that have been linked to the closely related compound, PFOA, in studies of populations with drinking water exposure, including cancer, have not been evaluated in humans exposed to PFNA.

- An unpublished 14 day study of Surflon S-111 (Wolterbeek, 2004; cited in Mertens et al., 2010, and Stump et al., 2008) found decreased serum cholesterol, phospholipids, and calcium in male rats at 0.1 mg/kg/day. Cholesterol and calcium were not affected at higher doses of Surflon S-111 in the 90 day rat subchronic study and phospholipids were not measured. These unpublished results raise uncertainties about the potential for Surflon S-111 and PFNA to cause these effects in rats, as well as uncertainty about other effects in the unpublished study that may not have been discussed by Stump et al. (2008) and Mertens et al. (2010).

- Histopathological changes in the liver, including necrosis, occurred in F_0 and F_1 males in the two-generation rat study (Stump et al., 2008) at a dose (Surflon S-111, 0.025 mg/kg/day; PFNA, 0.019 mg/kg/day) below the doses that caused increased liver weight in the same study. Thus, histopathological changes, including necrosis, were a more sensitive endpoint than increased liver weight in this rat study. No NOAEL was identified for these histopathological effects in liver in the two-generation rat study, and the LOAEL in male rats was 0.025 mg/kg/day Surflon S-111 (0.019 mg/kg/day PFNA). It is important to note that the histopathological changes in the liver reported by Stump et al. (2008), and Mertens et al. (2010) are not of the same nature as are those typically associated with PPAR-alpha activation (peroxisome proliferation and increased smooth endoplasmic reticulum), suggesting that PFNA causes liver toxicity independent of PPAR-alpha mediated effects.

  In part because numerical serum level data are not available, this study and endpoint cannot be used for quantitative risk assessment. Available graphical information from this study and the accompanying study (Mertens et al., 2010) suggests that the serum PFNA level in males at 0.025 mg/kg/day was well below the serum PFNA level at the lowest dose (1 mg/kg/day) in the Das et al. (2015) mouse developmental study used as the basis for quantitative risk assessment. However, histopathological changes in the maternal and pup liver were not evaluated by Das et al. (2015), and it is not known if these effects occurred in this study. Quantitative risk assessment based on liver histopathology could result in a significantly lower BMDL than the one based on Das et al. (2015).

- No chronic toxicology studies of cancer or other effects that may occur after longer exposures and/or in old age have been conducted. PFOA and PFOS, the only two PFCs for which chronic studies have been conducted, caused tumors in rats. Results of the subchronic (Mertens et al., 2010) and the two-generation (Stump et al., 2008) suggest that additional and/or more severe effects may occur as exposure duration increases.

- It is not known whether PFNA causes some effects seen in mice exposed to low doses of PFOA and/or other PFCs. PFOA causes specific developmental effects in mice at low doses (0.01 mg/kg/day) and serum levels, including delayed mammary gland development (reviewed in Post et al., 2012; Tucker et al., 2014). The serum level BMDLs for PFOA based on delayed mammary gland development endpoints in mice are 23-25 ng/ml (Post et al., 2012), more than two orders of magnitude lower than the BMDL for PFNA of 5300 ng/L based on maternal liver weight gain. Additionally, neonatal mice exposed to a single dose of <1 mg/kg of other persistent PFCs (PFOA, PFOS, PFHxS) exhibited permanent neurobehavioral effects accompanied by changes in critical brain proteins. These endpoints have not been evaluated for PFNA, which is closely related to PFOA and has a generally similar profile of toxicological effects.

- The subchronic (Mertens et al., 2010) and two-generation (Stump et al., 2008) rat studies used a mixture of PFCs with PFNA as the primary component. These are the only toxicology studies of PFNA with exposure durations greater than 21 days. As discussed above, the data suggest that PFNA was primarily responsible for the effects observed in these studies. Nonetheless, there is uncertainty about the contribution of the other PFCs that are minor components of the mixture to the effects that were observed.

- There is uncertainty about the serum:drinking water ratio of 200:1 for PFNA. Although this ratio is reasonable and not overly stringent based on the available toxicokinetic data from animals and humans, human information on the half-life of PFNA is limited, and scientific studies of serum levels in communities exposed to PFNA in drinking water are not available. Although upper percentile exposure assumptions are typically used in risk assessment, these values are intended to represent central tendency estimates, rather than upper percentile values.

- Uncertainties about the human relevance of effects seen in animals are inherent to all risk assessments based on animal data. The available information indicates that the effects of PFNA observed in experimental animals can be assumed to be relevant to humans for the purposes of risk assessment.

- Available information indicates that the target organs and modes of action are generally similar for PFNA and other PFCs, particularly PFOA. Therefore, the toxicity of PFNA and other PFCs may be additive. Although PFNA and other PFCs, including PFOA, are known to co-occur in some NJ public water supplies, the potential for additive toxicity of PFNA and other PFCs was not considered in development of the Health-based MCL.

HEALTH-BASED MCL RECOMMENDATION

The recommended Health-based MCL for PFNA is **13 ng/L or 0.013 µg/L.**

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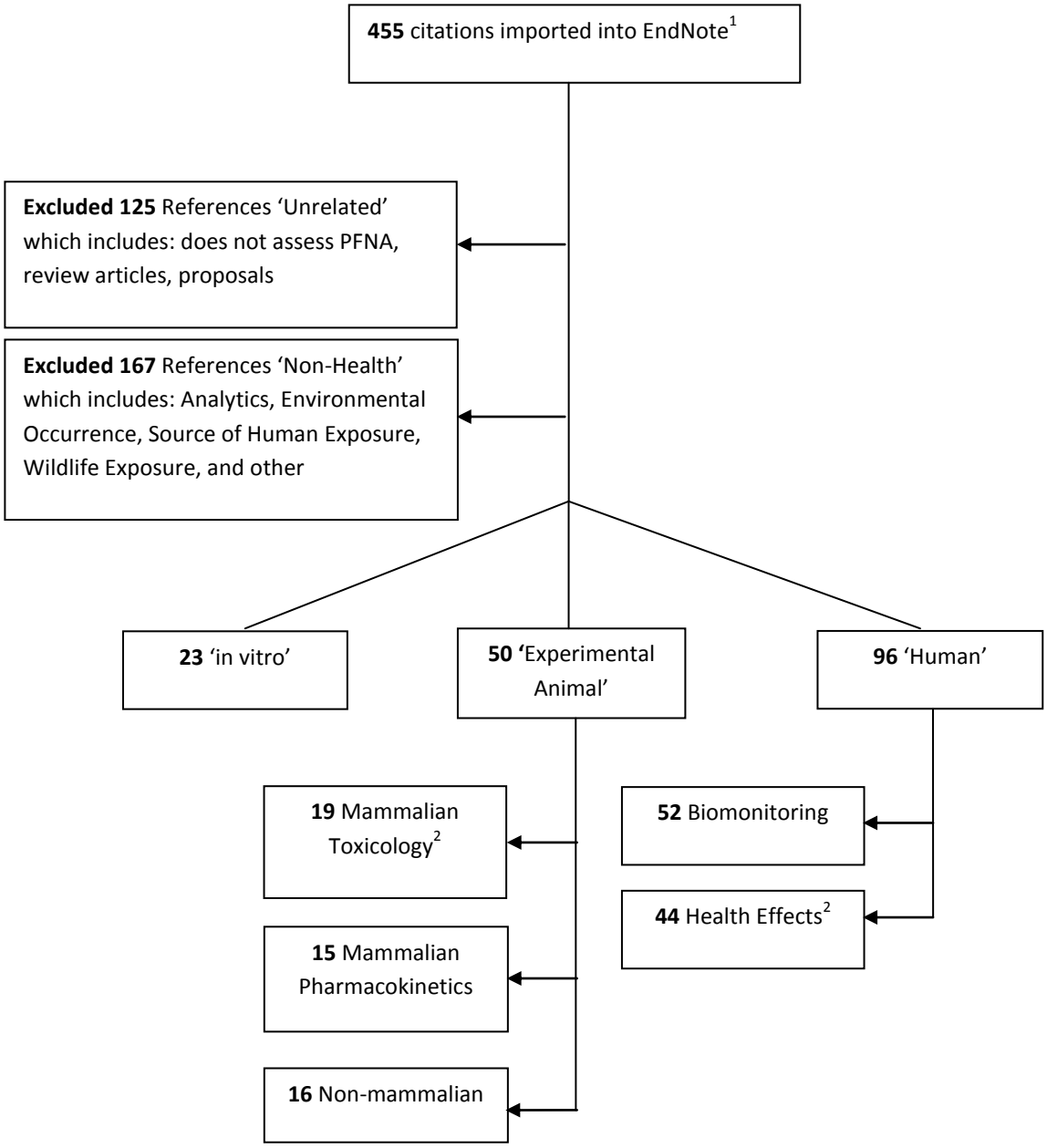
APPENDIX 1. Literature Search Criteria and Documentation

- **435 citations from PubMed** (November 5, 2014): search "375-95-1"[EC/RN Number] OR "4149-60-4"[EC/RN Number] OR pfna OR perfluorononanonic OR (perfluoro AND nonanoic) OR perfluorononanoate OR (perfluoro AND nonanoate) OR S-111-S-WB"; Limits: none
- **20 citations from Toxline** (November 6, 2014) – ***removed 7 duplicates***: search "375-95-1"[EC/RN Number] OR "4149-60-4"[EC/RN Number] OR pfna OR perfluorononanonic OR (perfluoro AND nonanoic) OR perfluorononanoate OR (perfluoro AND nonanoate) OR S-111-S-WB"; Limits: Include PubMed records = no

- **7 citations** identified from backward search



[1]Totals may exceed number of imported files if articles are placed into more than one category
[2]Researchers evaluated full text of each article to determine whether mammalian toxicology or human health effects were investigated in studies. All studies determined to be evaluating in vivo mammalian toxicology and human health effects are cited in the final report and other studies are cited as appropriate.

APPENDIX 2. Individual Study Tables for Epidemiologic Study of Human Health Effects and PFNA

Reference and Study Design	Exposure Measures	Results	Comment
Bloom et al., 2010 **Study Design:** Cross-sectional **Location:** New York, United States **Population:** Subgroup of 31 of 38 participants from Licensed New York State sportfish anglers and their partners (NYSACS) [n=18,082] who completed a Dioxin Exposure Substudy component, age 31 to 45 years. **Outcome Definition:** Questionnaire and a blood sample	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Geometric mean of PFNA 0.79 ng/mL (95% CI 0.68, 0.96)	**Stat Method:** Linear regression, covariates and confounders considered included age, gender, BMI, smoking, goiter or thyroid condition, race/ethnicity, use of thyroid medication, and self-reported consumption of sportfish caught from NY waters PFCs and TSH were log transformed. **Outcome:** ln-TSH (μIU/mL) **Major Findings:** β=0.09 (95% CI -0.60, 0.78) **Outcome:** free T4 (fg/dL) **Major Findings:** β=0.04 (95% CI -0.07, 0.15)	**Major Limitations:** Cross-sectional design prevents causal inference. Small sample size limited ability to control for potential covariates and confounders simultaneously, or other potential environmental compounds of interest or other PFCs.

Reference and Study Design	Exposure Measures	Results	Comment
Braun et al., 2014 **Study Design:** Prospective birth cohort **Location:** Cincinnati, OH **Population:** Pregnant women in the Health Outcomes and Measures of the Environment (HOME). Final study size included 175 mother-child pairs. **Outcome Definition:** Autistic behaviors measured as mother completed Social Responsiveness Scale (SRS) at 4 and 5 years of age of child. Higher scores indicate more autistic behaviors.	**Exposure Assessment:** Maternal serum @ 16-26 weeks of pregnancy **Population-Level Exposure:** PFNA median is 0.9 ng/ml.	**Stat Method:** Bayesian models were used and covariates and confounders considered include: maternal age at delivery, race, marital status, education, parity, insurance status, employment, and household income, and prenatal vitamin use, depressive symptoms during the second trimester, maternal full-scale IQ. Also used to two-stage Bayesian models to control for co-pollutants. Exposures were log-transformed **Outcome:** SRS total scores **Major Findings:** Negligible changes in SRS scores *note PFOA- found a protective association	**Major Limitations:** Modest sample size may have resulted in failure to detect associations. Possible confounding due to unmeasured variables and other environmental contaminants, including other PFCs.

Reference and Study Design	Exposure Measures	Results	Comment
Buck Louis et al., 2014 **Study Design:** Cross-sectional **Location:** Population from 16 counties in Michigan and Texas **Population:** 501 males partners of couples planning pregnancy **Outcome Definition:** 35 semen quality endpoints using baseline serum sample and follow-up one month later (used for sensitivity analysis)	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Median (IQR): Michigan=1.0 (0.75, 1.35) Texas=1.65 (1.2, 2.2)	**Stat Method:** Linear regression, covariates and confounders assessed include age, BMI, smoking, abstinence time, sample age (hours), and study site PFCs were natural log transformed **Outcome:** Volume, straw distance, sperm concentration, total count, hypo-osmotic swollen, 8 motility measures, 6 sperm head measures, 12 individual and 2 summary morphology measures, 2 sperm chromatin stability measures **Major Findings:** Only 'Morphology – Strict Criteria (%)' was statistically associated with PFNA [β=3.897 (95% CI 0.564, 7.231)]. The other 34 parameters were not statistically significant associated with PFNA.	**Comment:** PFNA serum concentrations are relatively different between mean from Michigan and Texas (possibly explainable by the different recruitment techniques in each state). Importantly analyses were not stratified by recruitment location other exposure ranges differed. **Major Limitations:** Lack of well-established norms for many individual parameters and a reliance on next day semen analysis and possible spurious associations. Absence of any reproductive hormone measurements. Possible confounding due to unmeasured variables. Cross-sectional design prevents causal inference.

Reference and Study Design	Exposure Measures	Results	Comment
Chen et al., 2012 **Study Design:** Prospective birth cohort **Location:** Taiwan **Population:** Study subjects from the Taiwan Birth Panel Study (TBPS), 2004-2005. Final study size 429 mother-infant pairs **Outcome Definition:** Mother interviews and medical record extraction	**Exposure Assessment:** Cord blood collected at delivery **Population-Level Exposure:** Geometric mean cord blood plasma PFNA 2.36 ng/mL (4.74 – geometric standard deviation)	**Stat Method:** Linear regression, covariates and confounders considered included maternal age at conception, prepregnancy BMI, educational level, ln-cord blood cotinine level, type of delivery, infant sex, and parity PFNA natural log transformed. Coefficients from most adjusted models shown below. **Outcome:** Gestation age (weeks) **Major Findings:** β=0.04 (95% CI -0.06, 0.14) **Outcome:** Birth weight (grams) **Major Findings:** β=6.07 (95% CI -16.6, 28.7) **Outcome:** Birth length (centimeters) **Major Findings:** **β=0.16 (95% CI 0.05, 0.27)** **Outcome:** Head circumference (cm) **Major Findings:** β=0.05 (95% CI -0.04, 0.13) **Outcome:** Ponderal index(gm/cm^3) **Major Findings:** **β=-0.02 (95% CI -0.03, -0.004)** **Outcome:** Preterm birth (weeks) **Major Findings:** OR=0.88 (95% CI 0.71, 1.11) **Outcome:** Low birth weight (kg) **Major Findings:** OR=0.76 (95% CI 0.47, 1.23) **Outcome:** Small for gestational age **Major Findings:** OR=0.97 (95% CI 0.74, 1.26)	**Major Limitations:** The sample size was not large enough to form conclusions on the impacts of PFCs on birth outcomes with low prevalence rates (e.g. low birth weight or small for gestational age). A lack of information concerning maternal diet habits could limit exploration of exposure sources.

<table>
<tr><th>Reference and Study Design</th><th>Exposure Measures</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Christensen et al., 2011

Study Design:
Nested Case-control

Location:
Avon, United Kingdom

Population:
448 girls born in 1991-1992 from mothers enrolled in a prospective cohort. Cases, girls reporting menarche before age 11.5 years of age n=218, and control girls reporting menarche at 11.5 years or after n=230.

Outcome Definition:
Follow-up responses</td>
<td>Exposure Assessment:
Maternal serum samples taken at delivery

Population-Level Exposure:
<table>
<tr><th>CACO</th><th>Median (ng/mL)</th><th>IQR</th></tr>
<tr><td>Case</td><td>0.7</td><td>0.5-0.8</td></tr>
<tr><td>Control</td><td>0.6</td><td>0.5-0.8</td></tr>
<tr><td>Total</td><td>0.6</td><td>0.5-0.8</td></tr>
</table>
</td>
<td>Stat Method:
Logistic regression, covariates and confounders considered include mother pre-pregnancy BMI, mother’s age at delivery, mother’s age at menarche, mother’s educational level, mother’s social class, child’s ethnic background, child’s birth order.

PFCs were natural log transformed. PFC treated continuously and dichotomized (above v. below median case serum concentration). Estimates from adjusted models presented here.

Outcome: Age at menarche (years)
Major Findings:
Continuous: OR=0.91 (95% CI 0.59,1.40)
Binary: OR=1.15 (95% CI 0.78,1.69)</td>
<td>Major Limitations:
Included a single measure of PFC exposure, lack of complete information on age of menarche for controls, and some missing information on covariates.

Participants may not be representative of cohort. Parents of non-respondents tended to be of a lower educational attainment, social class, more likely to be under the age of 25, and non-white race/ethnicity.

Did not control other unmeasured environmental pollutants, including other PFCs.</td>
</tr>
</table>

Reference and Study Design	Exposure Measures	Results	Comment
Dong et al., 2013 **Study Design:** Case-control **Location:** Taiwan **Population:** Children (10-15 years of age), 2009-2010 Asthmatic n=231 Non-asthmatic n=225 **Outcome Definition:** Asthma and immunological markers (absolute eosinophil count (AEC), IgE, eosinophilic cationic protein (ECP))	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Median PFNA serum concentration for cases was 1.0 ng/mL and for controls was 0.8 ng/mL	**Stat Method:** PFCs categorized using Wilcoxon rank-sum test. Logistic regression, confounders and covariates considered include parental education, body mass index, environmental tobacco smoke, and month of survey. Linear regression used to explore continuous outcomes. Results shown here for most adjusted model. **Outcome:** Asthma **Major Findings:** ↑ (P for trend <0.001) No trend looking as asthma severity **Outcome:** IgE (IU/mL) **Major Findings:** w/o asthma: NS (P for trend 0.084) w/ asthma: ↑ from lowest quartile (p for trend 0.001) **Outcome:** AEC (x 10^6 /L) **Major Findings:** w/o asthma: NS (P for trend 0.086) w/ asthma: ↑ (P for trend <0.001) **Outcome:** ECP (μg/L) **Major Findings:** w/o asthma: NS (P for trend 0.167) w/ asthma: ↑ (P for trend 0.003)	**Major Limitations:** Estimates may also be influenced by selection bias or uncontrolled confounding. Did not control for other co-occurring environmental contaminants including PFCs or other possibly important confounders.

<table>
<tr><th>Reference and Study Design</th><th>Exposure Measures</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Fu et al., 2014

Study Design:
Cross-sectional

Location:
Henan, China

Population:
133 participants, aged 0-88 years, randomly selected from people going for health check-up at Red Cross Hospital

Outcome Definition: Total cholesterol (TC), triglycerides, high-density lipoprotein cholesterol (HDLC), and LDLC</td>
<td>Exposure Assessment:
Serum concentrations

Population-Level Exposure:
Median PFNA serum level = 0.37 (0.02-4.18) ng/mL

<table>
<tr><th>Quartile</th><th>Mean (ng/mL)</th></tr>
<tr><td>1</td><td>0.14</td></tr>
<tr><td>2</td><td>0.30</td></tr>
<tr><td>3</td><td>0.45</td></tr>
<tr><td>4</td><td>1.02</td></tr>
</table>

Median (ng/ml)–
PFOS=1.47
PFOA=1.43</td>
<td>Stat Method: Linear regression, exposure modeled in quartiles with 1st quartile serving as referent group. Binary logistic regression of abnormal lipids by PFC quartile. Covariates and confounders considered include age, gender, and BMI were control.

Outcomes are based on a change in values.

Outcome: ln-Total cholesterol (TC) (mmol/L)
Major Findings: ↑ (p-value for trend 0.002)

Outcome: ln-triglycerides (mmol/L)
Major Findings: NS (p for trend 0.460)

Outcome: high-density lipoprotein cholesterol (HDLC) (mmol/L)
Major Findings: NS (p for trend 0.191)

Outcome: ln-LDLC (mmol/L)
Major Findings: ↑ (p for trend 0.004)</td>
<td>Major Limitations:
Did not take into account cholesterol-lowering medications or other environmental factors and contaminants including other co-occurring PFCs.

Cross-sectional design prevents causal inference</td>
</tr>
</table>

<table>
<tr><th>Reference and Study Design</th><th>Exposure Measures</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Gallo et al., 2013

Study Design:
Cross-sectional

Location:
United States – Ohio and West Virginia

Population:
Adults (age +50 years) who consumed water (for at least 1 year) from a water district with known PFOA contamination, n=21,024 were included in the analysis.

Outcome Definition:
Self-reported memory impairment</td>
<td>Exposure Assessment:
Serum concentrations

Population-Level Exposure:

<table>
<tr><th>Quintile</th><th>Range (ng/mL)</th></tr>
<tr><td>1</td><td>0.25-0.90</td></tr>
<tr><td>2</td><td>1.0-1.2</td></tr>
<tr><td>3</td><td>1.3-1.4</td></tr>
<tr><td>4</td><td>1.5-1.9</td></tr>
<tr><td>5</td><td>2.0-28.6</td></tr>
</table>
</td>
<td>Stat Method:
Logistic regression, covariates and confounders considered include age, race, gender and educational level, average household income, physical activity, alcohol consumption, smoking, BMI, and diabetes

PFNA was log-transformed, estimates are based on a doubling of PFNA

Outcome: Memory impairment (Age +65)
Major Findings:
Q2 v. Q1, OR=0.86 (95% CI 0.78, 0.96)
Q3 v. Q1, OR=0.87 (95% CI 0.77, 0.98)
Q4 v. Q1, OR=0.86 (95% CI 0.77, 0.95)
Q5 v. Q1, OR=0.89 (95% CI 0.80, 0.99)

Trend – 0.053
Ordinal regression – 0.97 (95% CI 0.94,1.01)</td>
<td>Major Limitations:
Cross-sectional design prevents causal inference.

Reverse causality

Possible confounding due to unmeasured variables and other environmental contaminants, including other PFCs.

Outcome definition depends on self-report</td>
</tr>
</table>

Reference and Study Design	Exposure Measures	Results	Comment
Gleason et al., 2015 **Study Design:** Cross-sectional **Location:** General U.S. Population **Population:** NHANES 2007-2010, n=4,333 individuals, aged >12 years **Outcome Definition:** Liver function biomarkers and uric acid	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Median PFNA 1.40 ng/mL	**Stat Method:** Linear regression, covariates and confounders considered include age, gender, race/ethnicity, BMI, poverty, smoking, and alcohol consumption and serum creatinine for uric acid. PFNA was natural log transformed for linear regression. Logistic regression was also performed by dividing PFNA into quartiles (no transformations). **Results are presented for models with most adjustment** **Outcome:** Uric acid (mg/dL) **Major Findings:** (linear)-**β=0.185 (95% CI 0.091, 0.280)** (logistic)- p-value=0.052 **Outcome:** ALT (µg/L) **Major Findings:** (linear)- **β=0.043 (95% CI 0.019, 0.067)** (logistic)- **p-value=0.042** **Outcome:** GGT (µg/L) **Major Findings:** (linear)- **β=0.050 (95% CI 0.017, 0.083)** (logistic)- p-value=0.126 **Outcome:** AST (µg/L) **Major Findings:** (linear)-β=0.013 (95% CI -0.005, 0.031) (logistic)- p-value=0.516 **Outcome:** ALP (µg/L) **Major Findings:** (linear)-β=-0.009 (95% CI -0.034, 0.016) (logistic)- p=value=0.097 **Outcome:** total bilirubin (mg/dL) **Major Findings:** (linear)-β=0.009 (95% CI -0.017, 0.034) (logistic)- p-value=0.614	**Major Limitations:** Cross-sectional design prevents causal inference. Possible confounding due to unmeasured variables and other environmental contaminants, including other PFCs

Reference and Study Design	Exposure Measures	Results	Comment
Granum et al., 2013 **Study Design:** Prospective birth-cohort **Location:** Norway **Population:** BraMat Cohort established 2007-2008 (recruited from the Norwegian Mother and Child (MoBa) Cohort) Children (n=99) **Outcome Definition:** Outcomes from blood samples from the children at 3 years-of-age and questionnaire given at child age 1, 2, and 3 years Serological outcomes: antibody levels specific for four vaccines (measles, rubella, tetanus, and Hib), using allergen-specific IgE. Clinical outcomes: from questionnaire include data about infectious diseases, allergy, and asthma	**Exposure Assessment:** Maternal serum concentrations collected at time of delivery **Population-Level Exposure:** The median PFNA serum concentration (n=99, 0.3 ng/mL)	**Stat Method:** Poisson regression used for health outcomes using count data. Confounders and covariates considered include maternal allergy, paternal allergy, maternal education, child's gender, and/or age at 3-year follow-up PFCs categorized into quartiles. **Outcome:** Rubella vaccine immune response (OD – optical density) **Major Findings:** **β=-1.38 (95% CI -2.35, -0.40)** (p-value 0.007) No significant associations were found between the PFNA concentration and other vaccine antibody levels (Measles, Tetanus, Haemophiluz influenza (Hib)). **Outcome:** Common Cold **Major Findings:** (No. of episodes) 3rd year **β=1.24 (95% CI 0.08, 2.40)** All years β=0.57(95% CI -0.10, 1.23) (Dichotomous) 3rd year OR=0.11 (95% CI 0.001-22.5) **Outcome:** Gastroenteritis **Major Findings:** No. of episodes: 3rd year β=-0.46 (95% CI -2.27,1.35) All years β=-0.10 (95% CI -1.36,1.17) Dichotomous: 3rd year OR=0.16 (95% CI 0.001,17.5) All years OR=0.06 (95% CI 0.00,171)	Other studies that found associations with PFCs and decreased vaccine response in children (Grandjean et al., 2012) and adults (Looker et al., 2013) did not evaluate PFNA. **Major Limitations:** Selection bias due to the low recruitment rate. Small study population. Multiple exposure-health outcome comparisons. Did not control for other co-occurring environmental contaminants including PFCs or other possibly important confounders.

		Outcome: Asthma, wheeze, eczema and itchiness, otitis media, atopic eczema **Major Findings:** Non-significant	

Reference and Study Design	Exposure Measures	Results	Comment
Gump et al., 2011 **Study Design:** Cross-sectional **Location:** Oswego County, NY **Population:** Subset of children aged 9-11 recruited from a mailed invitation (n=83). **Outcome Definition:** Impaired response inhibition was measured through performance in a 20 minute differential reinforcement of low rates (DRL) of responding task which requires children to learn that they need to wait 20 seconds before responding. Results are evaluated by inter-response times (IRT), with longer delays indicating better performance.	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** The mean, median, and range of PFNA were 0.82 ng/mL, 0.72 ng/mL, and 0.10-4.14 ng/mL	**Stat Method:** Covariates and confounders considered included child's, mother's, and father's age, family income, parent's education, parent's occupational class, child's, mother's and father's BMI, gender, race, history of chronic illness, blood lead levels, and blood mercury levels. PFNA and DRL was natural log transformed. **Outcome:** Median IRTs **Major Findings:** (by each time period) 0-5 min: -0.07 (95% CI -0.029, 0.14) **6-10 min: -0.24 (95% CI -0.46, -0.02)** 11-15 min: -0.15 (95% CI -0.38, 0.07) 16-20 min: -0.05 (95% CI -0.28, 0.18**)**	**Major Limitations:** Cross-sectional design prevents causal inference. Reverse causality Possible confounding due to unmeasured variables and other environmental contaminants including other PFCs. The study population was a subset of participants drawn from a larger study of volunteers and was disproportionately more male. Small sample size possibly limiting ability to detect associations.

Reference and Study Design	Exposure Measures	Results	Comment
Halldorsson et al., 2012 **Study Design:** Prospective birth-cohort **Location:** Denmark **Population:** Mother-offspring pairs (n=915 of 965 women), mothers recruited 1988-1989 and offspring followed at 20 years of age. N=345 for overweight and N=252 for other biomarkers of adiposity *Age is not clearly stated. **Outcome definition:** Anthropometric measures	**Exposure Assessment:** Prenatal PFC exposure assessed by PFCs in maternal serum samples from gestational week 30 **Population-Level Exposure:** The median maternal PFNA serum level was 0.3 ng/mL	**Stat Method:** Linear regression for continuous outcomes and log-Poisson regression for dichotomous outcomes. All analyses were performed for males and females separately. Confounders and covariates considered include maternal age, maternal education, maternal smoking, parity, prepregnancy BMI, infant birth weight, offspring age at follow-up. The main focus of this work was on PFOA concentrations and findings for additional associations with PFNA are not provided. **Outcome:** BMI (kg/m^2) **Major Findings:** In univariate analysis PFNA positively associated with female offspring BMI at age 20 (p for trend < 0.05). However, after adjustment for PFOA, the regression coefficients became nonsignificant.	**Major Limitations:** Losses during follow-up. Did not take into account other environmental factors and contaminants.

Reference and Study Design	Exposure Measures	Results	Comment
Hardell et al., 2014 **Study Design:** Case-Control **Location:** Sweden **Population:** Cases of prostate cancer admitted to hospital 2007-2011, n=201 Population-based controls (matched on age and geographical area), n=186 **Outcome definition:** Diagnosis of cancer with scheduled radiation or chemotherapy treatment	**Exposure Assessment:** Serum samples **Population-Level Exposure:** Cases median serum PFNA = 0.61 ng/mL Controls median serum PFNA=0.57 ng/mL	**Stat Method:** Wilcoxon rank sum test was used for calculation of p-values for comparisons between cases and controls. Unconditional logistic regression; covariates and confounders considered age, BMI, year of sampling to estimate as odds ratios and 95% CI. The median and 75 percentile concentration of PFNA used as cutoff values. Additionally OR and 95% CI by Gleason score and PSA level and examined interaction of PFNA and relation to heredity. **Outcome:** Prostate Cancer **Major Findings:** Blood concentrations between cases and controls were not statistically significantly different (p=0.03) OR=1.2 (95% CI: 0.8, 1.8) Stratified by Gleason Score Low- OR=1.4 (95% CI: 0.8, 2.5) High- OR=1.0 (95% CI: 0.6, 1.6) Stratified by PSA Low- OR=1.1 (95% CI 0.7, 1.8) High- OR=1.2 (95% CI 0.7, 2.1) Interaction with heredity p, interaction=0.92	**Major Limitations:** Small sample size, limits study power Did not control for other co-occurring environmental contaminants including PFCs or other possibly important confounders. **Comments:** Well performed study.

Reference and Study Design	Exposure Measures	Results	Comment
Hoffman et al., 2010 **Study Design:** Cross-sectional **Location:** General U.S. population **Population:** NHANES 1999-2000 & 2003-2004, children aged 12-15 years of age with PFC measurements n=571 (48 had ADHD) **Outcome Definition:** Parental report of medical diagnosis and/or parental report of medication use	**Exposure Assessment:** Serum concentration **Population-Level Exposure:** PFNA median serum concentration 0.6 ng/mL with a range of non-detect to 5.9 and an IQR of 0.5 ng/mL	**Stat Method:** Logistic regression, covariates and confounders considered include age, sex, race/ethnicity, sample cycle, maternal smoking during pregnancy, preschool attendance, NICU admittance, ETS, lead, PIR, Access to health care, health insurance coverage. Effect estimates provided for most adjusted model at 1 unit increase in serum level. **Outcome:** Attention Deficient /Hyperactivity Disorder (ADHD) **Major Findings:** Parental report, OR=1.32 (95% CI 0.86, 2.02) w/ prescription use, OR=1.57 (95% CI 0.67, 3.64)	**Major Limitations:** Reliance of parent report of outcome. Possible confounding due to unmeasured variables, and co-occurring environmental contaminants including other PFCs. Cross-sectional design prevents causal inference.

<table>
<tr><th>Reference and Study Design</th><th>Exposure Measures</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Humblet et al., 2014

Study Design:
Cross-sectional

Location:
U.S. population

Population:
General U.S. population aged 12-19 years
NHANES 1999-2000 & 2003-2008, n=1,877

Outcome Definition:
Questionnaire response</td>
<td>Exposure Assessment:
Serum concentrations

Population-Level Exposure:

<table>
<tr><th>Groups</th><th>Median (ng/mL)</th></tr>
<tr><td>Never asthma</td><td>0.8</td></tr>
<tr><td>Ever asthma</td><td>0.9</td></tr>
<tr><td>No wheezing</td><td>0.8</td></tr>
<tr><td>Wheezing</td><td>0.8</td></tr>
<tr><td>No current</td><td>0.8</td></tr>
<tr><td>Current</td><td>0.9</td></tr>
</table>
</td>
<td>Stat Method: Logistic regression; covariates and confounders considered included survey cycle, age, race/ethnicity, sex, poverty, smoking, health insurance, BMI. Effect modification for sex and race/ethnicity was explored.

Untransformed, ln-transformed, and tertiles of PFNA models are presented for the doubling of PFNA serum concentration

Outcome: Ever asthma
Major Findings:
Ln-linear: OR=0.99 (95% CI 0.88, 1.12)
Linear: OR=1.05 (95% CI 0.89, 1.23)
T2 v T1: OR=0.95 (95% CI 0.80, 1.12)
T3 v T1: OR=0.99 (95% CI 0.84, 1.17)

Outcome: Wheezing in past year
Major Findings:
Ln-linear: OR=0.99 (95% CI 0.84, 1.18)
Linear: OR=1.00 (95% CI 0.81, 1.22)
T2 v T1: OR=1.08 (95% CI 0.89, 1.32)
T3 v T1: OR=0.97 (95% CI 0.75, 1.25)

Outcome: Current asthma
Major Findings:
Ln-linear: OR=1.00 (95% CI 0.76, 1.33)
Linear: OR=1.02 (95% CI 0.81, 1.30)
T2 v T1: OR=0.90 (95% CI 0.71, 1.14)
T3 v T1: OR=1.05 (95% CI 0.82, 1.33)</td>
<td>Major Limitations:
Cross-sectional design prevents causal inference.

Duration of breastfeeding was not controlled for – a potential confounder.</td>
</tr>
</table>

Reference and Study Design	Exposure Measures	Results	Comment
Jain, 2013 **Study Design:** Cross-sectional **Location:** General U.S. population **Population:** NHANES 2007-2008, with exclusions for pregnancy, evidence of thyroid condition, and missing data (n=1,733), > 12 years of age. **Outcome Definition:** Laboratory measures	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Serum concentrations of PFCs for this study population were not provided.	**Stat Method:** Linear regression, covariates and confounders considered included age, gender, race/ethnicity, smoking, iodine status, C-reactive protein, BMI, fasting time, and caloric intake. PFCs and thyroid parameters were log-transformed. **Outcome:** TSH (µIU/mL), FT3 (pg/L), FT4 (fg/dL), thyroglobulin (ng/L), TT3 (fg/dL), and TT4 (pg/dL) **Major Findings:** PFNA not statistically significantly associated with any of the thyroid parameters. (Results not presented in paper).	**Major Limitations:** Did not control other unmeasured environmental pollutants, including other PFCs. Cross-sectional design prevents causal inference.

Reference and Study Design	Exposure Measures	Results	Comment
Ji et al., 2012 **Study Design:** Cross-sectional **Location:** Siheung, Korea **Population:** Recruited from cohort (n=633 >12 years of age) **Outcome Definition:** Thyroid hormones	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Median serum concentration of PFNA 2.09 ng/mL (IQR 1.49-2.74) PFOA- 2.74 ng/mL (IQR 2.04-3.64) PFOS- 7.96 ng/mL (IQR 5.58-12.10)	**Stat Method:** Linear regression, confounders and covariates considered include: age, sex, and BMI. PFCs were log-transformed. **Outcome:** log-TT4 (μg/dL) **Major Findings:** β=-0.005 (95% CI -0.034, 0.025) **Outcome:** log-TSH (μIU/mL) **Major Findings:** β=0.110 (95% CI -0.035, 0.225)	**Major Limitations:** Did not control other unmeasured environmental pollutants, including other PFCs. Cross-sectional design prevents causal inference.

Reference and Study Design	Exposure Measures	Results	Comment
Kim et al., 2011b **Study Design:** Prospective birth cohort **Location:** South Korea **Population:** Pregnant women recruited from three hospitals (n=44), mostly sampled during the third trimester of pregnancy, age > 25 years. Paired samples available for 26 mother-infant pairs. **Outcome Definition:** Serum concentration at 3rd trimester, cord blood at delivery, and breast milk during mother checkup-visit	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Median serum concentration of PFNA 0.44 ng/mL (IQR 0.23-0.39) – pregnant women Median cord serum concentration of PFNA 0.45 (IQR 0.23-0.66) – infants	**Stat Method:** Correlations between exposure and outcome calculated using Pearson correlation tests performed using the logarithms of thyroid hormones and PFCs with and with-out adjustment for influential covariates. The following covariate and confounders were considered maternal age, gestational age, and maternal BMI. **Outcome:** T3 (ng/dL), TSH (μIU/mL), TT4 (μg/dl) **Major findings:** No associations were found between thyroid hormones and maternal or cord blood concentrations for PFNA. (Results not presented in the paper). Also no associations with birth weight.	**Major Limitations:** Small sample size limited ability to control for potential covariates and confounders simultaneously, or other potential environmental compounds of interest. Possible reverse causality. Did not control other unmeasured environmental pollutants or other PFCs.

Reference and Study Design	Exposure Measures	Results	Comment
Leter et al., 2014 **Study Design:** Cross-sectional **Location:** Greenland, Poland, Ukraine **Population:** 262 partners of pregnant women (non-occupationally exposed and fertile, at least 18 years old) **Outcome Definition:** Sperm global methylation levels: 1). Average DNA methylation level in repetitive DNA sequences 2). Flow cytometric immunodetection	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** PFNA Average (SE) Greenland: 2.2 (0.2) Kharkiv: 1.1 (0.1) Warsaw: 1.4 (0.1) Combined: 1.6 (0.1)	**Stat Method:** Multivariate linear regression analysis, covariate and confounders assessed include age, BMI, cotinine, alcohol consumption, and abstinence time, and spillage PFCs were natural log transformed. **Outcome:** Global methylation levels - Flow cytometric (FCM) DGML **Major Findings:** Combined: β=-38.7 (95% CI -72.8, -4.6) **Outcome:** LINE-1 **Major Findings:** Combined: β=1.1 (95% CI -0.3, 2.5) **Outcome:** Alu **Major Findings:** Combined: β=-0.7 (95% CI -1.8, 0.3) **Outcome:** Satα **Major Findings:** Combined: β=1.7 (95% CI -1.6, 5.1)	**Major Limitations:** Study population included all degrees of subfertile men Possible confounding due to unmeasured variables, including other PFCs. Cross-sectional design prevents causal inference.

Reference and Study Design	Exposure Measures	Results	Comment
Lin et al., 2009 **Study Design:** Cross-sectional **Location:** General U.S. population **Population:** NHANES 1999-00 & 2003-04, Adolescents, 12-20 year n=474 Adults, >20 years, n=969 **Outcome Definition:** Glucose homeostasis and metabolic syndrome/metabolic syndrome components (WC, glucose, HDL, and triglycerides)	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Mean PFNA concentrations for adolescents=0.70 ng/mL and adults=0.81 ng/mL	**Stat Method:** Linear regression, covariates and confounders considered include age, sex, race, smoking, alcohol consumption, household income, waist measurement, CRP, insulin/glucose/HOMA, current medications. Logistic regression used to examine metabolic syndrome. PFCs are log-transformed. Findings shown here are for most adjusted models. **Outcome:** Blood glucose (mmol/l) **Major Findings:** Adolescents β= 0.07 ± 0.04, Adults β= 0.00 ± 0.04 **Outcome:** log-Insulin (pmol/l) **Major Findings:** Adolescents **β= -0.10 ± 0.05***, Adults β= -0.04 ± 0.03 **Outcome:** log HOMA-IR **Major Findings:** Adolescents β= -0.08 ± 0.04, Adults β= -0.04 ± 0.04 **Outcome:** log β-cell function **Major Findings:** Adolescents **β= -0.12 ± 0.06***, Adults β= -0.04 ± 0.03 **Outcome:** Metabolic syndrome **Major Findings:** <u>Adolescents</u> OR=0.37 (95% CI 0.21,0.64) WC OR=1.09 (95% CI 0.61,1.95) Glucose OR=3.16 (95% CI 1.39, 7.16) HDL **OR=0.67 (95% CI 0.45, 0.99)** Trigly OR=0.71 (95% CI 0.37, 1.34) <u>Adults</u> OR=0.92 (95% CI 0.69, 1.24) WC OR=1.34 (95% CI 0.93, 1.92) Glucose OR=0.86 (95% CI 0.66, 1.12) HDL OR=0.81 (95% CI 0.65, 1.00) Trigly OR=0.99 (95% CI 0.81, `1.19)	**Major Limitations:** Cross-sectional design prevents causal inference Did not take into account other environmental factors and contaminants including other co-occurring PFCs.

Reference and Study Design	Exposure Measures	Results	Comment
Lin et al., 2010 **Study Design:** Cross-sectional **Location:** General U.S. Population **Population:** NHANES 1999-2000 & 2003-2004, n=2,216. Individuals, who fasted less than 6 hours, were hepatitis B or C virus carriers were excluded. **Outcome Definition:** Liver function biomarkers	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Median PFNA 0.70 ng/mL	**Stat Method:** Linear regression, covariates and confounders considered included age, gender, race/ethnicity, smoking, alcohol consumption, education level, BMI, HOMA-IR, metabolic syndrome, and iron saturation status. PFNA was modeled separately and included in a composite analysis with PFOS, PFOA, and PFHxS. PFCs assessed as quartiles and natural log transformed. Model estimates are shown for most adjusted. **Outcome:** ALT (U/l) **Major Findings:** Quartiles of PFNA (unadjusted), no trend (p-value=0.16). Separated: β=0.84 (p-value=0.13) Composite: β= -0.19 (p-value=0.77) **Outcome:** log-GGT (U/l) **Major Findings:** Quartiles of PFNA (unadjusted), no trend (p-value=0.07) Separated: β=-0.00 (p-value=0.86) Composite: β= -0.03 (p-value=0.25) **Outcome:** total bilirubin (μM) **Major Findings:** Quartiles of PFNA (unadjusted), **increasing trend (p-value=0.014)** **Separated: β=0.49 (p-value=0.05)** **Composite: β=0.75 (p-value=0.004)**	**Major Limitations:** Cross-sectional design prevents causal inference. Reverse causality. No control for other environmental chemicals or medications.

Reference and Study Design	Exposure Measures	Results	Comment
Lin et al., 2011 **Study Design:** Cross-sectional **Location:** Taiwan **Population:** n=287 Taiwanese adolescents and young adults, aged 12-30 years recruited from a hypertension cohort **Outcome definition:** Serum samples	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** The median PFNA serum level was 1.68 ng/mL	**Stat Method:** The relation of PFC variables to categorical variables was tested using the Mann-Whitney U test or Kruskal-Wallis test. Linear regression was used for continuous variables. Covariates and confounders considered include age, gender, smoking, alcohol consumption, household income, waist measurement, systolic blood pressure (sBP), total cholesterol, HOMA-IR, creatinine. Associations studied over categories of PFNA. Results shown here for most adjusted model. Outcome, Parameters related to glucose metabolism **Outcome:** ln-adiponectin (ng/mL) **Major Findings:** ↑ (p-value <0.01) **Outcome:** glucose (mg/dL) **Major Findings:** NS **Outcome:** ln-insulin (pmol/L) **Major Findings:** NS **Outcome:** log-HOMA-IR **Major Findings:** NS **Outcome:** HDL (mg/dL) **Major Findings:** NS **Outcome:** log-TG (mg/dL) **Major Findings:** NS **Outcome:** log-CRP (mg/L) **Major Findings:** NS	**Major Limitations:** Cross-sectional design prevents causal inference The study population is made up of adolescents and young adults with abnormal urinalysis in childhood. Did not take into account medications or other environmental factors including other co-occurring PFCs.

<table>
<tr><th>Reference and Study Design</th><th>Exposure Measures</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Lin et al., 2013a

Study Design:
Cross-sectional

Location:
Taiwan

Population:
n=664 individuals with abnormal urinalysis results (246 with elevated blood pressure and 398 with normal blood pressure) aged 12-30 years, who had been originally recruited from a population-based mass urine screening in Taiwan

Outcome definition:
Serum samples, socio-demographic data collected during interview. Clinical outcomes were determined from clinical serum measures.

Carotid artery intima-media thickness (CIMT) is a marker of subclinical atherosclerosis.

Related studies:
Lin et al., 2011</td>
<td>Exposure Assessment:
Serum concentrations

Population-Level Exposure:
Median PFNA serum level = 0.38 ng/mL* (range 0.38-25.4)
*most likely an error

<table>
<tr><th>Percentile</th><th>Mean (ng/mL)</th></tr>
<tr><td>≤60th</td><td>≤1.58</td></tr>
<tr><td>60th-90th</td><td>≤6.78</td></tr>
<tr><td>>90th</td><td>>6.78</td></tr>
</table>

Males (ng/mL) – 1.19 (95% CI 0.56-3.92)
Females (ng/mL) – 1.00 (95% CI 0.24-1.01)</td>
<td>Stat Method:
Linear regression and logistic regression, covariates and confounders considered include age, gender, smoking status, alcohol consumption, and BMI to estimate association with cardiovascular risk factors, and additionally, systolic blood pressure (sBP), BMI, LDL, CRP, TG, and HOMA-IR for Carotid intima-associated thickness (CIMT).

Logistic regression analysis was conducted to examine the odds ratios of thicker CIMT for PFOS only. Investigators performed a composite analysis with four PFCs modeled together.

Outcome: systolic blood pressure (SBP) (mm Hg)
Major Findings: NS (P for Trend 0.321)

Outcome: BMI (kg/m^2)
Major Findings: NS (P for Trend 0.043)

Outcome: LDL (mg/dL)
Major Findings: NS (P for Trend 0.811)

Outcome: log-TG (mg/dL)
Major Findings: NS (P for Trend 0.593)

Outcome: uric acid (UA) (mg/dL)
Major Findings: NS (P for Trend 0.689)

Outcome: log-HOMA-IR
Major Findings: NS (P for Trend 0.009)

Outcome: CIMT
Major Findings: CIMT decreased insignificantly with increasing levels of PFNA.</td>
<td>Major Limitations:
Cross-sectional design prevents causal inference

The study population is made up of adolescents and young adults with abnormal urinalysis in childhood.

Did not take into account medications or other environmental factors.</td>
</tr>
</table>

<table>
<tr><th>Reference and Study Design</th><th>Exposure Measures</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Lin et al., 2013b

Study Design:
Cross-sectional

Location:
Taiwan

Population:
n=551 individuals with abnormal urinalysis results (221 with elevated blood pressure and 310 with normal blood pressure) aged 12-30 years, who had been originally recruited from a population-based mass urine screening in Taiwan

Outcome Definition:
Laboratory, examination, and survey information.</td>
<td>Exposure Assessment:
Serum concentrations

Population-Level Exposure:
Geometric mean of PFNA, 1.01 ng/mL. PFNA was categorized into three percentile cutoff groups.

<table>
<tr><th>Percentile Groups</th><th>Median (ng/mL)</th></tr>
<tr><td><60th</td><td><1.2</td></tr>
<tr><td>60th-90th</td><td>≤6.46</td></tr>
<tr><td>>90th</td><td>>6.46</td></tr>
</table>
</td>
<td>Stat Method:
Linear regression and logistic regression, covariates and confounders considered include age, gender, smoking, and alcohol consumption.

TSH was natural log transformed.

PFNA was explored against different levels of BMI, smoking, and current hypertension.

PFOS, PFHxS, PFOA, and PFNA are put into model. (Did not alter results)

Outcome: Free T4 (ng/dl)
Major Findings:
Mean=1.07, 1.06, 1.12 (P for Trend <0.05)

The association between FT4 and PFNA was significant for active smokers and those with higher BMI, but tests for interaction were insignificant.

Outcome: ln-TSH (m IU/l)
Major Findings:
Mean=0.43, 0.40, 0.58 (P for Trend NS)

No differences were found between exposure to PFNA related to the OR of being hypothyroid.</td>
<td>Major Limitations:
Cross-sectional design prevents causal inference.

The study population is composed of 12-30 yr olds with abnormal urinalysis results in childhood living in the Taipei area.

Did not control for medications that could be potential confounders, and other unmeasured environmental pollutants.</td>
</tr>
</table>

<table>
<tr><th>Reference and Study Design</th><th>Exposure Measures</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Lind et al., 2013

Study Design:
Cross-sectional

Location:
Sweden

Population:
Adults aged 70 years or older, n=1,016, 2001-2004

Outcome definitions:
Participant response or laboratory measure</td>
<td>Exposure Assessment:
Serum concentrations

Population-Level Exposure:

<table>
<tr><th>Percentile</th><th>Median (ng/mL)</th></tr>
<tr><td>25th</td><td>0.5</td></tr>
<tr><td>50th</td><td>0.7</td></tr>
<tr><td>75th</td><td>1.0</td></tr>
</table>
</td>
<td>Stat Method:
Logistic regression to evaluated association with prevalent diabetes, with PFNA (log transformed) treated linearly and squared (for non-linear effects). Covariates and confounders considered include sex, cholesterol, triacylglycerol, BMI, smoking, exercise habits, energy and alcohol intakes, and education level. Linear regression used to evaluate association with proinsulin/insulin ratio and HOMA-IR and restricted to non-diabetic participants.

Results shown here for most adjusted model. Diabetes defined as having a history of diabetes or a fasting glucose value >7.0 mmol/l

Outcome: Diabetes
Major Findings: (linear) OR=1.30 (95% CI 0.85-1.97); (quadratic) OR=1.25 (95% CI 1.08-1.44)

Outcome: Proinsulin/insulin ratio
Major Findings: β=0.043 (95% CI -0.015-0.102)

Outcome: HOMA-IR
Major Findings: β=0.004 (95% CI -0.059-0.066)</td>
<td>Major Limitations:
Cross-sectional design prevents causal inference.

Confounding due to medications may be occurring, although participants underwent a fasting period.

Did not take into account other environmental factors and contaminants including other co-occurring PFCs.</td>
</tr>
</table>

<table>
<tr><th>Reference and Study Design</th><th>Exposure Measures</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Lopez-Espinosa et al., 2012

Study Design:
Cross-sectional (for analysis involving PFNA)

Location:
United States – Ohio and West Virginia

Population:
Children (age 1-17 years) who consumed water (for at least 1 year) from a water district with known PFOA contamination, n=10,725 were included in the analysis.
Side note: Subsample of children matched to mothers for modeled in utero PFOA exposure n=4,713

Outcome Definition:
Serum samples of TSH, TT_4, categorized into subclinical hypothyroidism and hyperthyroidism. Also parent self-reported thyroid disease and thyroid disease related medication use</td>
<td>Exposure Assessment:
Serum concentrations of PFNA.

Side note: Historical PFOA exposures estimated through environmental, exposure, and pharmacokinetic modeling to estimate annual PFOA exposure.

Population-Level Exposure:

<table>
<tr><th>Age Group</th><th>Median (ng/mL)</th></tr>
<tr><td>1-5 yr</td><td>1.4</td></tr>
<tr><td>6-10 yr</td><td>1.8</td></tr>
<tr><td>>10 yr</td><td>1.4</td></tr>
<tr><td>1-17 yr</td><td>1.5</td></tr>
</table>
</td>
<td>Stat Method:
Linear and logistic regression, covariates and confounders considered include age, sex, race/ethnicity, BMI, month of sampling, average household family income, smoking, and alcohol consumption.

TSH and PFNA were log-transformed (linear regression), and PFNA also analyzed in quartiles (logistic regression) and estimates presented as percent change for continuous outcomes and odds ratio for binary outcomes presented as an IQR shift.

Outcome: ln-TSH (μIU/mL)
Major Findings:
Q2 v. Q1: 0.4 (95% CI -2.6, 3.5)
Q3 v. Q1: -0.3 (95% CI -4.2, 1.7)
Q4 v. Q1: 1.5 (95% CI -1.6, 4.6)
IQR: 0.8 (95% CI -0.4, 2.0)

Outcome: TT_4 (μg/dL)
Major Findings:
Q2 v. Q1: 0.8 (95% CI -0.3, 1.8)
Q3 v. Q1: 1.7 (95% CI 0.7, 2.8)
Q4 v. Q1: 2.7 (95% CI 1.7, 3.8)
IQR: 1.1 (95% CI 0.7, 1.5)

Outcome: Thyroid Disease
Major Findings:
Reported: OR=1.05 (95% CI 0.78, 1.41)

Outcome: Hypothyroidism
Major Findings:
Reported: OR=1.11 (95% CI 0.77, 1.60)
Subclinical: OR=0.99 (95% CI 0.88, 1.12)

Outcome: Hyperthyroidism
Major Findings:
Subclinical: OR=0.78 (95% CI 0.61, 1.01)</td>
<td>Major Limitations:
Cross-sectional design prevents causal inference.

Lack of measurement of additional childhood thyroid hormones.

Reliance on recall for thyroid disease

No control for other environmental chemicals including other PFCs.</td>
</tr>
</table>

<table>
<tr><th>Reference and Study Design</th><th>Exposure Measures</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Louis et al., 2012

Study Design:
Case-control

Location:
Salt Lake City or San Francisco

Population:
1. Operative sample (OS):495 women aged 18-44 years scheduled for laparoscopy/laparotomy at one of 14 participating clinical sites, 2007-2009. (190 cases of endo and 283 none)

2. Population sample (P): Population-based sample consisting of 131 women matched to the operative sample on age and residence within a 50-mile radius of participating clinics (14 cases Endo and 113 none)

Controls from referent population matched on age and residence.

Outcome Definition:
Endometriosis defined through surgical visualization (in the operative sample) or magnetic resonance imaging (in the population sample)</td>
<td>Exposure Assessment:
Serum concentrations

Population-Level Exposure:

<table>
<tr><th>Tertile</th><th>PFNA</th></tr>
<tr><td>1^{s}</td><td>-0.21, 0.53</td></tr>
<tr><td>2nd</td><td>0.53, 0.84</td></tr>
<tr><td>3rd</td><td>0.84, 4.1</td></tr>
</table>

<table>
<tr><th>Groups</th><th>GM (95% CI)</th></tr>
<tr><td>OS/Endo</td><td>0.69 (0.63, 0.77)</td></tr>
<tr><td>OS/None</td><td>0.58 (0.53, 0.63)</td></tr>
<tr><td>P/None</td><td>0.71 (0.55, 0.92)</td></tr>
<tr><td>P/Endo</td><td>0.64 (0.55, 0.74)</td></tr>
</table>
</td>
<td>Stat Method: Logistic regression, covariates and confounders considered include age, BMI, and parity

PFCs were natural log transformed

Outcome: Endometriosis
Major Findings:
OS: OR (unadj)=2.75 (95% CI 1.30, 5.80)
OS: OR (ajdA)=2.20 (95% CI 1.02, 4.75)
OS: OR (ajdB)= 1.99 (95% CI 0.91, 4.33)

P: OR (unadj)= 1.31 (95% CI 0.14, 12.0)
P: OR (ajdA)=1.52 (95% CI 0.15, 15.1)
P: OR (ajdB)=1.63 (95% CI 0.16, 16.9)

*restricted to stage 3 and 4 of endometriosis.
OS: OR=0.99 (95% CI 0.27, 3.65)

*comparison group restricted to postoperative diagnosis of a normal pelvis
OS: OR=1.18 (95% CI 0.46, 3.05)</td>
<td>Major Limitations:
Bidirectional errors reportedly associated with endometriosis staging.

Model dependent results.

Possible confounding due to unmeasured variables, and co-occurring environmental contaminants including other PFCs.

Small sample size – especially for population sample with only 14 cases of endometriosis – results in very wide confidence intervals.</td>
</tr>
</table>

Reference and Study Design	Exposure Measures	Results	Comment
Monroy et al., 2008 **Study Design:** Nested Prospective birth cohort **Location:** Canada **Population:** 101 mother:infant pairs from large cohort study **Outcome Definition:** Measured and recorded at birth	**Exposure Assessment:** Maternal serum concentration at second trimester and delivery, and cord serum concentration **Population-Level Exposure:** Maternal serum at 24-28 weeks – Median PFNA concentration 0.86 ng/mL and range 0.58-0.96 Maternal serum at delivery – Median PFNA concentration 0.80 ng/mL and range 0.54-0.87 Umbilical cord blood – Median PFNA concentration 0.94 and range 0.61-0.80	**Stat Method:** Paired t-tests and linear regression, covariates and confounders considered included parity, gestational length, birth weight, and gender, maternal BMI, **Outcome:** Gestational length (cm), Birth weight (kg) **Major Findings:** No association was found between PFNA in maternal serum and cord blood at delivery and birth weight. (Results presented in a figure)	**Major Limitations:** Small sample size. No control for maternal exposures or other potential confounders including other PFCs.

Reference and Study Design	Exposure Measures	Results	Comment
Mundt et al., 2007 **Study Design:** Occupational: Cross-sectional and retrospective cohort **Location:** U.S. factory **Population:** 630 individuals employed, actively and formerly, at a polymer production facility using PFNA surfactant blend (Surflon S-111) at any time between 1 January 1989 and 1 July 2003. Final sample size was 592 (518 men and 74 women) **Outcome Definition:** Laboratory test results (32 clinical parameters) were abstracted from annual medical examination records.	**Exposure Assessment:** Detailed work histories using to categorize into exposure groups. **Population-Level Exposure:** Exposure categories: no exposure, low exposure, and high exposure. Women classified as only exposed or not exposed. No serum concentrations.	**Stat Method:** Cross-sectional analysis to evaluate pairwise differences in average values of clinical parameters at the five time points (1976, 1989, 1995, 1998, and 2001) across exposure groups, additional cross sectional analyses of mean laboratory values by exposure groups, and longitudinal analysis accounting for multiple measurements in the same individual. Covariates and confounders considered include age and BMI. Longitudinal analysis also included age at entry into cohort, exposure category in the month before blood sample was taken, weighted cumulative intensity score **Outcome:** Pairwise comparisons in average clinical parameters **Major Findings:** LDH: NS AST: NS ALT: **Men (1976 -High v. Low; 2001 High v. None)** Bilirubin: NS GGT:NS Alkaline phosphatase: **Men (1998 High v. None)** Cholesterol (total): **Men (1976 High v. Low, Low v. None; 1989 High v. Low, Low v. None)** Triglycerides: NS HDL: NS LDL: NS VLDL: NS No significant findings reported in women. Data for electrolytes, BUN, creatinine, thyroid hormones (TSH, T4, T3 uptake, and free thyroxine uptake), and uric acid are not shown. Led to assume non-significant findings. **Outcome:** Extended cross-sectional analysis **Major Findings:** Data for the extended cross-sectional analysis is not presented. It is reported that values fluctuated slightly across exposure groups over the years and that no group mean was consistently increased or decreased over time. **Outcome:** Longitudinal analysis of 7 clinical parameters (men only) **Major Findings:** total cholesterol, GGT, AST, ALT, alkaline phosphatase, bilirubin, triglycerides) – no significant increase or decrease.	**Major Limitations:** Data are not presented for some findings are discussed. Small percentage of subjects in high and no exposure groups compared to low exposure groups. Limited data for women. No serum concentration data. Further: Exposure in the least exposed groups may be well above the population exposure range.

<table>
<tr><th>Reference and Study Design</th><th>Exposure Measures</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Nelson et al., 2010

Study Design:
Cross-sectional

Location:
General U.S. population

Population:
NHANES 2003-2004, < 80 years old
N=416 or 860 depending on parameter

Outcome definition:
Serum samples, and anthropometric measures</td>
<td>Exposure Assessment:
Serum concentrations

Population-Level Exposure:
Median PFNA serum level = 1.0 ng/mL
Range 0.1-10.3 ng/mL

<table>
<tr><th>Quartiles</th><th>Median (ng/mL)</th></tr>
<tr><td>1</td><td>0.4</td></tr>
<tr><td>2</td><td>0.7</td></tr>
<tr><td>3</td><td>1.0</td></tr>
<tr><td>4</td><td>2.0</td></tr>
</table>
</td>
<td>Stat Method:
Regression, covariates and confounders considered include age, sex, race/ethnicity, socioeconomic status, saturated fat intake, exercise, TV time, alcohol consumption, smoking, and parity in women.

Effect estimates of each quartile to the lowest quartile. Test for trend performed.

Outcome: TC (mg/dL)
Major Findings: ↑ Test for trend p=0.04

Outcome: HDL (mg/dL)
Major Findings: ↓ Test for trend p=0.31

Outcome: non-HDL (mg/dL)
Major Findings: ↑ Test for trend p=0.04

Outcome: LDL (mg/dL)
Major Findings: ↑ Test for trend p=0.08

Outcome: BMI (kg/m^2), waist circumference (WC) (cm), HOMA-IR (insulin resistance assessed as Homeostatic Model Assessment)
Major Findings: PFNA was not associated with BMI, WC, or HOMA (results not presented in paper)</td>
<td>Major Limitations:
Cross-sectional design prevents causal inference.

The authors note that correlation with PFOA and/or PFOS could partially explain the results, although PFNA was only moderately correlated with them (r=0.5).</td>
</tr>
</table>

Reference and Study Design	Exposure Measures	Results	Comment
Ode et al., 2014 **Study Design:** Matched case-control (prospective) **Location:** Malmo, Sweden **Population:** Children born between 1978-2000 that were followed up until 2005. Cases were children with ADHD (n=206). Controls selected from same study base, matched on year of birth and maternal country of birth (n=206). **Outcome Definition:** Clinician diagnosed ADHD	**Exposure Assessment:** PFC concentrations measured in umbilical cord serum samples. For PFNA concentrations above the level of detection (0.2 ng/mL) were compared to those above. **Population-Level Exposure:** PFNA concentrations not provided. PFOA in Cases – 1.80 ng/mL PFOA in Controls – 1.83 ng/mL PFOS in Cases – 6.92 ng/mL PFOS in Controls – 6.77 ng/mL	**Stat Method:** Differences in PFC concentrations between cases and controls were compared using the Wilcoxon's paired test. Conditional logistic regression was used to evaluate possible threshold effects. Confounders and covariates considered include smoking during pregnancy, parity, and gestational age at birth. PFNA categorized as high v. low. **Outcome:** ADHD **Major Findings:** No difference between cases and control PFNA concentration (p-value 0.48) OR adj=1.1 (95% CI 0.75, 1.7) No significant associations between cord blood PFC concentrations and ADHD.	**Major Limitations:** Small study size – significant loss of potential cases. Possible confounding due to unmeasured variables and other environmental contaminants, including other PFCs.

Reference and Study Design	Exposure Measures	Results	Comment
Power et al., 2013 **Study Design:** Cross-sectional **Location:** General U.S. population **Population:** NHANES 1999-2000 & 2003-2008, adults aged 60-85 years of age with PFC measurements n=1,766 **Outcome Definition:** Cognitive ability was measured by the main outcome, self-reported difficulty due to remembering or periods of confusion, and secondarily the outcomes self-reported difficulty with activities of daily-living due to senility and performance on the Digit-Symbol Substitution Task (DSST) was investigated.	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Geometric mean of PFNA was 1.01 ng/mL.	**Stat Method:** Logistic regression, covariates and confounders considered include age, age-squared, race/ethnicity, gender, cycle, education, poverty-income ratio, food security, health insurance, social support, physical activity, smoking, and alcohol consumption and diabetes assessed as an effect modifier. PFNA natural log transformed, estimates based on a doubling of PFNA. **Outcome:** Difficulty remembering or periods of confusion **Major Findings:** OR=0.91 (95 % CI 0.79, 1.04) **Outcome:** Senility **Major Findings:** OR=0.92 (95% CI 0.59, 1.44) **Outcome:** DSST **Major Findings:** OR=0.29 (95% CI -1.69, 2.26)	**Major Limitations:** Cross-sectional design prevents causal inference. Reverse causality Possible confounding due to unmeasured variables and other environmental contaminants, including other PFCs Outcome definition depends on self-report.

Reference and Study Design	Exposure Measures	Results	Comment
Specht et al., 2012 **Study Design:** Cross-sectional **Location:** Greenland, Poland, Ukraine **Population:** 604 fertile male partners of pregnant women (199 from Greenland, 197 from Poland, 208 from Ukraine) **Outcome Definition:** DNA damage in spermatozoa by sperm chromatin structure assay (SCSA) and in situ terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay, apoptotic markers in semen, and reproductive hormones in serum	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Median PFNA (ng/mL) and Range Greenland: 1.4 (0.5-12) Poland: 1.2 (0.5-6) Ukraine: 1.0 (0.2-4)	**Stat Method:** General linear models. Covariates and confounders considered include sexual abstinence period, age, BMI, caffeinated drinks, cotinine, fever during past 3 months, self-reported genital infections, and testicular disorders, and spillage of semen sample. All analyses were stratified by region **Outcome:** DNA damage in sperm **Major Findings:** Estimates for associations with PFNA not provided in paper: An association of PFNA with sperm DNA fragmentation was not found in any of the three regions in uncorrected analyses, and similar results were obtained after adjustment for potential confounders." PFNA was not associated with TUNEL-positivity No other associations between PFNA and apoptotic markers were consistent across regions or in models within regions. PFNA were not consistently related to SHBG concentrations and associations were not consistent across region for testosterone, estradiol, and gonadotropins.	**Major Limitations:** Serum levels of the individuals PFCs were highly and significantly correlated. Cross-sectional design prevents causal inference. Varying participation rates. Blood samples were collected approximately a year before the semen samples. Long half-lives make it unlikely that a skewed sampling is unlikely.

<table>
<tr><th>Reference and Study Design</th><th>Exposure Measures</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Starling et al., 2014a

Study Design:
Nested case-control

Location:
Norway

Population:
Nulliparous pregnant women (466 cases of preeclampsia, 510 non-cases) selected from a prospective pregnancy cohort (MoBa), 16-44 years.

Outcome Definition:
Medical record review - validated</td>
<td>Exposure Assessment:
Serum concentrations

Population-Level Exposure:

<table>
<tr><th>Percentile</th><th>Median (ng/mL)</th></tr>
<tr><td>25th</td><td>0.39</td></tr>
<tr><td>50th</td><td>0.54</td></tr>
<tr><td>75th</td><td>0.74</td></tr>
</table>
</td>
<td>Stat Method:
Proportional hazards model, covariates and confounders considered include maternal age at delivery, pre-pregnancy BMI, maternal educational level, smoking at mid-pregnancy, plasma creatinine, cystatin C, HDL cholesterol.

PFNA categorized into quartiles and log transformed continuous. Adjusted estimates presented here.

Outcome: Preeclampsia
Major Findings:
Q2 v. Q1, HR=0.85 (95% CI 0.60, 1.22)
Q3 v. Q1, HR=0.92 (95% CI 0.64, 1.21)
Q4 v. Q1, HR=0.80 (95% CI 0.56, 1.15)
Continuous, HR=0.84 (95% CI 0.66, 1.07)</td>
<td>Major Limitations:
Correlations with other PFCs makes it difficult to tease out the impact of PFNA independently.

Variation in exposure in exposure concentrations.

Possible confounding due to unmeasured variables.

Possible selection bias, participation rate in cohort was 39%</td>
</tr>
</table>

<table>
<tr><th>Reference and Study Design</th><th>Exposure Measures</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Starling et al., 2014b

Study Design:
Cross-sectional

Location:
Norway

Population:
Pregnant women (n=891), enrolled in the Norwegian Mother and Child (MoBa) Cohort Study, 2003-2004

Outcome definition:
Serum samples</td>
<td>Exposure Assessment:
Non-fasting plasma samples taken at mid-pregnancy

Population-Level Exposure:
Median PFNA serum level = 0.39 ng/mL

<table>
<tr><th>Percentile</th><th>Median (ng/mL)</th></tr>
<tr><td>5th</td><td>0.17</td></tr>
<tr><td>25th</td><td>0.29</td></tr>
<tr><td>50th</td><td>0.39</td></tr>
<tr><td>75th</td><td>0.51</td></tr>
<tr><td>95th</td><td>0.27</td></tr>
</table>
</td>
<td>Stat Method:
Linear regression, covariates and confounders considered include maternal age, pre-pregnancy BMI, nulliparous or most recent inter-pregnancy interval, duration of breastfeeding most recent child, maternal years of education, current smoking at mid-pregnancy, gestational weeks at blood draw, and amount of oily fish consumed daily, and weight gain, and albumin.

PFNA treated as continuous ln-PFNA and as quartiles, and effect estimates for quartiles, log unit increase, and IQR increase

Outcome: TC (mg/dL)
Major Findings:
Q2 v. Q1 β=-5.28 (95% CI -12.75, 2.19)
Q3 v. Q1 β=-3.84 (95% CI -11.55, 3.86)
Q4 v. Q1 β=2.22 (95% CI -6.47, 10.90)
Ln-unit β=0.01 (95% CI -5.98, 6.00)
IQR β=0.01 (95% CI -3.51, 3.52)

Outcome: HDL (mg/dL)
Major Findings:
Q2 v. Q1 β=-0.06 (95% CI -2.60, 2.47)
Q3 v. Q1 β=0.48 (95% CI -2.09, 3.06)
Q4 v. Q1 β=3.26 (95% CI 0.47, 6.05)
Ln-unit β=2.84 (95% CI 0.97, 4.71)
IQR β=1.66 (95% CI 0.57, 2.76)

Outcome: LDL (mg/dL)
Major Findings:
Q2 v. Q1 β=-5.04 (95% CI -11.78, 1.70)
Q3 v. Q1 β=-3.82 (95% CI -10.71, 3.07)
Q4 v. Q1 β=-0.81 (95% CI -8.30, 6.69)
Ln-unit β=-2.51 (95% CI -7.31, 3.02)
IQR β=-1.26 (95% CI -4.29, 1.77)

Outcome: ln-triglycerides (mg/dL)
Major Findings:
Q2 v. Q1 β=-0.03 (95% CI -0.10, 0.04)
Q3 v. Q1 β=-0.02 (95% CI –0.09, 0.05)
Q4 v. Q1 β=-0.02 (95% CI -0.09, 0.06)
Ln-unit β=-0.02 (95% CI -0.07, 0.03)
IQR β=-0.01 (95% CI -0.04, 0.02)</td>
<td>Major Limitations:
Cross-sectional design prevents causal inference

Did not take into account other environmental factors and contaminants including other co-occurring PFCs.</td>
</tr>
</table>

<table>
<tr><th>Reference and Study Design</th><th>Exposure Measures</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Taylor et al., 2014

Study Design:
Cross-sectional

Location:
General U.S. population

Population:
NHANES 1999-2000 & 2003-2010, women aged 20-65 years of age with PFC measurements n=2,732

Outcome Definition:
Premenopausal v. post-menopausal categorized by questionnaire responses</td>
<td>Exposure Assessment:
Serum concentrations

Population-Level Exposure:

<table>
<tr><th>Category</th><th>Median (ng/mL)</th><th>IQR</th></tr>
<tr><td>Pre menopause</td><td>0.90</td><td>0.60, 1.40</td></tr>
<tr><td>Menopause</td><td>1.20</td><td>0.80, 1.80</td></tr>
<tr><td>Hysterectomy</td><td>1.30</td><td>0.80, 1.20</td></tr>
</table>
</td>
<td>Stat Method:
Proportional hazard modeling, covariates and confounders considered include age, race, parity, education,

Hazard ratios calculated as the onset of natural menopause as a function of age and serum PFNA concentration. Premenopausal women were censored at the time of their survey.

Side note: Also look at both the association of PFC and hysterectomy and whether rate of natural menopause predicts serum concentrations to assess reverse causality. HR calculated with increasing tertiles of PFNA.

Outcome: Menopause
Major Findings:
T2 v. T1, HR=1.43 (95% CI 1.08, 1.87)
T3 v. T1, HR=1.47 (95% CI 1.14, 1.90)

Outcome: Hysterectomy
Major Findings:
T2 v. T1, HR=1.39 (95% CI 1.08, 1.80)
T3 v. T1, HR=1.78 (95% CI 1.33, 2.37)

Assessing reverse causality:
Positive associations between PFNA and the rate of hysterectomy, and PFNA increased with time since natural menopause.</td>
<td>Major Limitations:
PFC measures based on a single measurement.

Correlations with other PFCs makes it difficult to tease out the impact of PFNA independently.

Reverse causality may explain association of PFNA and earlier age at menopause.

Cross-sectional design prevents causal inference.</td>
</tr>
</table>

<table>
<tr><th>Reference and Study Design</th><th>Exposure Measures</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Toft et al., 2012

Study Design:
Cross-sectional

Location:
Greenland, Poland, and Ukraine

Population:
588 partners of pregnant women (Greenland n=196, Poland n=189, Ukraine n=203)

Outcome Definition:
Semen volume, sperm concentration, total sperm count, motility and morphology</td>
<td>Exposure Assessment:
Serum concentrations

Population-Level Exposure: PFNA (ng/mL)
<table>
<tr><th colspan="2">Greenland</th></tr>
<tr><th>Estimates</th><th>PFNA</th></tr>
<tr><td>Median</td><td>1.7</td></tr>
<tr><td>33rd per.</td><td>1.3</td></tr>
<tr><td>66th per.</td><td>2.4</td></tr>
</table>
<table>
<tr><th colspan="2">Poland</th></tr>
<tr><th>Estimates</th><th>PFNA</th></tr>
<tr><td>Median</td><td>1.2</td></tr>
<tr><td>33rd per.</td><td>1.0</td></tr>
<tr><td>66th per.</td><td>1.3</td></tr>
</table>
<table>
<tr><th colspan="2">Ukraine</th></tr>
<tr><th>Estimates</th><th>PFNA</th></tr>
<tr><td>Median</td><td>1.0</td></tr>
<tr><td>33rd per.</td><td>0.8</td></tr>
<tr><td>66th per.</td><td>1.2</td></tr>
</table>
<table>
<tr><th colspan="2">All</th></tr>
<tr><th>Estimates</th><th>PFNA</th></tr>
<tr><td>Median</td><td>1.2</td></tr>
<tr><td>33rd per.</td><td>1.0</td></tr>
<tr><td>66th per.</td><td>1.5</td></tr>
</table>
</td>
<td>Stat Method:
Multivariate linear regression analysis, covariate and confounders assessed include age, BMI, cotinine, alcohol consumption, abstinence time, spillage, urogenital infections.

Additionally used generalized-linear model to allow for possibly non-linear associations.

PFCs natural log transformed

Analyses were stratified by population type

Outcome: Sperm concentration
Major Findings:
Adj. DiffT2-T1: -1 (95% CI, -19, 18)
Adj. DiffT3-T1: 7 (95% CI, -13, 28)
p-value=0.53

Outcome: Volume
Major Findings:
Adj. DiffT2-T1: 0 (95% CI, -11, 12)
Adj. DiffT3-T1: -5 (95% CI, -17, 7)
p-value=0.34

Outcome: Total count
Major Findings:
Adj. DiffT2-T1: 2 (95% CI, -21, 24)
Adj. DiffT3-T1: 5 (95% CI, -19, 29)
p-value=0.93

Outcome: Percent motile sperm
Major Findings:
Adj. DiffT2-T1: 0 (95% CI, -12, 11)
Adj. DiffT3-T1: -1 (95% CI, -13, 11)
p-value=0.34

Outcome: Percent normal cells
Major Findings:
Adj. DiffT2-T1: -12 (95% CI, -26, 2)
Adj. DiffT3-T1: -8 (95% CI, -23, 7)
p-value=0.36</td>
<td>Major Limitations:

Measured PFCs are highly correlated; mutual adjustments are presented as subanalyses.

Male semen quality is known to vary considerably from day to day.

Cross-sectional design prevents causal inference.</td>
</tr>
</table>

Reference and Study Design	Exposure Measures	Results	Comment
Wang et al., 2011 **Study Design:** Prospective birth cohort **Location:** Taiwan **Population:** Children of pregnant women enrolled in study and had cord blood collected at delivery, n=244 children after exclusions **Outcome Definition:** Atopic dermatitis and IgE levels in cord blood and serum concentrations at 2 years of age	**Exposure Assessment:** Serum concentrations in cord blood at delivery **Population-Level Exposure:** Median PFNA 2.30 (0.38-63.87) ng/mL	**Stat Method:** Linear regression for IgE; covariates and confounders considered included gender, gestational age, parity, maternal age, and pre-natal ETS exposure. Logistic regression as used to analyze atopic dermatitis: covariates and confounders considered included gender, gestational age, maternal age, maternal history of atopy, duration of breast feeding, and pre-natal ETS exposure. Outcomes and exposure were log-transformed. Most adjusted model results are shown. **Outcome:** log-serum IgE (KU/l) **Major Findings:** (Cord Blood): β=0.024 (p-value 0.91) (@ 2 years): β=0.039 (p-value 0.84) **Outcome:** Atopic dermatitis **Major Findings:** (Cord Blood): Q2 v. Q1 OR=1.46 (95% CI 0.35, 6.07) Q3 v. Q1 OR= 1.53 (95% CI 0.59, 3.93) Q4 v. Q1 OR=0.72 (95% 0.23, 2.21)	**Major Limitations:** Blood PFC levels were not measured at 2 years of age. Did not control for other co-occurring environmental contaminants including PFCs or other possibly important confounders.

<table>
<tr><th>Reference and Study Design</th><th>Exposure Measures</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Wang et al., 2013

Study Design:
Cross-sectional

Location:
Norway

Population:
903 pregnant women, recruited from a case-control study in a subset of the MoBa cohort, who had blood sample and had a live birth. [950 women from case-control study, 400 subfecund women selected randomly and 550 control women selected at random], 18 to 44 years

Outcome Definition:
Blood sample taken at 17-18 weeks of gestation and self-reported.</td>
<td>Exposure Assessment:
Serum concentrations

Population-Level Exposure:
Geometric mean of PFNA 0.37 (95% 0.36, 0.39)

<table>
<tr><th>Percentile</th><th>Median (ng/L)</th></tr>
<tr><td>25th</td><td>0.28</td></tr>
<tr><td>50th</td><td>0.39</td></tr>
<tr><td>75th</td><td>0.51</td></tr>
</table>
</td>
<td>Stat Method:
Linear and logistic regression, covariates and confounders considered included age, gestational age at blood draw, pre-pregnancy BMI, parity, smoking during pregnancy, interval between birth and current pregnancy, duration of breast-feeding a previous child, total seafood intake, HDL, and albumin, and also consumption of fatty fish and thyroid hormone affecting medication.

TSH log transformed. Stratified by subfecund and control group. Findings for adjusted models shown.

Outcome: ln-TSH (μIU/mL)
Major Findings:
β=0.165 (95% CI -0.023, 0.353)

No significant associations found between PFNA and dichotomized TSH in logistic models.

Outcome: thyroid disease
Major Findings:
No association with self-reported thyroid disease when PFNA treated continuously and categorical/

Stratification was non-significant.</td>
<td>Major Limitations:
Cross-sectional design prevents causal inference.

Reverse causation.

TSH levels change throughout pregnancy so a single measurement may not adequately characterized thyroid homeostasis during pregnancy.

Did not control other unmeasured environmental pollutants.

Participation in MoBa was low.</td>
</tr>
</table>

<table>
<tr><th>Reference and Study Design</th><th>Exposure Measures</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Wang et al., 2014a

Study Design:
Cross-sectional and prospective birth cohort

Location:
Central Taiwan

Population:
Subjects from a longitudinal birth cohort study of pregnant women and children, n=285 pregnant women (mean age 28.8 years) and 116 neonates.

Outcome Definition:
Four thyroid hormones in pregnant women (third trimester) and cord serum thyroid hormones in neonates</td>
<td>Exposure Assessment:
Maternal provided blood samples for serum concentration of PFNA

Population-Level Exposure:

<table>
<tr><th>Percentile</th><th>ng/mL</th></tr>
<tr><td>25th</td><td>0.85</td></tr>
<tr><td>50th</td><td>1.51</td></tr>
<tr><td>75th</td><td>2.51</td></tr>
<tr><td>95th</td><td>6.20</td></tr>
</table>
</td>
<td>Stat Method:
Linear regression; covariates and confounders considered included maternal age, maternal education, previous live births, family income, maternal pregnancy body mass index, and maternal fish consumption during pregnancy

Estimated effect size is presented as the percent of β to the mean hormone concentration with 95% confidence interval and corresponding p-value (which is presented here. Estimates calculated with and without log-transformation of thyroid hormones.

Outcome: maternal free T4 (ng/dL)
Major Findings: ↓ ($p < 0.001$)

Outcome: maternal total T4 (ng/dL)
Major Findings: ↓ ($p < 0.01$)

Outcome: maternal total T3 (µg/dL)
Major Findings: NS

Outcome: maternal TSH (µIU/mL)
Major Findings: NS

Outcome: cord free T4 (ng/dL)
Major Findings: NS

Outcome: cord total T4 (ng/dL)
Major Findings: ↓ ($p < 0.05$)

Outcome: cord total T3 (µg/dL)
Major Findings: ↓ ($p < 0.01$)

Outcome: cord TSH (µIU/mL)
Major Findings: NS</td>
<td>Major Limitations:
PFNA was highly correlated with two other PFCs making it difficult to distinguish individual associations.

Also cross-sectional design prevents causal inference.</td>
</tr>
</table>

Reference and Study Design	Exposure Measures	Results	Comment
Watkins et al., 2013 **Study Design:** Cross-sectional **Location:** United States – Ohio and West Virginia **Population:** Children aged 1 to <18 years of age from a community exposed to PFOA contaminated drinking water **Outcome Definition:** Serum creatinine from blood sample and height (used to calculate estimated glomerular filtration rate (eGFR)) – which is a measure of kidney function.	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** PFNA median 1.5 ng/mL: PFNA interquartile range (0.51 ng/mL) *side note: Historical serum PFOA concentrations were estimated	**Stat Method:** Linear regression, covariates and confounders considered include age, sex, race, smoking, and household income, and also regular exercise and BMI, and total cholesterol. PFNA was log-transformed. Quartiles of PFNA were also considered and a test for trend was performed. Estimates from most adjusted models are provided here. **Outcome:** change in eGFR **Major Findings:** **-0.88 (-1.41, -0.36) p-value=0.001** **Linear trend of PFNA quartiles p-value=0.005**	**Major Limitations:** Cross-sectional design prevents causal inference. Possible reverse causation (at least partly explains results) – decreased GFR could lead to increased PFC serum concentrations – slower elimination of PFCs. Possible confounding due to unmeasured variables and other environmental contaminants, including other PFCs

Reference and Study Design	Exposure Measures	Results	Comment
Watkins et al., 2014 **Study Design:** Cross-sectional **Location:** United States – Ohio and West Virginia **Population:** Subset of C8 Health Survey participants (consumed water (for at least 1 year) from a water district with known PFOA contamination). Participants were 20-60 years of age, agreed to follow-up in 2010. Participants were ineligible is they had cancer, were taking anti-inflammatory medication, or had or have an active infection. Present analysis: n=685 **Outcome Definition:** % LINE-1 DNA methylation (epigenetic mechanism- hypomethylation of LINE-1 elements has been associated with genomic instability, risk of cancer, cerebrovascular outcomes, and serum lipids) in peripheral blood leukocytes at follow-up	**Exposure Assessment:** Averaged serum concentrations from 2005-2006 survey collection and 2010 follow-up **Population-Level Exposure:** The geometric mean PFNA serum level was: @05-06- 0.3 ng/mL @2010- 1.3 ng/mL @average- 1.4 ng/mL The GM of additional PFCs (averaged – ng/mL): PFOA=57.9 PFOS=14.1 PFHxS=2.6	**Stat Method:** Linear regression with PFCs modeled as linearly, as natural log transformed and as tertiles. Confounders and covariates considered include age, gender, BMI, smoking, and current alcohol consumption. Also total cholesterol and LDL-C were assessed as confounders. Stratification by gender also performed. Estimates presented are absolute differences in outcome associate with IQR increases. **Outcome:** Line-1 DNA methylation **Major Findings:** Unadjusted Difference- 0.102 (p-value 0.03) Adjusted Difference- 0.064 (p-value 0.19)	**Major Limitations:** Cross-sectional design prevents causal inference. Did not control for other co-occurring environmental contaminants including PFCs or other possibly important confounders. Possibly confounding by leukocyte type not. Imprecise measurement of DNA methylation (a more precise measure of changes associated with PFCs may have been to focus on promoter regions of specific genes associated with PPARs -if DNA methylation is indeed affected by PFCs through activation of PPARs).

Reference and Study Design	Exposure Measures	Results	Comment
Webster et al., 2014 **Study Design:** Prospective cohort study **Location:** Vancouver, Canada **Population:** Euthyroid pregnant women (n=152) enrolled in the Chemicals, Health and Pregnancy (CHirP) study [participants had to be non-smokers, singleton birth, and conceived naturally, no prior diagnosis of thyroid or endocrine conditions, no thyroid medication, and ≥ 19 years of age] **Outcome Definition:** Repeated measures of maternal thyroid hormones (15 and 18 week of gestation)	**Exposure Assessment:** Maternal serum concentrations in early 2nd trimester of pregnancy **Population-Level Exposure:** Median serum concentration of PFNA 0.60ng/mL (range <0.5-1.8) – pregnant women Median: PFHxS=1.0 ng/mL PFOA=1.7 ng/mL PFOS=4.8 ng/mL	**Stat Method:** Mixed effects linear models with random intercept for individual. Confounders and covariates considered include maternal age, ethnicity, education, household income, current stress levels, smoking, environmental tobacco smoke exposure, drug use, alcohol use, and the use of iodized salt and prenatal vitamins containing iodine. PFCs and hormones not transformed. Sensitivity analysis stratified by high v. normal TPOAb (thyroid peroxidase antibody levels – a marker of autoimmune hypothyroidism). Estimates presented for most adjusted models and an IQR increase in PFNA. **Outcome:** Free thyroxine (fT4) (pmol/L) **Major Findings:** Normal TPOAb:β=0.004 (95% CI -0.2, 0.2) High TPOAb: β=-0.3 (95% CI -1.0, 0.5) **Outcome:** total thyroxine (T4) (pmol/L) **Major Findings:** No associations found, estimates not presented in paper **Outcome:** TSH (mIU/L) **Major Findings:** Normal TPOAb: β=0.1 (95% CI -0.05, 0.3) **High TPOAb: β=0.6 (95% CI 0.1, 1.0)**	**Major Limitations:** Small sample size, especially in TPOAb group (n=14) Did not assess co-exposures Study population is not representative of general population Reverse causation *PFOS and PFOA were strongly correlated; all other PFCs were poorly to moderately correlated. The PFCs were poorly correlated with other suspected thyroid disrupting compounds in the same serum sample (PBDEs, PCBs, and organochlorine pesticides)

Reference and Study Design	Exposure Measures	Results	Comment
Wen et al., 2013 **Study Design:** Cross-sectional **Location:** General U.S. population **Population:** NHANES 2007-2010 adults >20 years of age, n=1,180 **Outcome Definition:** Laboratory measures	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Geometric mean of PFNA 1.54 (95% CI 1.48-1.59) ng/mL	**Stat Method:** Linear regression and logistic regression, covariates and confounders considered included age, gender, race, alcohol consumption, smoking, and urinary iodine. Modeled separately for men and women. Free T4, free T3, TSH, thyroglobulin, urinary iodine, and PFNA were natural log-transformed. PFCs were modeled separately and in a composite model. Most adjusted results shown below. **Outcome:** total T4 (μg/mL) **Major Findings:** Men: β=-0.164 (95% CI -0.361, 0.033) Women: β=-0.097 (95% CI -0.251, 0.445) **Outcome:** ln- free T4 (ng/dL) **Major Findings:** Men: β=--0.021 (95% CI -0.050, 0.007) Women: β=-0.015 (95% CI -0.038, 0.008) **Outcome:** total T3 (ng/dL) **Major Findings:** Men: β=-2.946 (95% CI -6.073, 0.181) Women: β=2.434 (95% CI -1.964, 6.832) **Outcome:** ln-free T3 (pg/mL) **Major Findings:** Men: β=0.002 (95% CI -0.011, 0.016) Women: β=0.014 (95% CI -0.001, 0.030) **Outcome:** ln-TSH (mIU/L) **Major Findings:** Men: β=-0.030 (95% CI -0.111, 0.051) Women: β=-0.093 (95% CI –0.189, 0.003) **Outcome:** ln-TG (ng/mL) **Major Findings:** Men: β=-0.072 (95% CI -0.192, 0.048) Women: β=0.086 (95% CI –0.057, 0.230)	**Major Limitations:** Cross-sectional design prevents causal inference. A common physiology could influence serum PFCs and thyroid functions independent of exposure. Did not control for medications that could be potential confounders, and other unmeasured environmental pollutants. Serum thyroid measures collected at a single time point for each participant, although previous reports demonstrated that measures of thyroid function in an individual are maintained with relatively narrow limits over time.

		Outcome: Subclinical hypothyroidism **Major Findings:** Men: OR=1.30 (95% CI 0.65, 2.60) Women: OR=2.54 (95% CI 0.40, 16.05) **Outcome:** Subclinical hyperthyroidism **Major Findings:** Men: OR=2.41 (95% CI 0.48, 12.04) Women: OR=1.91 (95% CI 0.83, 4.38)	

APPENDIX 3. Individual Study Tables of Toxicological Studies of PFNA

Reference and Study Design	Results	Comment
Das et al. (2015). Developmental toxicity of perfluorononanoic acid in mice. **Species and strain:** Timed-pregnant CD-1 mice **Group size:** 19-27 per dose group, subdivided as follows: - Sacrificed for maternal and fetal examination on GD 17 (8-10 per dose group). - Allowed to give birth (11-17 per dose group). **Test article and vehicle:** PFNA (97% pure, stated by supplier to be primarily linear) in water. **Route of exposure:** Oral gavage. **Exposure levels:** 0, 1, 3, 5, or 10 mg/kg/day. **Exposure regimen:** *Mice sacrificed on GD 17*: GD 1-16. *Mice allowed to give birth and sacrificed on PND 28*: GD 1-17	**Severe maternal toxicity at 10 mg/kg/day** Substantial weight loss starting at GD 8. All mice in this dose group were sacrificed on GD 13, and all pregnant mice had full litter resorptions. **PFNA concentration in serum and liver** ● Data presented graphically. ● Liver and serum PFNA ↑ with dose in: -GD 17 pregnant and non-pregnant adult females -GD 17 fetuses (livers only, serum not analyzed) -Dams at post-weaning on PND 28 -Pups followed from PND 1 to PND 70. ***Serum PFNA*** (numerical data obtained from investigator, C. Lau) ● On GD 17, serum PFNA generally ~2x higher in non-pregnant than in pregnant adult female mice. Lower PFNA serum levels in pregnant mice are presumed to be due to transfer to the fetal compartment. ● In pups, similar in males and females from PND 1 to PND 70. ● In pups soon after birth (PND 1), similar to maternal serum levels on GD 17. ● In pups, serum PFNA ↓ at PND 70 to about 4-7% of PND 1 levels. ● In pups, serum PFNA persisted at low levels at 43 weeks (10 months), the last time point assessed. In males, levels were about 1% of those at PND 1, and in females, about 0.2-0.4% of PND 1 (Numerical data provided by C. Lau.) ***Liver PFNA*** ● In pregnant, non-pregnant, and post-weaning (PND 28) female adults, PFNA in liver was about 5-10 times higher than serum PFN levels in the same dose groups. Liver levels generally paralleled serum levels. ● PFNA levels In fetal livers on GD 17 were similar to maternal PFNA serum levels at the same time point. ● In the livers of pups, PFNA levels declined more slowly than serum levels over time. PFNA in liver was about 3-fold higher than PFNA serum levels at PND 1. At PND 70, PFNA liver PFNA concentrations were about 12-18% of PND 1 levels. ● In pups, PFNA persisted at low levels in liver until 43 weeks (10 months), the last time point assessed. In male offspring at age 10 months, PFNA in liver was about 2-4% of PND 1 levels, and in females, about 0.1-0.3% of PND 1 levels (Numerical data obtained from C. Lau).	Serum PFNA levels were measured in adults on GD 17 and PND 28, and in offspring on days 1, 10, 24, 42, and 70. Histopathological examination was not performed on liver or other organs. Mice that had no live or dead fetuses at sacrifice on GD 17 were considered to be non-pregnant **Gene expression in fetal and pup liver** Real time PCR analysis was used to study the expression of genes of interest in livers from fetuses (GD 17) and pups on PND 1, 24, 42, and 70. PFNA clearly caused expression of genes associated with PPAR-alpha activation in fetal and pup liver. Gene changes associated with PPAR-alpha persisted until PND 42, although the effects were weaker after PND 24.

Reference and Study Design	Results	Comment
Das et al. (2015) *(continued).* Developmental toxicity of perfluorononanoic acid in mice	**<u>Maternal weight gain, pregnancy outcome, and fetal abnormalities (1, 3, 5 mg/kg/day)</u>** ● *Maternal body weight gain through GD 17*: No effect. ● *Pregnancy outcome*: No significant effects on full litter resorptions, # of implants, # or % live fetuses, prenatal litter loss, or fetal weight. ● *Fetal abnormalities (skeletal and visceral):* No effect. **<u>Postnatal mortality</u>** ● Data presented graphically. ● Not affected at 1 and 3 mg/kg/day. ● Severely affected at 5 mg/kg/day. Survival at PND 21: < 20% compared to > 80% in controls. - Neonatal mortality was gradual, with a sharp ↑ during PND 2-10. - Pups were weak and failed to thrive, although lack of maternal care was not observed. - Milk was present in stomachs of pups after death, indicating that they were able to suckle and swallow (C. Lau, personal communication). **<u>Offspring body weight</u>** ● Data presented graphically. ● On PND 1-24, dose-related ↓ at all doses, with statistical significance at 3 and 5 mg/kg/day. ● At weaning, 3 mg/kg/day and 5 mg/kg/day groups about 27% and 50% lower than the controls, respectively. ● Decrements persisted until PND 287 (9 months of age) and were significant in males at this time point. **<u>Markers of post-natal development (day of eye opening, day of vaginal opening, and day of preputial separation)</u>** ● Data presented graphically. ● Dose-dependent delays, with statistically significant for all three endpoints at 3 and 5 mg/kg/day.	

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr><td>Das et al. (2015) (continued).
Developmental toxicity of perfluorononanoic acid in mice</td><td>
<u>Liver weight in adults, fetuses, and pups</u>
● Adult and pup data presented graphically; fetal data presented in table.
● Pregnant and non-pregnant females on GD 17 and post-weaning (PND 28): Dose-related ↑ in absolute and relative liver weights, statistically significantly at all doses.
- Serum levels and liver weight assessed at the same time point (GD 17), one day after the last dose was administered.
- Dose-related ↑ (absolute and relative) persisted 4 weeks after dosing ended (PND 28). Significant at 3 and 6 mg/kg/day.

<table>
<tr><th colspan="5">Liver Weight (Pregnant females, GD 17)</th></tr>
<tr><td>Dose (mg/kg/day)</td><td>0</td><td>1</td><td>3</td><td>5</td></tr>
<tr><td>Serum level (ug/ml)</td><td>0.013</td><td>12.4</td><td>18.3</td><td>57.1</td></tr>
<tr><td>Absolute weight (g)</td><td>2.24</td><td>3.29</td><td>4.36</td><td>5.26</td></tr>
<tr><td>Relative weight (%)</td><td>7.12</td><td>9.76</td><td>12.43</td><td>15.27</td></tr>
</table>

● Fetuses: Dose-related ↑ in absolute and relative liver weights, statistically significantly at all doses, except that ↑ in absolute liver weight not significant at 5 mg/kg/day. Magnitude of the increases was similar in all dose groups.

● Pups: Dose-related ↑ in relative liver weights on PND 1 through PND 70; significant at all doses on PND 1, 10 and 24, and at 3 and 5 mg/kg/day on PND 42.
</td><td></td></tr>
</table>

Reference and Study Design	Results	Comment
Fang et al. (2008). Immunotoxic effects of perfluorononanoic acid on BALB/c mice. **Species and strain:** Male BALB/c mice. 6-8 weeks old. **Group size:** 6 per group. **Test article and vehicle:** PFNA; vehicle not stated. **Route of exposure:** Oral gavage. **Exposure levels:** 0, 1, 3, 5 mg/kg/day. **Exposure regimen:** 14 days. **Related studies:** Fang et al. (2010)	**Body weight** **Body weight gain (g) after 14 days PFNA dosing** mg/kg/day: *0* | *1* | *3* | *5* +0.6 | -0.1 | -2.5 | -3.1 Decreased at all doses. p<0.01 at 3 and 5 mg/kg/day **Effects on thymus** ● Data presented graphically. Complete numerical data not provided. ● *Thymus weight*: At 3 and 5 mg/kg/day, ↓ relative and absolute (33% and 44%) weight (p<0.01). ● *T cell subsets*: Dose-related ↓ in immature ($CD4^+CD8^+$) and ↑ in mature ($CD4^+CD8^-$; $CD4^-CD4^+$). p<0.01 at 5 mg/kg/day. ● *Cell cycle*: Dose related ↑ in % in G0/G1 (p<0.01 at 5 mg/kg/day) and ↓ in % in G2/M (p<0.01 at 3 and 5 mg/kg/day). % in S ↓ at 5 mg/kg/day (p<0.01). ● *Apoptosis*: ↑ at 5 mg/kg/day (p<0.01). **Effects on spleen** ● Data presented graphically. Complete numerical data not provided. ● *Spleen weight*: ↓ absolute weight (10% and 13%; p<0.01) at 3 and 5 mg/kg/day; ↓ relative weight (p<0.01) at 5 mg/kg/day. ● *Innate splenic immune cells*: Two types of cells significantly ↓ at all doses; one cell type significantly ↓ at 3 and 5 mg/kg/day. ● *T-cell subsets:* Percentages of three types evaluated were nearly unchanged. ● *Cell cycle*: ↑ in % in G0/G1 (sig. at 3 and 5 mg/kg/day) and ↓ in % in G2/M (sig. at all doses). % in S ↓ at 5 mg/kg/day (p<0.01). ● *Apoptosis*: ↑ at 5 mg/kg/day (p<0.01). ● *Cytokine secretion by T lymphocytes*: Dose-related ↓ in IL-4 at all doses, and IFN-gamma ↓ at 5 mg/kg/day (p<0.01). **Serum hormone levels** ● Data presented graphically. Complete numerical data not provided. ● ACTH (5 mg/kg/day) and c ortisol (3 and 5 mg/kg/day) ↑ significantly. At 5 mg/kg/day, ACTH ↑ 53% and cortisol ↑ 51%. **Gene expression** ● Data presented graphically. Numerical data not provided. ● *PPAR-alpha & PPAR-gamma*: ↑ (p<0.01) at low dose (1 mg/kg/day) only. ● *Glucocorticoid receptor*: No effect. ● *NF-κB-signaling pathway (modulator of inflammatory and immune response)*: IL-1β (target gene for this pathway) sig. ↑ at low dose (1 mg/kg/day) only. Two other genes involved with this pathway not affected.	Serum levels of PFNA were not measured in this study. In preliminary study, 50% mortality in mice given 10 mg/kg/day for 14 days. The *in vitro* response of splenic lymphocytes from PFNA treated mice to the mitogen Con-A did not differ from controls.

[insert endpoint] *incidence (percent)*

Reference and Study Design	Results	Comment
Fang et al. (2009). Alterations of cytokines and MAPK signaling pathways are related to the immunotoxic effect of perfluorononanoic acid. **Species and strain:** Male Sprague-Dawley rats, 320-340 g. **Group size:** 6 per group. (10 per group were dosed, but 4 per group were used for future proteomics analysis not part of this study). **Test article and vehicle:** PFNA (97% pure) in 0.5% Tween-20. **Route of exposure:** Oral gavage. **Exposure levels:** 0, 1, 3, 5 mg/kg/day. **Exposure regimen:** 14 days.	**Body weight** ● Data shown graphically and discussed in text. ● Dose-related ↓ at 3 mg/kg/day (18%)) and 5 mg/kg/day (39%). At both doses, $p<0.01$. **Thymus weight (absolute and relative to body weight)** ● Data shown graphically and discussed in text. ● At 1 mg/kg/day, 24% ↑ in absolute weight; relative weight also ↑ ($p<0.01$). ● At higher doses, dose-related ↓ in absolute weight at 3 mg/kg/day (20%) and 5 mg/kg/day (87%). At both doses, $p<0.01$ for ↓ absolute and relative liver weight. **Thymus histopathology** ● Results provided in text and photos. Quantitative data not presented. ● Text states that dose-related effects included ↑ cortex:medulla ratio, ↑ apoptotic lymphocytes, and ↑ tangible body macrophages (macrophages that have ingested apoptotic cells). Implied but not stated that these effects occur at doses below the highest (5 mg/kg/day). **Serum levels of cytokines and cortisol** ● Data shown graphically and discussed in text. ● *IL-1*: Dose-related ↑ ($p<0.01$) at 3 mg/kg/day (3.15-fold) and 5 mg/kg/day (3.67-fold). ● *IL-4*: ↑ 2.1-fold at 5 mg/kg/day ($p<0.01$). ● *IL-2:* Dose-related ↓, significant ($p<0.01$) at 3 mg/kg/day (29%) and 5 mg/kg/day (40%). ● *Cortisol:* ↑ 1.67 fold at 5 mg/kg/day ($p<0.05$).	PFNA serum levels were not measured in this study. Other components of this study evaluated changes in gene and protein expression. PPAR-alpha and PPAR-gamma genes were ↑ by PFNA, similar to what occurred in mice (Fang et al. 2008) The increases in cytokines caused by PFNA may result in multiple effects impacting the immune system, including increased cortisol and activation of genes and proteins involved with apoptosis in thymus.

Reference and Study Design	Results	Comment
Fang et al. (2010). Perfluorononanoic acid-induced apoptosis in rat spleen involves oxidative stress and the activation of caspase-independent death pathway. **Species and strain:** Male Sprague-Dawley rats. 220-230 g. Age not stated. **Group size:** 6 per group. **Test article and vehicle:** PFNA (acid, 97% pure) in 0.5% Tween-20 in water. **Route of exposure:** Oral gavage. **Exposure levels:** 0, 1, 3, 5 mg/kg/day. **Exposure regimen:** 14 days. **Related studies:** Fang et al. (2008).	**Spleen weight** ● Data presented graphically. Complete numerical data not provided. ● *Absolute weight:* ↓ ($p<0.01$) at all doses. ● *Relative weight:* ↓ ($p<0.01$) only at 5 mg/kg/day, by 8.5%. **Apoptosis of lymphoid cells in spleen** ● Results provided in text and photos. Quantitative data not presented. ● Evaluated by TUNEL assay for DNA fragmentation. ● *Apoptotic cell:* ↑ at 3 and 5 mg/kg/day. No effect at 1 mg/kg/day. **Levels of cytokines, H_2O_2, and superoxide dismutase in spleen** ● Data presented graphically. Complete numerical data not provided. ● *Pro-inflammatory cytokines*: Three cytokines ↑ and two ↓ significantly at 5 mg/kg/day. No effects at other doses. ● *H_2O_2*: ↑ ($p<0.05$) at 5 mg/kg/day only. ● *Superoxide dismutase*: ↓ ($p<0.01$) at 3 and 5 mg/kg/day. **PPAR gene expression in spleen** ● Data presented graphically. Complete numerical data not provided. ● *PPAR alpha*: ↑ 2.6-fold at 3 mg/kg/day and 3.5 fold at 5 mg/kg/day ($p<0.01$). No effect at 1 mg/kg/day. ● *PPAR gamma*: ↑ 2.3-fold at 3 mg/kg/day and 2.1 fold at 5 mg/kg/day ($p<0.05$). No effect at 1 mg/kg/day. **Protein expression in spleen** See comments.	Serum levels of PFNA were not measured in this study. The splenic levels of 9 proteins related to apoptotic signaling pathways were measured. The data are presented graphically as ratio of proteins/beta-actin. PFNA caused dose-related changes in levels of some, but not all, of these proteins. Although statistically significant changes compared to controls are indicated, the data for controls are not shown. The role of these proteins and the potential toxicological significance of the changes caused by PFNA are complex and relate to the potential MOA(s) of PFNA-induced apoptosis.

Reference and Study Design	Results	Comment
Fang et al. (2012b). In vitro and in vivo studies of the toxic effects of perfluorononanoic acid on rat hepatocytes and Kupffer cells. **Species and strain:** Male Sprague-Dawley rats, 6-8 weeks old. **Group size:** 6 per group **Test article and vehicle:** PFNA (97% pure). Vehicle not stated. **Route of exposure:** Oral, assumed to be by gavage. **Exposure levels:** 0, 0.2, 1, and 5 mg/kg/day. **Exposure regimen:** 14 days. Note: Study also includes an *in vitro* component. See Comments. **Related studies:** Fang et al. (2012c).	**Liver histopathology** ● Results provided in text and photos. Quantitative data not presented. ● Terminology different than in other studies presenting liver histopathology data. ● Focal vacuolar degeneration, indistinct hepatocyte borders, and lipid accumulation at 5 mg/kg/day only; no effect at lower doses. **Expression of hepatic genes related to lipid metabolism** ● Data presented graphically. Complete numerical data not provided. ● Expression of 6 of 7 genes assessed was affected by PFNA (↓ or ↑) in a dose-related manner, with significance at one or more doses. ● PFNA effects on expression of these genes in hepatocytes *in vitro* was similar to *in vivo* for some genes and different for others. **Effects on hepatic cytokines** ● Three pro-inflammatory cytokines were significantly ↓ (1.4-2.4 fold) at 0.2 mg/kg/day and ↑ (1.3-1.9 fold) at 5 mg/kg/day; no effect at 1 mg/kg/day. ● Gene expression for one of these three cytokines ↑ in a dose-related fashion, and two at 5 mg/kg/day. ● *In vitro* gene expression changes were generally consistent with *in vivo.*	Serum levels of PFNA were not measured in this study. Additional *in vitro* studies showed that PFNA did not decrease hepatocyte or Kupffer cell viability at concentrations up to 50 uM. Cell viability was increased at 5 and 10 uM PFNA. Hepatocyte viability was decreased at 100 uM. Release of liver enzymes (ALT and AST) was not increased by PFNA in hepatocytes cultured with or without Kupffer cells.

Reference and Study Design	Results	Comment
Fang et al. (2012c). Kupffer cells suppress perfluorononanoic acid-induced hepatic peroxisome proliferator-activated receptor α expression by releasing cytokines. Arch. Toxicol. 86, 1515-25. **Species and strain:** Male Sprague-Dawley rats, age not stated; 120-130 g. **Group size:** 20 per PFNA dose group. Half of each dose group treated with gadolinium chloride ($GdCl_3$, an inactivator of Kupffer cells). **Test article and vehicle:** PFNA (97% pure). **Route of exposure:** PFNA – oral gavage. $GdCl_3$ – intraperitoneal injection. **Exposure levels:** PFNA – 0, 0.2, 1, 5 mg/kg/day. $GdCl_3$ – 10 mg/kg **Exposure regimen:** PFNA – daily for 14 days. $GdCl_3$ – 2 times per week. At the end of dosing, liver and blood was collected from 6/10 rats/group for analysis. Hepatocytes and Kupffer cells were isolated from the livers of the other rats in each group. **Related studies:** Fang et al. (2012b).	Data presented graphically. Complete numerical data not provided. **Effect of Kupffer cell inactivation on hepatic PFNA accumulation and toxicity** ● Hepatic PFNA levels after 14 days of dosing were not affected by inactivation of Kupffer cells by $GdCl_3$. ● *Body weight:* ↓ at 5 mg/kg/day. Not affected by inactivation of Kupffer cells with $GdCl_3$. ● *Relative liver weight:* dose-related ↑. Not affected by inactivation of Kupffer cells with $GdCl_3$. ● *Liver triglycerides:* Dose-related ↑ ($p<0.01$ at 5 mg/kg/day). Slightly, but significantly, smaller ↑ when Kupffer cells were inactivated with $GdCl_3$. ● *Serum triglycerides and cholesterol:* Dose-related ↓ ($p<0.01$ at 5 mg/kg/day). Not affected by inactivation of Kupffer cells with $GdCl_3$. ● *Liver enzymes in serum:* 4 enzymes ↑ at 5 mg/gk/day. Inactivation of Kupffer cells with $GdCl_3$ significantly reduced the ↑ serum levels for 3 of 4 enzymes. **Effect of Kupffer cell inactivation on hepatic genes involved with lipid metabolism** ● *Hepatic expression of PPAR-alpha:* ↑ slightly but significantly at 5 mg/kg/day. PPAR-alpha target gene expression also ↑ at 1 and 5 mg/kg/day (0.2 mg/kg/day not tested). ● PFNA-induced increases in PPAR-alpha were much greater with inactivation of Kupffer cells. Expression of 2 of 3 target genes was also greater with Kupffer cell inactivation with $GdCl_3$. **Effect of Kupffer cell inactivation on cytokine release in liver homogenate from PFNA-treated rats** ● *Cytokine release:* Dose-dependent ↑ in release of cytokines TNF-alpha and IL-1 beta in liver homogenates from PFNA-treated rats (significant at 0.02, 1, and 5 mg/kg/day). ● PFNA did not cause ↑ cytokine release when Kupffer cells were inactivated with $GdCl_3$.	Serum levels of PFNA were not measured in this study. Additional *in vitro* portions of this study further investigated the mechanisms of the effects observed in the *in vivo* studies.

Reference and Study Design	Results	Comment
Fang et al. (2012a). Exposure of perfluorononanoic acid suppresses the hepatic insulin signal pathway and increases serum glucose in rats. **Species and strain:** Male Sprague-Dawley rats, age not stated. 120-130 g. **Group size:** 6 per group **Test article and vehicle:** PFNA (97% pure), in water. **Route of exposure:** Oral gavage **Exposure levels:** 0, 0.2, 1, and 5 mg/kg/day **Exposure regimen:** 14 days	Data presented graphically. Complete numerical data not provided. **<u>Serum glucose, HDL, LDL</u>** ● *Serum glucose:* Dose-dependent ↑ (significant at 1 and 5 mg/kg/day). ● *HDL:* Dose-dependent ↓ (significant at all doses). ● *LDL*: Significantly ↑ only at 5 mg/kg/day. ● *HDL/LDL ratio*: Dose-dependent ↓ (significant at 1 and 5 mg/kg/day). **<u>Liver glycogen</u>** ● Dose-dependent ↑. Significant (1.88-fold change) at highest dose (5 mg/kg/day). **<u>Hepatic markers of oxidative stress</u>** ● H_2O_2: significantly ↑ 1.71-fold at highest dose (5 mg/kg/day). ● *Malondialdehyde:* significantly ↑ 1.50-fold at highest dose (5 mg/kg/day). **<u>Hepatic expression of genes related to glucose metabolism</u>** ● Genes for glucose-6-phosphatase and glucose transporter ↑ and gene for gluokinase ↓ at 5 mg/kg/day; PI3Kca ↓ at all doses. **<u>Hepatic proteins involved with insulin signaling pathway</u>** ● Four proteins significantly ↓ (two at all doses; two at 1 and 5 mg/kg/day). ● One protein, p-GSK3-beta, significantly ↑ at all doses. ● Significance of these findings discussed in text.	Serum levels of PFNA were not measured in this study.

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Feng et al. (2009).
Perfluorononanoic acid induces apoptosis involving the Fas death receptor signaling pathway in rat testis.

Species and strain:
Male Sprague-Dawley rats, 7 weeks old

Group size:
6 per group

Test article and vehicle:
PFNA (97% pure) in 0.2% Tween-20. Dosing volume – 6 ml/kg.

Route of exposure:
Oral gavage

Exposure levels:
0, 1, 3, 5 mg/kg/day

Exposure regimen:
14 days

Related studies:
Feng et al. 2010.
Effects of PFNA exposure on expression of junction–associated molecules and secretory function in rat Sertoli cells.</td>
<td><u>Hormone levels in serum</u>
● Data presented graphically. Complete numerical data not provided.
● Estradiol: No significant effect at 1 and 3 mg/kg/day; ↑ by 104% (p<0.01) at 5 mg/kg/day.
● Testosterone: ↑ by 87.5% at 1 mg/kg/day (p<0.01); no change at 3 mg/kg/day; ↓ by 85.4% at 5 mg/kg/day (p<0.01).
● FSH and LH: No effect.

<u>Histological examination of testes</u>
● Results provided in text and photos. Quantitative data not presented.
● 5 mg/kg/day – Disorganization and atrophy of the seminiferous tubules, with germ cells sloughed into the lumen, crescent chromatin condensation and chromatin margination in the germ cells.

<u>DNA fragmentation in testes (TUNEL assay)</u>
(Terminal deoxynucleotidyl transferase dUTP nick end labeling [TUNEL] is a method for visualizing DNA fragmentation by labeling the terminal end of nucleic acids. TUNEL positive cells are considered to be apoptotic.)
● Results provided in text and photos. Quantitative data not presented.
● Dose-dependent increase in TUNEL positive cells, mainly spermatocytes and spermatogonia with sharp increase in 3 and 5 mg/kg/day groups.

<u>Flow cytometry of testicular cells</u>
● Data presented graphically. Numerical results were estimated from graphs.
● Percent apoptotic cells: Dose-dependent increase. Not significant at 1 mg/kg/day. Sharp increase at 3 and 5 mg/kg/day (p<0.01).

<table>
<tr><td colspan="5">Estimated % Apoptotic Testicular Cells</td></tr>
<tr><td>mg/kg/day</td><td>0</td><td>1</td><td>2</td><td>3</td></tr>
<tr><td></td><td>1.5%</td><td>3%</td><td>7%</td><td>9%</td></tr>
</table>

<u>Expression of genes involved in apoptosis pathways in testes</u>
● Mitochondrial-dependent pathway genes: Bax: ↑ 35.7% in 5 mg/kg/day (p<0.05); not significant in other groups. Bcl-2: ↓ in 3 and 5 mg/kg/day (p<0.05).
● Death receptor pathway: Fas: ↑ at all doses. Not significant at 1 and 3 mg/kg/day. At 5 mg/kg/day, ↑ by 90% (p<0.05).

<u>Levels of testicular proteins involved in apoptotic pathways</u>
● Data presented graphically. Complete numerical data not provided.
● Caspase-8 (involved in death receptor pathway): Dose-related ↑ at all doses; 1 mg/kg.day – not significant. 3 and 5 mg/kg/day - p<0.05. 5 mg/kg/day, ~2x control (estimated from graph).
● Caspase-9 (involved in mitochondrial-dependent pathway): Not affected by PFNA.</td>
<td>Serum levels of PFNA were not measured in this study.

In preliminary study, all rats given 20 mg/kg/day for 14 days died.</td>
</tr>
</table>

Reference and Study Design	Results	Comments
Feng et al. (2010). Effects of PFNA exposure on expression of junction–associated molecules and secretory function in rat Sertoli cells. **Species and strain:** Male Sprague-Dawley rats, 7 weeks old **Group size:** 6 per group **Test article and vehicle:** PFNA (97% pure) in 0.2% Tween-20 **Route of exposure:** Oral gavage **Exposure levels:** 0, 1, 3, 5 mg/kg/day *Some endpoints not evaluated for 1 mg/kg/day group.* **Exposure regimen:** 14 days **Related studies:** Feng et al. 2009. Perfluorononanoic acid induces apoptosis involving the Fas death receptor signaling pathway in rat testis.	**Ultrastructure of rat seminiferous tubule** • Results provided in text and photos. Quantitative data not presented. • Vacuoles between Sertoli cells and spermatogonia in 3 and 5 mg/kg/day, but not controls; more numerous and larger in 5 mg/kg/day. • Increasing germ cell degeneration in 5 mg/kg/day. • 1 mg/kg/day not evaluated for this endpoint. **Testicular Wilms Tumor Protein (WT1) and Transferrin protein** • Data presented graphically. Numerical data not provided. • *WT1:* ↑ at all doses ($p<0.05$, 5 m/kg/day; $p<0.01$, 1 and 3 mg/kg/day). • *Transferrin:* Dose related ↓. 1 mg/kg/day -; $p<0.05$; 3 and 5 mg/kg/day - $p<0.01$. • Consistent with gene expression changes *in vitro* (see Comments). **Serum Mullerian Inhibiting Substance (MIS) and Inhibin B** • Data presented graphically. Numerical data not provided. • *MIS:* dose-related ↑ (not significant at 1 and 3 mg/kg/day; $p<0.05$ at 5 mg/kg/day). • *Inhibin B:* dose-related ↓ (1 mg/kg/day, $p<0.05$; 3 and 5 mg/kg/day, $p<0.01$). • Consistent with gene expression changes *in vitro* (see comments).	Serum levels of PFNA were not measured in this study. Both *in vivo* (presented in "Results" column) and *in vitro* (presented below to complement the *in vivo* data) studies were reported. Summary of *in vitro* studies: Primary cultures of Sertoli cells were exposed to 0, 1, 10, 25, 50, and 75 µM PFNA (0.464 – 34.8 mg/L). These concentrations were not cytotoxic. ● Expression not affected for genes related to: tight junctions, adherens junctions, components of the seminiferous tubule basement membrane, and Sertoli cell products sertolin and testin. ● Expression upregulated (at higher concentrations) for gene related to an intermediate filament protein. ● The effect of PFNA on gene expression for factors related to germ cell development secreted by Sertoli cells was evaluated. Results for the two factors also assessed *in vivo* (MIS & WT1) were qualitatively consistent with the *in vitro* data as follows: Expression of MIS gene was increased at ≥10 µM, and WT1 gene expression was dramatically increased at all PFNA concentrations. Expression of transferrin and inhibin B genes were significantly decreased at higher concentrations. Results for several other genes are also presented. ●Levels of two cytoskeleton-associated proteins involves in formation of adherens junctions were not affected by PFNA *in vitro.*

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Kennedy (1987).
Increase in mouse liver weight following feeding of ammonium perfluorooctanoate and related fluorochemicals.

Species and strain:
Male and female Crl:CD-1 mice. 40-45 days old.

Group size: 5 per group.

Test article and vehicle:
Ammonium perfluorononanoate, 99% pure.

Route of exposure: Diet

Exposure levels:
0, 3, 10, 30, 300, 3000 ppm. (All mice died at 300 and 3000 ppm). Based on assumed food consumption of 1.5 g/10 g body weight/ day (University of Wisconsin, 2014), the doses are estimated as 3 ppm - 0.45 mg/kg/day, 10 ppm -1.5 mg/kg/day, and 30 ppm - 4.5 mg/kg/day.

Exposure regimen: 14 days

The PFNA results are part of a larger study that also included PFOA, Telomer B ammonium sulfate, and WG-111.</td>
<td>
<table>
<tr><th colspan="6"><u>Liver: body weight ratio (percent)</u>*</th></tr>
<tr><td></td><td colspan="5">Dietary exposure level (ppm)</td></tr>
<tr><td></td><td>0</td><td>3</td><td>10</td><td>30</td><td>300**</td></tr>
<tr><td>Male</td><td>5.4, 6.0</td><td>8.4</td><td>13.7</td><td>17.1</td><td>19.0</td></tr>
<tr><td>Female</td><td>5.7, 5.7</td><td>7.7</td><td>13.7</td><td>16.7</td><td>25.5</td></tr>
</table>
*All dosed groups were significantly different from controls (p>0.05).
**It was stated that all mice fed 300 and 3000 ppm died before the end of the study, but data for 300 ppm are provided.

<u>Other observations</u>
Weight loss and generalized weakness seen at 30 ppm.
</td>
<td>Serum levels of PFNA were not measured in this study.

The authors state that PFNA appears to more toxic than PFOA, based on observations in this study.</td>
</tr>
</table>

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Kinney et al. (1989).
Acute inhalation toxicity of ammonium perfluorononanoate.

Species and strain:
Male Crl:CD BR rats, age not stated. 234-298 g.

Group size:
10 per group for 2 lowest dose groups.
6 per group for 4 highest dose groups.

Test article and vehicle:
Ammonium perfluorononanoate (≥99% pure).

Route of exposure:
Inhalation of dust, nose only.

Exposure levels:
Mean concentrations: 67, 590, 620, 910, 1600, and 4600 mg/m^3. (Ranges and standard deviations provided.)

Exposure regimen:
One 4 hour exposure.
Two lowest dose groups (n=10) were sacrificed 5 days (n=5) and 12 days (n=5) after exposure ended.
Other groups were followed for 14 days post-exposure.</td>
<td><u>Mortality</u>
<table><tr><td colspan="7">Exposure level (mg/m^3)*</td></tr><tr><td></td><td>67**</td><td>590**</td><td>620</td><td>910</td><td>1600</td><td>4600</td></tr><tr><td>Mortality</td><td>0/10</td><td>1/10</td><td>0/6</td><td>4/6</td><td>6/6</td><td>6/6</td></tr><tr><td>Post-exposure days when deaths occurred</td><td>--</td><td>12</td><td>--</td><td>9-11</td><td>4-8</td><td>During exposure.</td></tr></table>*No mortality occurred in control groups.
**Five sacrificed on day 5, and five on day 12.

<u>Body weight</u>
● One day post-exposure, ↓ 1-9% at 67 mg/m^3 and 6-15% at higher doses.
● At 12-14 days post-exposure, most surviving rats at 590, 620, and 910 mg/m^3 lost 29-46% of initial weight.

<u>Clinical signs and autopsy observations</u>
● At ≥590 mg/m^3, dose-related signs including hunched posture; ruffled/discolored fur; discharge from eyes, nose, mouth; stained perineum; pallor; lung noise/labored breathing; lethargy; limpness; hair loss were seen during the post-exposure period.
● At 590 mg/m^3, gross lesions in liver at sacrifice on days 5 and 12.

<u>Liver: body weight ratio (percent)*</u>
<table><tr><td></td><td>0
(Two control groups)</td><td>67</td><td>590</td></tr><tr><td>5 days</td><td>4.09, 4.95</td><td>5.24</td><td>5.80</td></tr><tr><td>12 days</td><td>4.68, 4.65</td><td>6.27</td><td>7.00</td></tr></table>*p<0.05 for all treated groups.</td>
<td>Serum levels of PFNA were not measured in this study.</td>
</tr>
</table>

Reference and Study Design	Results	Comment
Mertens et al. (2010). Subchronic toxicity of S-111-S-WB in Sprague Dawley rats. **Species and strain:** Crl:CD (SD) IGS BR rats, approximately 45 days old. **Group size:** ● *Main 90 day study*: 10 per sex per dose. Additional groups; ● *60 day recovery period after 90 day dosing*: 5 per sex per dose for control and 0.6 mg/kg/day only. ● *Peroxisome proliferation in liver after 10 days of dosing:* 5 per sex per dose. ● *Toxicokinetics:* 5 per sex per dose. **Test article and vehicle:** Surflon S-111 in water (see comments for composition). **Route of exposure:** Oral gavage. **Exposure levels:** Surflon S-111: 0, 0.025, 0.125, or 0.6 mg/kg/day. PFNA (estimated; see comments): 0, 0.019, 0.09, and 0.44 mg/kg/day. **Exposure regimen:** Daily for 90 days **Related study:** Stump et al. (2008).	**Survival**: All rats survived until scheduled necropsy. **Clinical signs**: 2/10 high dose (0.6 mg/kg/day) males exhibited clinical signs, stated to be associated with decreased body weight and food consumption, beginning in week 10. **Body weight and food consumption**: ● Shown graphically and discussed in text. ● *Weight loss or ↓ weight gain:* Statistically significant in high dose (0.6 mg/kg/day) males beginning in weeks 2 to 3; weight ↓ to 24% below controls at day 90. ● Body weight in the high dose (0.6 mg/kg/day) males remained 12.5% below controls after 60 day recovery period. ● No effects in females. ● Body weight effects not attributable to ↓ food consumption. **Hematology** ● Tabular data for all doses at Week 13, and for control and high dose (0.6 mg/kg/day) for Week 21 (post recovery). ● Statistically significant effects in 0.6 mg/kg/day males only: ● Week 13: ↑ prothrombin time; ↓ reticulocytes ($p<0.05$). ● Week 21 (post recovery): ↓ hemoglobin, hematocrit ($p<0.05$); red cells ($p<0.01$); ↑ lymphocytes ($p<0.05$). **Clinical Chemistry** ● Tabular data for all doses at Week 13, and for control and high dose (0.6 mg/kg/day) for Week 21 (post recovery). ● *Alkaline phosphatase and albumin/globulin ratio:* ↑ in 0.125 mg/kg/day and 0.6 mg/kg/day males and females at 13 weeks, and in recovery group males at 21 weeks.. ● *Total protein and globulin*: ↓ in 0.6 mg/kg/day males at 13 weeks. ● *BUN, bilirubin, and chloride (chloride data not shown):* ↑ at 13 weeks in 0.6 mg/kg/day males. **Liver Weight** ● Tabular data for all doses at Week 13, and for control and high dose (0.6 mg/kg/day) for Week 21 (post recovery). ● At 13 weeks, dose-related ↑ (absolute, and relative to body weight and brain weight); significant in 0.125 mg/kg/day males and 0.6 mg/kg/day males and females. Liver:body weight increase similar in mid dose (0.125 mg/kg/day) males and high dose (0.6 mg/kg/day) females. ● *Recovery group at week 21 (60 days post-dosing):* liver weight parameters remained increased in males, but not females, at 0.6 mg/kg/day.	Surflon S-111 is a commercial mixture of linear perfluorinated carboxylic acids containing primarily PFNA. The specific composition of the Surflon S-111 used in this study is not reported; this information has been requested but not provided from the study sponsors to date. The composition of Surflon S-111 by weight was reported by Prevedouros et al. (2006) as: PFNA, 74%; perfluoroundecanoic acid (C11), 20%; perfluorotridecanoic acid (C13), 5%; PFOA (C8), 0.78%; perfluorodecanoic acid (C10), 0.37%; and perfluorododecanoic acid (C12), 0.1%. This composition was used to estimate the PFNA doses in this study. Data on serum levels of PFOA, PFNA, C11, and C13 in males and females in each dose group over time were presented graphically by Mertens et al. (2010). However, it is not possible to accurately estimate the serum values at lower dose levels from the graphs due to their scale. The numerical serum data have been requested from the study sponsors but have not been provided to date.

Reference and Study Design	Results	Comment
Mertens et al. (2010) (continued). Subchronic toxicity of S-111-S-WB in Sprague Dawley rats.	**Hepatic beta-oxidation (marker of peroxisome proliferation)** ● Tabular data for all doses at 10 days, 90 days (week 13), and for control and high dose (0.6 mg/kg/day) for Week 21 (60 days post dosing). ● 10 days: Significant ↑ in 0.6 mg/kg/day males only. ● 90 days: Significant ↑in 0.125 mg/kg/day males, and 0.6 mg/kg/day males and females. ● Week 21 (60 days post dosing): Significant ↑ in 0.6 mg/kg/day males only. **Liver histopathology** ● Tabular data for males at all doses at Week 13, and for control and high dose (0.6 mg/kg /day) for Week 21 (60 days post dosing). ● At 0.125 and 0.6 mg/kg/day at Week 13, dose-related incidence of hepatocellular hypertrophy and eosinophilic foci in males. ● At 0.6 mg/kg/day at Week 13, some male rats had acute inflammation, degeneration, and necrosis. The incidence of necrosis was 2/10 (minimal grade). ● In recovery group (60 days post dosing) 0.6 mg/kg/day males, similar effects were seen as at the end of dosing (week 13), with hypertrophy persisting in all animals. ● In females, only the control and high dose (0.6 mg/kg/day) were evaluated (data not shown). No effects observed. **Gastrointestinal histopathology** ● Tabular data for males at all doses at Week 13, and for control and high dose (0.6 mg/kg /day) for Week 21 (60 days post dosing). ● Inflammation, ulceration, erosion, and hyperplasia were observed in the duodenum and stomach of some 0.6 mg/kg/day males at week 13. ● Minimal stomach erosion persisted in one recovery group 0.6 mg/kg/day male, 60 days after dosing ended. ● In females, only the control and high dose (0.6 mg/kg/day) were evaluated (data not shown). No effects observed. **Other parameters (data not shown)** ● No treatment-related macroscopic changes. No effects on weights of 9 organs other than liver. No histopathological effects (except liver and gastrointestinal). No effects on functional observational battery and locomotor activity assessments, ophthalmic examinations, or urinalysis.	Notably, no effects were seen on kidney weight or histopathology. The same doses of Surflon S-111 caused effects on these endpoints in rats in the longer duration (18-21 week) two-generation study (Stump et al., 2008).

Reference and Study Design	Results	Comment
Rockwell et al. (2013). Acute immunotoxic effects of perfluorononanoic acid (PFNA) in C57BL/6 mice. **Species and strain:** Male and female C57BL/6 mice, 8 weeks old. **Group size:** 5 per group (male) 4 per group (female) **Test article and vehicle:** PFNA (97% pure) in propylene glycol:water, 1:1. **Route of exposure:** Intraperitoneal injection **Exposure levels:** 46.4 mg/kg (stated as 0.1 mmol/kg) **Exposure regimen:** One dose, followed by sacrifice 2 weeks later.	**Body weight and organ weights** ● *Body weight:* ↓ 31% (M), ↓ 38% (F) ($p<0.05$). ● *Relative spleen weight:* ↓ 60-70% ($p<0.05$). ● *Relative kidney weight:* Little or no effect. ● *Relative liver weight:* ↑ ~300% ($p<0.05$). **Viabilities of leukocytes and RBCs in spleen, and thymocyes** ● *Splenic leukocyte counts:* ↓ 87.5%(M), ↓ 93%(F) ($p<0.05$). ● *Splenic red blood cell counts:* ↓ 95%(M), ↓ 89%(F) ($p<0.05$). ● Control viability was >99%. ↓ to 46.7%(M), ↓ to 71.5%(F) ($p<0.05$). **Effects on cell populations in spleen and thymus** ● *Spleen T cells:* ↑ $CD4^+$ (F) and $CD8^+$ (M, F) ($p<0.05$). ● *Spleen B cells:* ↓ $CD19^+$ (marker for B cells) in females ($p<0.05$). ● *Spleen phagocytes:* ↓ $CD14^+$ (marker for phagocytes) in M and F ($p<0.05$). ● *Thymus cells:* Severe ↓ in immature ($CD4^+CD8^+$) from 76-80% in controls to 1% in treated; accompanied by ↑ in mature ($CD4^+CD8^-$; $CD4^-CD4^+$) and $CD4^-CD4^-$. ($p<0.05$ for all). **Tumor necrosis factor alpha (TNF-alpha)** ● TNF-alpha production (mediator of inflammation produced by macrophages) in response to lipopolysaccharide (LPS) was ↑ ~6-fold in PFNA-treated compared to controls. ($p<0.05$; data shown only for males).	Serum levels of PFNA were not measured in this study. Although this study indicates the potential for PFNA to cause immune toxicity, the dose used was high enough to cause overt toxicity, as demonstrated by the severe weight loss seen in treated animals. Also, the route of administration, i.p. injection, is not relevant to human exposure.

Reference and Study Design	Results	Comment
Rogers et al. (2014). Elevated blood pressure in offspring of rats exposed to diverse chemicals during pregnancy. **Species and strain:** Pregnant Sprague-Dawley rats **Group size:** Not stated (data appears to have been inadvertently omitted) **Test article and vehicle:** PFNA in water **Route of exposure:** Oral gavage **Exposure levels:** 5 mg/kg/day **Exposure regimen:** GD 1-20	**Maternal body weight gain** ● Presented graphically and discussed in text. ● ↓ ($p<0.05$) on GD 4-19. **Pup weight at birth** ● Presented graphically and discussed in text. ● ↓ ($p<0.05$) in males and females. **Postnatal growth** ● Discussed in text; data not shown. ● No significant effects at weaning (PND 21) or until 56 weeks of age. **Systolic blood pressure in offspring** ● Presented graphically and discussed in text. ● ↓ ($p<0.05$) in males and females on PND 10, but not PND 26 or 56. **Nephron endowment in renal glomeruli** ● Presented graphically and discussed in text. ● ↓ ($p<0.05$) in males on PND 22. No effect in females. ● Not associated with changes in body weight or kidney weight.	PFNA serum levels were not measured in this study. The number of pregnant dams dosed with PFNA is not provided. There were 21 animals in the control group and 12-21 animals in groups dosed with other chemicals. Only one dose level of PFNA was used in this study. Renal glucocorticoid mRNA at birth and aldosterone on PND 28 were not affected by PFNA in this study.

Reference and Study Design	Results	Comment
Stump et al. (2008). An oral two-generation reproductive toxicity study of S-111-S-WB in rats. **Species and strain:** Crl:CD (SD) 6 weeks of age (F0 generation) **Group size:** ● *F0 and F1adults:* 30/gender/group. ● Eight F0 females/dosed group for toxicokinetic evaluation. ● *F1 and F2 litters:* 22-30 litters per dose group. **Test article and vehicle:** Surflon S-111 in deionized water. (see comments for composition). **Route of exposure:** Oral gavage, dose volume 2 ml. **Exposure levels:** Surflon S-111: 0, 0.025, 0.125, or 0.6 mg/kg/day. PFNA (estimated; see comments): 0, 0.019, 0.09, and 0.44 mg/kg/day **Exposure regimen:** ● *F0 males and females*: Starting at 6 weeks, for at least 70 days prior to mating, throughout mating, gestation, and lactation, and until euthanasia. Total duration not stated, but graphical data indicate dosing period was 18 weeks. ● *F1 males and females*: Dosed for at least 70 days prior to mating, throughout mating, gestation, and lactation, until euthanasia. The age at which dosing began and the duration of dosing are not explicitly stated. Data presented indicates that dosing began at 4 or 6 weeks and continued for 21 weeks. **Related studies:** Mertens et al. (2010).	**Clinical observations/survival** One high dose (0.6 mg/kg/day) F1 male with 171 g body loss was euthanized *in extremis* after 14 weeks of dosing. **Body weight** ● Shown graphically and discussed in text. ● Statistically significant weight loss or ↓ weight gain in high dose F0 and F1 high dose (0.6 mg/kg/day) males beginning in weeks 7-8; F0 weight ↓ to 24.8% below controls at week 18. ● No effects in females. ● Effects not attributable to ↓ food consumption. **Reproductive parameters** ● F0 and F1 data shown in tables. ● *Fertility index:* significantly ↓ (from 90% to 73%) only in low dose (0.025 mg/kg/day) F0 males and females. ● *Other reproductive parameters in F0 or F1:* not affected. **Spermatogenic endpoints** ● F0 and F1 data shown in tables. ● *Sperm motility and progressive motility:* significantly ↓ in high dose (0.6 mg/kg/day) F1 males. ● Text states that this effect is not test related because reproductive organ weights were not affected. However, data tables show significantly ↓ left epididymis weight in high dose F0 and F1 males, and significantly ↓ left epididymis sperm concentration in high dose F0 males. **Hepatic effects (F0 and F1 adult)** ***Liver weight*** ● Data shown in tables and discussed in text. ● *Liver weights (absolute and relative to body weight):* significantly ↑ in mid (0.125 mg/kg/day) males and high (0.6 mg/kg/day) dose males and females.	Surflon S-111 is a commercial mixture of linear perfluorinated carboxylic acids containing primarily PFNA. The specific composition of the Surflon S-111 used in this study is not reported; this information has been requested but not provided from the study sponsors to date. The composition of Surflon S-111 by weight was reported by Prevedouros et al. (2006) as: PFNA, 74%; perfluoroundecanoic acid (C11), 20%; perfluorotridecanoic acid (C13), 5%; PFOA (C8), 0.78%; perfluorodecanoic acid (C10), 0.37%; and perfluorododecanoic acid (C12), 0.1%. This composition was used to estimate the PFNA doses in this study. Exposure duration (18-21 weeks) in this study was longer than in the 90 day (13 week) subchronic study (Mertens et al., 2010).

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Stump et al. (2008) (continued).
An oral two-generation reproductive toxicity study of S-111-S-WB in rats.</td>
<td>
<u>Liver histopathology</u>
● Data shown in tables and discussed in text.
● Assessed in all F0 and F1 groups except 0.025 and 0.125 (low and mid dose) F1 females.
● Hepatocellular hypertrophy (males): High frequency in all treated groups with dose-related increases in frequency and severity. Not seen in controls.

<table>
<tr><td colspan="5">Incidence (%) of hepatocellular hypertrophy in F0 and F1 males</td></tr>
<tr><td></td><td colspan="4">Exposure level (mg/kg/day Surflon S-111)</td></tr>
<tr><td></td><td>0</td><td>0.025</td><td>0.125</td><td>0.6</td></tr>
<tr><td>F0</td><td>0/30 (0%)</td><td>21/30 (70%)</td><td>30/30 (100%)</td><td>29/30 (97%)</td></tr>
<tr><td>F1</td><td>0/30 (0%)</td><td>23/30 (77%)</td><td>29/29 (100%)</td><td>30/30 (100%)</td></tr>
</table>
● Hepatocellular necrosis (males): Dose-related ↑ in frequency and severity. Not seen in controls.

<table>
<tr><td colspan="5">Incidence (%) of hepatocellular necrosis in F0 and F1 males</td></tr>
<tr><td></td><td colspan="4">Exposure level (mg/kg/day Surflon S-111)</td></tr>
<tr><td></td><td>0</td><td>0.025</td><td>0.125</td><td>0.6</td></tr>
<tr><td>F0</td><td>0/30 (0%)</td><td>2/30 (7%)</td><td>5/30 (17%)</td><td>5/30 (17%)</td></tr>
<tr><td>F1</td><td>0/30 (0%)</td><td>3/30 (10%)</td><td>4/29 (14%)</td><td>8/30 (27%)</td></tr>
</table>
● Other changes (males): Subacute inflammation, clear cell foci, and vacuolation in all dosed group, with severity and/or incidence increasing with dose. Minimal inflammation in only one control F0 male.
● Females: Hepatocellular hypertrophy occurred in 5 of 30 high dose F0 (0.6 mg/kg/day) females; not seen in F1.
</td>
<td></td>
</tr>
</table>

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr><td>Stump et al. (2008) (continued).
An oral two-generation reproductive toxicity study of S-111-S-WB in rats.</td><td>
<u>Renal effects (F0 and F1 adult)</u>

<u>Kidney weight</u>
● Data shown in tables and discussed in text.
● Kidney weights (absolute and relative to body weight): significantly ↑ in the mid (0.125 mg/kg/day) and high (0.6 mg/kg/day) dose F0 and F1 males, and in high dose (0.6 mg/kg/day) F0 females.

<u>Kidney histopathology</u>
● Data shown in tables and discussed in text.
● Assessed in all F0 and F1 groups except 0.025 and 0.125 (low and mid dose) F1 females.
● Renal tubule cell hypertrophy (males): Incidence ↑ with dose in F0 and F1: severity ↑ with dose in F0.
<table>
<tr><td colspan="5">Incidence (%) of renal tubule cell hypertrophy in F0 & F1 males</td></tr>
<tr><td></td><td colspan="4">Exposure level (mg/kg/day Surflon S-111)</td></tr>
<tr><td></td><td>0</td><td>0.025</td><td>0.125</td><td>0.6</td></tr>
<tr><td>F0</td><td>0/30 (0%)</td><td>0/30 (0%)</td><td>5/30 (17%)</td><td>28/30 (93%)</td></tr>
<tr><td>F1</td><td>0/30 (0%)</td><td>0/30 (0%)</td><td>0/29 (0%)</td><td>30/30 (100%)</td></tr>
</table>
● Other changes (F0 males): Renal inflammation (1/30 mid dose and 1/30 low dose), brown pigment (2/30 high dose), and capsular fibrosis (1/30 high dose).
● Females: Renal tubule cell hypertrophy in 8 of 30 high dose (0.6 mg/kg/day) F0.

<u>Litter data (F1 and F2 pups)</u>
● Data shown in tables and discussed in text.
● Parameters not affected: Number of pups born, live litter size, postnatal survival, and pup weight through weaning in F1 or F2; nor age at vaginal opening and preputial separation in F1 (not assessed in F2).
● Relative liver weights (PND 21): Significantly ↑ in mid (0.125 mg/kg/day) and high (0.6 mg/kg/day) dose F1 males and females, and high dose F2 males and females.
</td><td></td></tr>
</table>

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Wang et al. (2014b). Integrated proteomic and miRNA transcriptional analysis reveals the hepatotoxicity mechanism of PFNA exposure in mice.

Species and strain:
Male BALB/c mice; 6-8 weeks old.

Group size:
8 per group.

Test article and vehicle:
PFNA (97% pure)

Route of exposure:
Oral gavage

Exposure levels:
0, 0.2, 1, 5 mg/kg/day

Exposure regimen:
14 days</td>
<td><u>PFNA levels in serum and liver</u>
● Numerical data provided.
● Assessed at the end of dosing period.
● PFNA levels in serum and liver ↑ with dose.
● Levels in liver were 2.1-fold (0.2 m/kg/day) to 1.1-fold (5 mg/kg/day) higher than in serum.

<u>Body weight</u>
● Presented graphically; numerical data not provided.
● ↓ (p<0.01) at highest dose, 5 mg/kg/day.

<u>Liver weight</u>
● Presented graphically and discussed in text. Some numerical data estimated from graph.
● Relative liver weight ↑ (p<0.01) at all doses.

<table>
<tr><th colspan="5">Relative Liver Weight*</th></tr>
<tr><td>Dose (mg/kg/day)</td><td>0</td><td>0.2</td><td>1</td><td>5</td></tr>
<tr><td>Serum level (ug/ml)</td><td>---</td><td>11.5</td><td>38.5</td><td>156.1</td></tr>
<tr><td></td><td>1</td><td>1.2</td><td>1.6</td><td>2.6</td></tr>
</table>
*Relative liver weight for 1 and 5 mg/kg/day estimated from graph.

<u>Serum and liver triglycerides (TG) and total cholesterol (TCH)</u>
● Presented graphically and discussed in text.
● Serum TG and TCHO : ↓ (p<0.01) at 5 mg/kg/day.
● Liver TG and TCHO: ↑ (p<0.01) at 0.2 and 1 mg/kg/day, but not at 5 mg/kg/day.

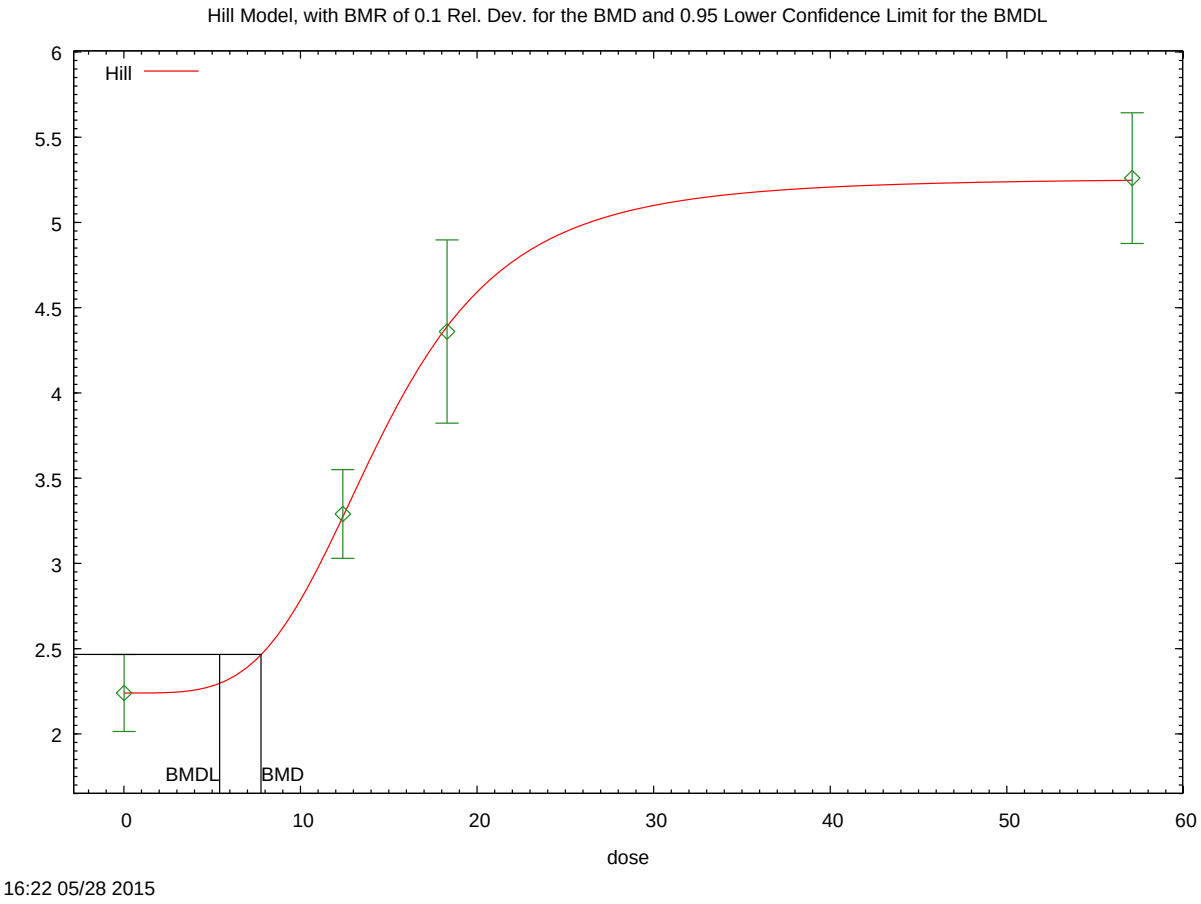
<u>Serum levels of liver enzymes (ALT and AST)</u>
● Presented graphically. Some numerical data estimated from graph.
● ALT and AST: ↑ (p<0.01) 4-8 fold at 5 mg/kg/day.</td>
<td>Serum PFNA levels were measured at the end of the dosing period, the same time point at which the toxicological endpoints were assessed.

Other components of this study evaluated the effects of PFNA on expression of genes and proteins related to PPAR-alpha (which increases fatty acid oxidation/lipolysis, and decreases hepatic and serum lipids) and sterol regulatory element-binding proteins (SREBPs) which are related to lipid biosynthesis. Although PPAR-alpha was activated, SREBP genes involved with lipid synthesis were even more strongly activated, at doses below the highest dose.

The authors conclude that PFNA upregulates PPAR-alpha-mediated lipid breakdown, but also upregulates SREBP mediated lipid biosynthesis, and that the effects on serum and hepatic lipids represent a balance between these effects. The increased hepatic lipids at the lower doses may occur because the lipid synthesis effect predominates over the PPAR-alpha mediated lipid breakdown effect at the lower doses, while the PPAR-alpha stimulation of lipolysis is more dominant at the highest dose.</td>
</tr>
</table>

Reference and Study Design	Results	Comment
Wolf et al. (2010). Developmental effects of perfluorononanoic acid in the mouse are dependent on peroxisome proliferator-activated receptor-alpha. **Species and strain:** Female wild-type (WT) 129S1/SvlmJ mice and PPARα knockout (KO) mice on a 129S1/SvlmJ background; mated to males of same strain. **Group size:** 9-18 pregnant females per group. **Test article and vehicle:** PFNA (97% pure) in water. **Route of exposure:** Oral gavage, total volume 10 ml/kg. **Exposure levels:** 0, 0.83, 1.1, 1.5, or 2 mg/kg/day. **Exposure regimen:** GD 1-18. Adults and pups sacrificed on PND 21.	**Pregnancy outcome and maternal weight gain** ● Maternal weight gain, number of uterine implants, and number of live plus dead pups per litter were not affected by PFNA. ● *Pregnancy rate: S*ignificantly ($p<0.001$) ↓ at all doses of PFNA in KO mice compared to control KO mice; no effect in WT mice. ● *% litter loss:* Non-significant ↑ in high dose (2 mg/kg/day) WT to 35.3% as compared to 14.3% in control WT. No effect in KO. ● *# of live pups at birth:* ↓ at all doses in WT mice; significant at 1.1 and 2.0 mg/kg/day. No effect in KO. **Pup survival from birth to weaning** ● Dose-related ↓ in WT groups; significant at two highest doses. • Most pup deaths in first few postnatal days. • At PND 21, survival was 36% at 1.5 mg/kg/day and 31% at 2 mg/kg/day compared to about 75% in controls. ● No effect in KO mice. **Pup weight gain and eye opening** ● *Pup weight at birth:* Not affected. ● *Weight gain from birth until weaning:* - WT: ↓ in male and female WT pups at 2 mg/kg/day; no effect at lower doses. - KO: No effects at any dose. ● *Eye opening:* - WT: significantly delayed in 2 mg/kg/day, no effect at lower doses. - KO: No effects at any dose **Adult and pup relative liver weight 23 days after last dose (PND 21).** ● *Non-pregnant WT and KO adult females:* ↑ at all doses; significant in all groups except low dose (0.83 mg/kg/day) KO. ● *WT mice that had given birth:* ↑ significantly increased at all doses in WT; no significant ↑ in KO. ● *KO mice that had given birth:* ↑ significantly increased at all doses in WT; no significant ↑ in KO. • Serum levels in the KO much lower than in the WT for reason(s) that were not determined. ● *Pups at weaning (PND 21:* - WT: ↑ at all doses. - KO: ↑ only at the highest dose (2 g/kg/day).	Serum PFNA (presented numerically) was measured in all adult females and in 2 pups per litter 23 days after the last dose. Histopathological examination was not performed on liver or other organs.

APPENDIX 4. Detailed Benchmark Dose Modeling Results for 10% Increase in Maternal Liver Weight on GD 17 (Das et al., 2015)



```
================================================================================
        Hill Model. (Version: 2.17;  Date: 01/28/2013)
        Input Data File: C:/BMDS260/Data/hil_Lau maternal liver wt without resorption
5-28-15_Opt.(d)
        Gnuplot Plotting File:  C:/BMDS260/Data/hil_Lau maternal liver wt without
resorption 5-28-15_Opt.plt
                                                Thu May 28 16:22:18 2015
 ================================================================================

 BMDS Model Run
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   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   Power parameter restricted to be greater than 1
   The variance is to be modeled as Var(i) = exp(lalpha  + rho * ln(mean(i)))

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 500
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                    Default Initial Parameter Values
                           lalpha =       -1.504
                              rho =            0
                        intercept =         2.24
                                v =         3.02
                                n =           18
                                k =      21.6636


           Asymptotic Correlation Matrix of Parameter Estimates

                lalpha          rho    intercept            v            n            k

    lalpha           1        -0.97        -0.14         0.15       -0.079        0.051

       rho       -0.97            1         0.12        -0.16        0.081       -0.053

 intercept       -0.14         0.12            1        -0.42         0.13         0.27

         v        0.15        -0.16        -0.42            1        -0.43         0.49

         n      -0.079        0.081         0.13        -0.43            1        -0.38

         k       0.051       -0.053         0.27         0.49        -0.38            1
```

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	-4.46569	1.02676	-6.4781	-2.45327
rho	2.03532	0.764172	0.537573	3.53307
intercept	2.24197	0.08613	2.07315	2.41078
v	3.016	0.211426	2.60162	3.43039
n	3.9983	0.938654	2.15857	5.83802
k	14.5893	0.829097	12.9643	16.2143

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0.013	8	2.24	2.24	0.27	0.244	-0.0228
12.4	8	3.29	3.28	0.311	0.359	0.107
18.3	8	4.36	4.39	0.643	0.483	-0.175
57.1	10	5.26	5.25	0.536	0.579	0.0811

Warning: Likelihood for fitted model larger than the Likelihood for model A3.

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	10.695847	5	-11.391693
A2	14.716640	8	-13.433281
A3	13.772953	6	-15.545906
fitted	13.772953	6	-15.545906
R	-24.175471	2	52.350943

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)

Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	77.7842	6	<.0001
Test 2	8.04159	3	0.04516
Test 3	1.88738	2	0.3892
Test 4	-8.91731e-013	0	NA

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

NA - Degrees of freedom for Test 4 are less than or equal to 0. The Chi-Square test for fit is not valid

Benchmark Dose Computation

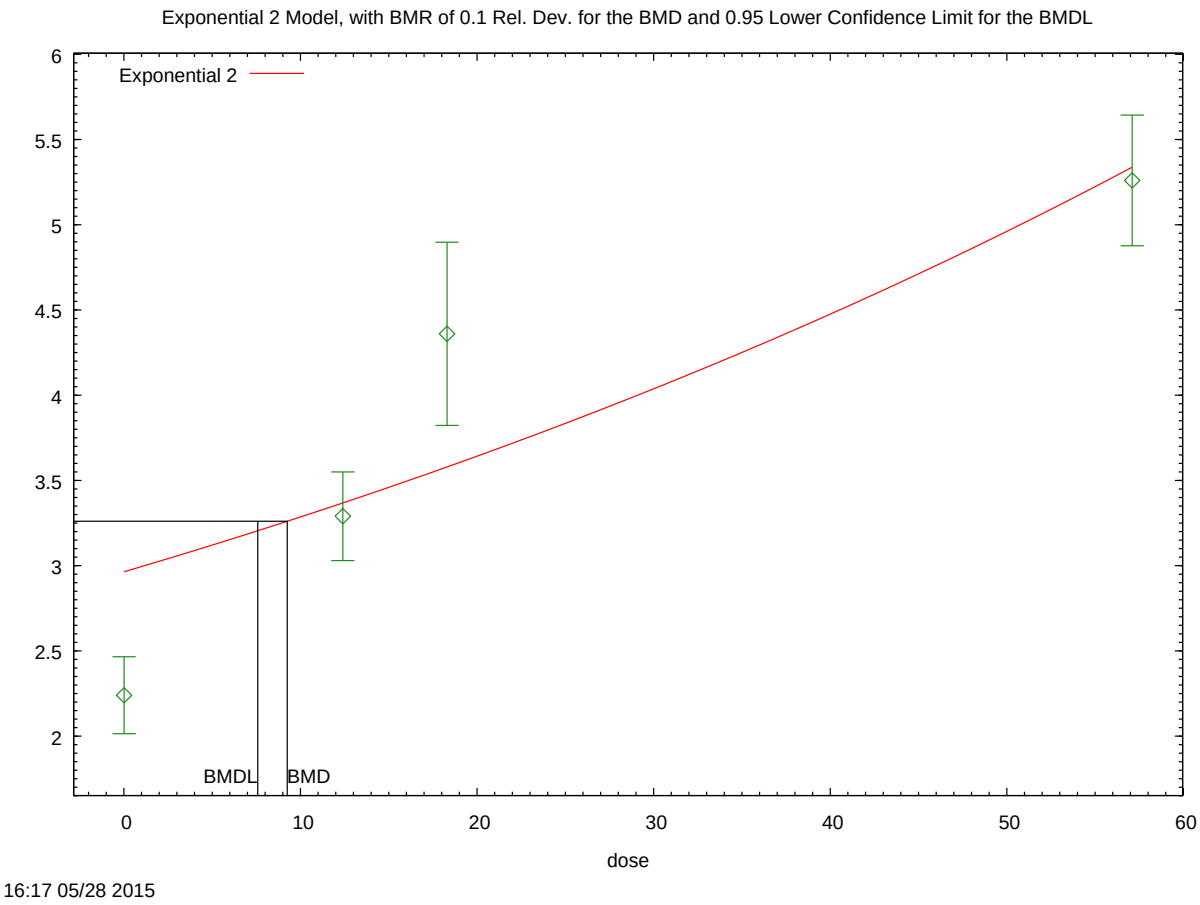
Specified effect = 0.1

Risk Type = Relative deviation

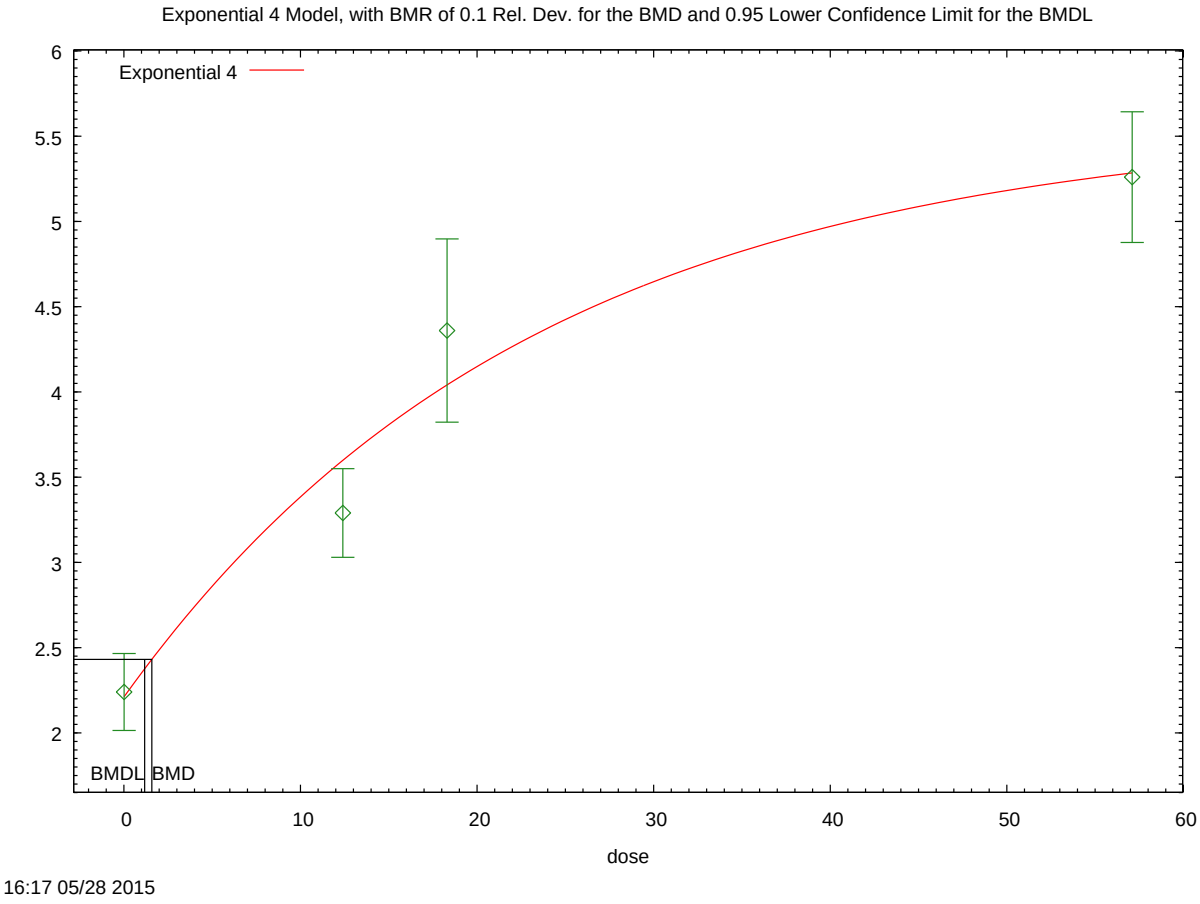
Confidence level = 0.95

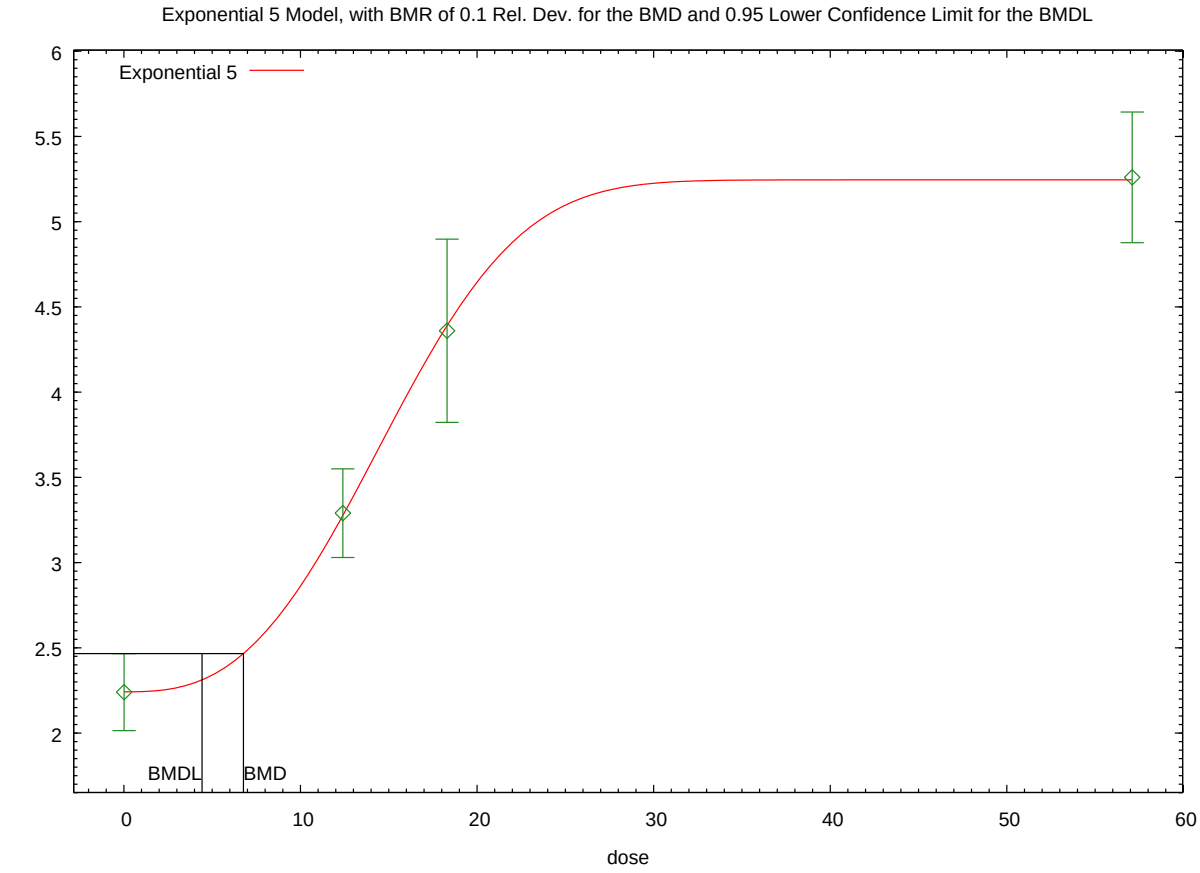
BMD = 7.7643

BMDL = 5.42534



(NOTE: Exponential Model 3 is identical to Exponential Model 2)


Exponential 4 Model, with BMR of 0.1 Rel. Dev. for the BMD and 0.95 Lower Confidence Limit for the BMDL
Exponential 4
BMDL
BMD
6
5.5
5
4.5
4
3.5
3
2.5
2
0
10
20
30
40
50
60
dose
16:17 05/28 2015


Exponential 5 Model, with BMR of 0.1 Rel. Dev. for the BMD and 0.95 Lower Confidence Limit for the BMDL
Exponential 5
BMDL
BMD
dose
16:17 05/28 2015

```
================================================================
        Exponential Model. (Version: 1.10;  Date: 01/12/2015)
        Input Data File: C:/BMDS260/Data/exp_Lau maternal liver wt without resorption
5-28-15_Opt.(d)
        Gnuplot Plotting File:
                                                  Thu May 28 15:51:05 2015
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 BMDS Model Run
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   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.


   Dependent variable = Mean
   Independent variable = Dose
   Data are assumed to be distributed: normally
   Variance Model: exp(lnalpha +rho *ln(Y[dose]))
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 500
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

   MLE solution provided: Exact


                              Initial Parameter Values

    Variable          Model 2            Model 3            Model 4            Model 5
    --------          -------            -------            -------            -------
    lnalpha          -4.31867           -4.31867           -4.31867           -4.31867
        rho            1.9863             1.9863             1.9863             1.9863
          a           2.71116            2.71116              2.128              2.128
          b         0.0127943          0.0127943          0.0455732          0.0455732
          c                 0 *                0 *            2.59539            2.59539
          d                 1 *                1                  1 *                1

    * Indicates that this parameter has been specified
```

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	0.825765	0.825768	-4.04657	-4.46569
rho	-1.21492	-1.21492	1.92163	2.03532
a	2.96415	2.96415	2.21016	2.24197
b	0.0103013	0.0103013	0.042932	0.0592793
c	--	--	2.52223	2.33953
d	--	1	--	2.80096

-- Indicates that this parameter does not appear in model

Std. Err. Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	NA	1.77774	1.21057	1.02676
rho	NA	1.30924	0.90747	0.764172
a	NA	0.177514	0.0991425	0.08613
b	NA	0.00129421	0.0091977	0.00366332
c	NA	NA	0.188129	0.121848
d	NA	NA	NA	0.584725

NA - Indicates that this parameter was specified (by the user or because of the model form)
or has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0.013	8	2.24	0.27
12.4	8	3.29	0.311
18.3	8	4.36	0.643
57.1	10	5.26	0.536

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
2	0.013	2.965	0.7809	-2.624
	12.4	3.368	0.7227	-0.3054
	18.3	3.579	0.6965	3.171
	57.1	5.338	0.5464	-0.4497
3	0.013	2.965	0.7809	-2.624
	12.4	3.368	0.7227	-0.3054
	18.3	3.579	0.6965	3.171
	57.1	5.338	0.5464	-0.4497
4	0.013	2.212	0.2835	0.279
	12.4	3.599	0.4526	-1.931
	18.3	4.041	0.5058	1.784
	57.1	5.285	0.6546	-0.1189
5	0.013	2.242	0.2438	-0.02281
	12.4	3.276	0.3587	0.1075
	18.3	4.39	0.4832	-0.1754
	57.1	5.245	0.5791	0.08113

Other models for which likelihoods are calculated:

```
  Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

  Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
           Var{e(ij)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	10.69585	5	-11.39169
A2	14.71664	8	-13.43328
A3	13.77295	6	-15.54591
R	-24.17547	2	52.35094
2	-3.485372	4	14.97074
3	-3.485372	4	14.97074
4	9.115981	5	-8.231961
5	13.77295	6	-15.54591

Additive constant for all log-likelihoods = -31.24. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

Test 7a: Does Model 5 fit the data? (A3 vs 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	77.78	6	< 0.0001
Test 2	8.042	3	0.04516
Test 3	1.887	2	0.3892
Test 4	34.52	2	< 0.0001
Test 5a	34.52	2	< 0.0001

Test 5b	-5.739e-011	0	N/A
Test 6a	9.314	1	0.002274
Test 6b	25.2	1	< 0.0001
Test 7a	-1.044e-012	0	N/A
Test 7b	34.52	2	< 0.0001
Test 7c	9.314	1	0.002274

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 4 is less than .1. Model 2 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5a is less than .1. Model 3 may not adequately describe the data; you may want to consider another model.

Degrees of freedom for Test 5b are less than or equal to 0. The Chi-Square test for fit is not valid.

The p-value for Test 6a is less than .1. Model 4 may not adequately describe the data; you may want to consider another model.

The p-value for Test 6b is less than .05. Model 4 appears to fit the data better than Model 2.

Degrees of freedom for Test 7a are less than or equal to 0. The Chi-Square test for fit is not valid.

The p-value for Test 7b is less than .05. Model 5 appears to fit the data better than Model 3.

The p-value for Test 7c is less than .05. Model 5 appears to fit the data better than Model 4.

Benchmark Dose Computations:

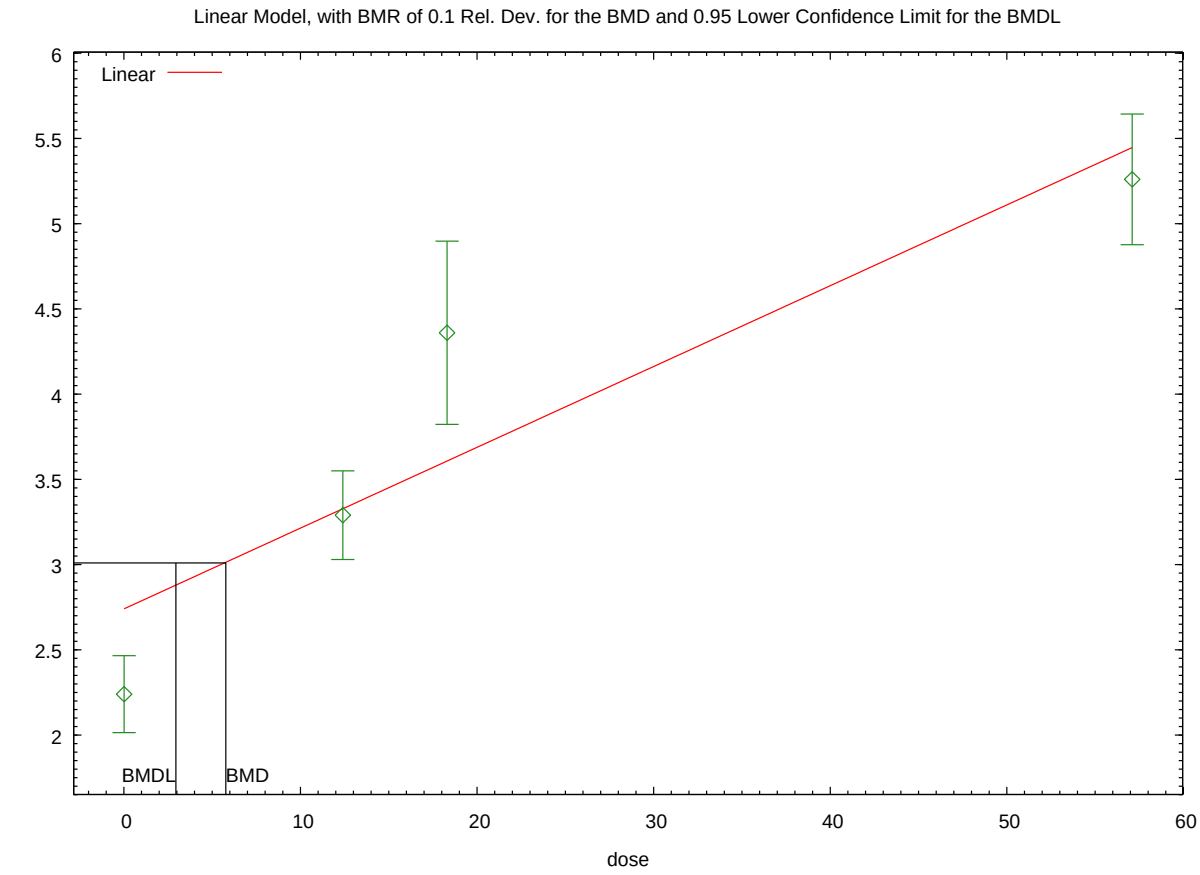
Specified Effect = 0.100000

Risk Type = Relative deviation

Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL
2	9.25227	7.58073
3	9.25226	7.58073
4	1.58274	1.17748
5	6.77218	4.42541



16:47 05/28 2015

```
================================================================
        Polynomial Model. (Version: 2.20;  Date: 10/22/2014)
        Input Data File: C:/BMDS260/Data/ply_Lau maternal liver wt without resorption
5-28-15_Opt.(d)
        Gnuplot Plotting File:  C:/BMDS260/Data/ply_Lau maternal liver wt without
resorption 5-28-15_Opt.plt
                                              Thu May 28 16:47:25 2015
 ================================================================

 BMDS Model Run
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   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Mean
   Independent variable = Dose
   Signs of the polynomial coefficients are not restricted
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 500
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                        lalpha =       -1.504
                           rho =            0
                        beta_0 =      2.72234
                        beta_1 =    0.0485194


          Asymptotic Correlation Matrix of Parameter Estimates

               lalpha          rho       beta_0       beta_1

    lalpha          1        -0.99         0.63         -0.7

       rho      -0.99            1        -0.63          0.7

    beta_0       0.63        -0.63            1        -0.85

    beta_1       -0.7          0.7        -0.85            1


                              Parameter Estimates

                                                    95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
        lalpha       -0.833995          2.03328           -4.81914             3.15115
           rho       -0.0645824          1.52989           -3.06311             2.93395
        beta_0          2.73608         0.207163            2.33004             3.14211
        beta_1        0.0474058       0.00678799          0.0341015             0.06071
```

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0.013	8	2.24	2.74	0.27	0.638	-2.2
12.4	8	3.29	3.32	0.311	0.634	-0.151
18.3	8	4.36	3.6	0.643	0.632	3.38
57.1	10	5.26	5.44	0.536	0.624	-0.927

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	10.695847	5	-11.391693
A2	14.716640	8	-13.433281
A3	13.772953	6	-15.545906
fitted	-1.373442	4	10.746883
R	-24.175471	2	52.350943

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	77.7842	6	<.0001
Test 2	8.04159	3	0.04516
Test 3	1.88738	2	0.3892
Test 4	30.2928	2	<.0001

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance

model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears
 to be appropriate here

The p-value for Test 4 is less than .1. You may want to try a different
model

Benchmark Dose Computation

Specified effect = 0.1

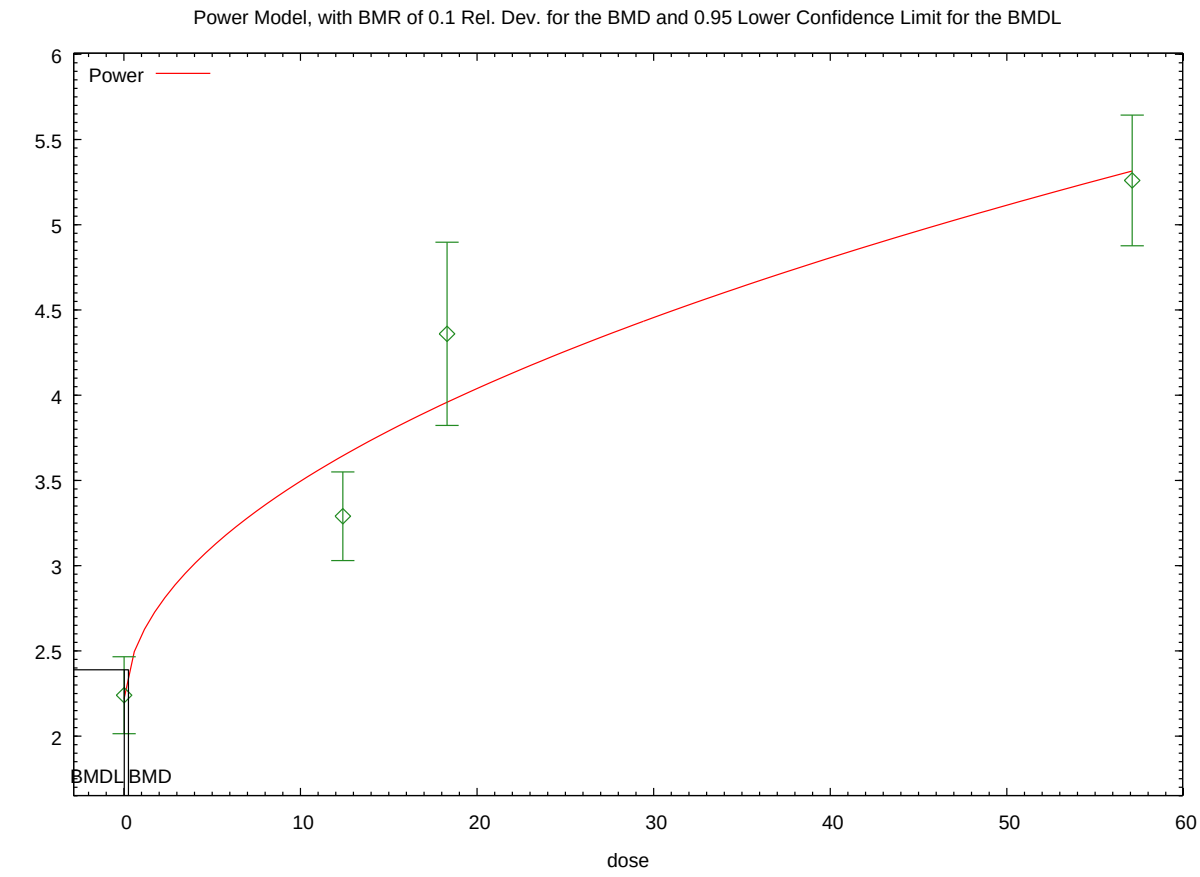
Risk Type = Relative deviation

Confidence level = 0.95

BMD = 5.77161

BMDL = 2.94582

BMDL computation failed for one or more point on the BMDL curve.
 The BMDL curve will not be plotted


Power Model, with BMR of 0.1 Rel. Dev. for the BMD and 0.95 Lower Confidence Limit for the BMDL
Power
6
5.5
5
4.5
4
3.5
3
2.5
2
BMDL BMD
0
10
20
30
40
50
60
dose
11:02 05/29 2015

```
================================================================================
        Power Model. (Version: 2.18;  Date: 05/19/2014)
        Input Data File: C:/BMDS260/Data/pow_Lau maternal liver wt without resorption
5-28-15_Opt.(d)
        Gnuplot Plotting File:  C:/BMDS260/Data/pow_Lau maternal liver wt without
resorption 5-28-15_Opt.plt
                                                Fri May 29 11:02:48 2015
 ================================================================================

 BMDS Model Run
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   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = Mean
   Independent variable = Dose
   The power is not restricted
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 500
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                         lalpha =       -1.504
                            rho =            0
                        control =         2.24
                          slope =     0.260376
                          power =        -9999


           Asymptotic Correlation Matrix of Parameter Estimates

                 lalpha          rho      control        slope        power

    lalpha            1        -0.98        -0.16        0.054       -0.023

       rho        -0.98            1         0.15       -0.058        0.024

   control        -0.16         0.15            1        -0.63         0.53

     slope        0.054       -0.058        -0.63            1        -0.98

     power       -0.023        0.024         0.53        -0.98            1
```

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	-3.96586	1.265	-6.44521	-1.48651
rho	1.93299	0.949011	0.0729611	3.79301
control	2.17191	0.120445	1.93584	2.40798
slope	0.422924	0.144482	0.139745	0.706103
power	0.495715	0.0886516	0.321961	0.669469

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0.013	8	2.24	2.22	0.27	0.298	0.18
12.4	8	3.29	3.65	0.311	0.481	-2.09
18.3	8	4.36	3.96	0.643	0.52	2.18
57.1	10	5.26	5.31	0.536	0.692	-0.241

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	10.695847	5	-11.391693
A2	14.716640	8	-13.433281
A3	13.772953	6	-15.545906
fitted	7.468885	5	-4.937769
R	-24.175471	2	52.350943

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	77.7842	6	<.0001
Test 2	8.04159	3	0.04516
Test 3	1.88738	2	0.3892
Test 4	12.6081	1	0.0003841

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is less than .1. You may want to try a different model

Benchmark Dose Computation

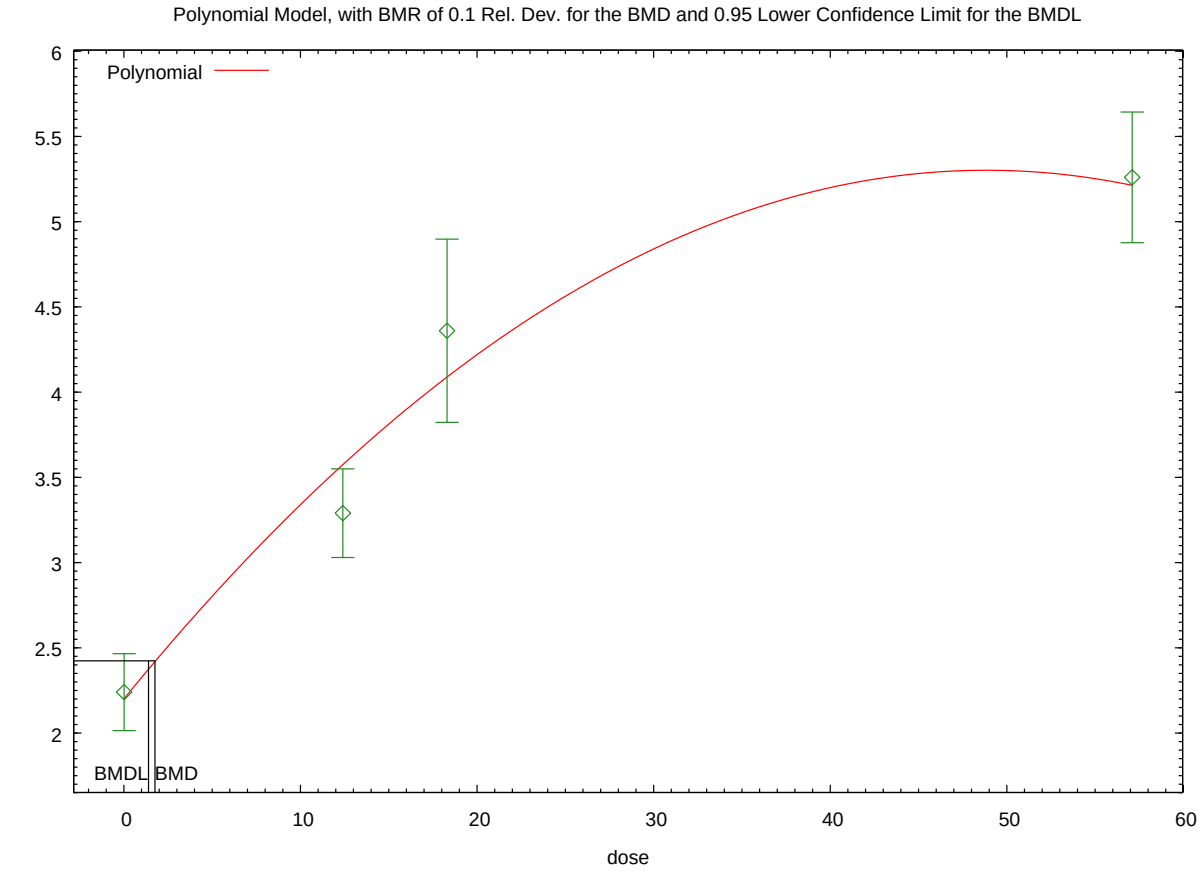
Specified effect = 0.1

Risk Type = Relative deviation

Confidence level = 0.95

BMD = 0.260709

BMDL = 0.0210435


Polynomial Model, with BMR of 0.1 Rel. Dev. for the BMD and 0.95 Lower Confidence Limit for the BMDL
Polynomial
BMDL
BMD
6
5.5
5
4.5
4
3.5
3
2.5
2
0
10
20
30
40
50
60
dose
15:43 05/28 2015

```
================================================================
        Polynomial Model. (Version: 2.20;  Date: 10/22/2014)
        Input Data File: C:/BMDS260/Data/ply_Lau maternal liver wt without resorption
5-28-15_Opt.(d)
        Gnuplot Plotting File:  C:/BMDS260/Data/ply_Lau maternal liver wt without
resorption 5-28-15_Opt.plt
                                            Thu May 28 15:43:34 2015
 ================================================================

 BMDS Model Run
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   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Mean
   Independent variable = Dose
   Signs of the polynomial coefficients are not restricted
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 500
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                        lalpha =       -1.504
                           rho =            0
                        beta_0 =      2.16211
                        beta_1 =     0.132156
                        beta_2 =  -0.00136126


          Asymptotic Correlation Matrix of Parameter Estimates

               lalpha          rho       beta_0       beta_1       beta_2

    lalpha          1        -0.98        -0.15         0.13       -0.089

       rho        -0.98            1         0.15        -0.12        0.071

    beta_0        -0.15         0.15            1        -0.61         0.49

    beta_1         0.13        -0.12        -0.61            1        -0.96

    beta_2       -0.089        0.071         0.49        -0.96            1


                                Parameter Estimates

                                                      95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
         lalpha         -4.07867          1.15936            -6.35097            -
1.80638
```

```
            rho          1.90932         0.867406            0.209232
3.6094
         beta_0           2.2034        0.0983742             2.01059
2.39621
         beta_1         0.127329        0.0136347            0.100606
0.154053
         beta_2      -0.00129539      0.000236025         -0.00175799        -
0.000832786
```

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0.013	8	2.24	2.21	0.27	0.277	0.357
12.4	8	3.29	3.58	0.311	0.44	-1.88
18.3	8	4.36	4.1	0.643	0.5	1.47
57.1	10	5.26	5.25	0.536	0.634	0.0479

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	10.695847	5	-11.391693
A2	14.716640	8	-13.433281
A3	13.772953	6	-15.545906
fitted	9.944650	5	-9.889301
R	-24.175471	2	52.350943

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value

Test 1	77.7842	6	<.0001
Test 2	8.04159	3	0.04516
Test 3	1.88738	2	0.3892
Test 4	7.6566	1	0.005656

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is less than .1. You may want to try a different model

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Relative deviation

Confidence level = 0.95

BMD = 1.76206

BMDL = 1.39649

BMDL computation failed for one or more point on the BMDL curve.
The BMDL curve will not be plotted