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DRAFT TECHNICAL SUPPORT DOCUMENT: INTERIM SPECIFIC GROUND WATER CRITERION FOR PERFLUORONONANOIC ACID (PFNA, C9)

(CAS #: 375-95-1; Chemical Structure: $CF_3(CF_2)_7COOH$)

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ABSTRACT

A draft interim specific ground water criterion for perfluorononanoic acid (PFNA, C9) was developed based on chronic (lifetime) drinking water exposure. The criterion is based on modeling of PFNA levels in blood serum that caused increased maternal liver weight from 16 days of exposure in a mouse developmental study and is further supported by data on effects in the offspring in the same study and on other effects in studies from other laboratories. Appropriate uncertainty factors were applied to account for extrapolation from animals to humans, to protect sensitive human subpopulations, and to account for chronic exposure and gaps in the toxicology database. Based on available toxicokinetic data from animal and humans, an estimated factor relating PFNA intake to increased PFNA in blood serum of 0.085 (ng/kg/day)/(ng/L) was developed. This corresponds to a blood serum:drinking water ratio of 200:1 for humans with ongoing drinking water exposure to PFNA. Using this information, a water concentration protective of chronic drinking water exposure of 17 ng/L was derived. As ground water criteria are rounded to one significant figure, the recommended interim specific ground water criterion for PFNA is 20 ng/L (0.02 µg/L).

BACKGROUND INFORMATION

Development of an interim specific ground water criterion for perfluorononanoic acid (PFNA, C9) was requested of the New Jersey Department of Environmental Protection (NJDEP) Office of Science by the NJDEP Site Remediation Program under N.J.A.C 7:9C. Interim specific ground water criteria are intended to be protective for chronic drinking water exposure.

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 end 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 perfluorooctanoate, 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 from production of 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 (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 that is produced and used in commercial and industrial products (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 are part of the voluntary stewardship agreement with USEPA.

Data provided to NJDEP shows that the 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.

Other Guidelines, Regulations, and Standards

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

ENVIRONMENTAL SOURCES, FATE, AND OCCURRENCE

Because of the extreme stability of their carbon−fluorine bonds, PFCs are highly persistent in the environment. PFCs are highly water-soluble in comparison with other well-studied persistent and bioaccumulative organic pollutants such as polychlorinated dioxins and PCBs that have low water solubility (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 such as 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 source of PFNA and other PFCs in the environment is the breakdown of 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 and 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 are 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 contributions of each of these pathways are not known. The first pathway involves the atmospheric transport of volatile precursors, such as fluorotelomer alcohols (FTOH), followed by oxidation to PFCs (e.g. PFNA) which are then deposited onto the land or the water. The second pathway involves long-range aqueous transport of perfluorinated carboxylates such as 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 pathway of migration of a ground water plume that has been contaminated either directly from surface spills or by contaminated surface water mixing with ground water drawn in by pumping wells. Air emission has been established as an additional 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 about 20 miles from the emission source. This occurred by 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 a similar pathway. This pathway, discussed further below, is being investigated as a possible source of PFNA in drinking water wells in the vicinity of a 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 treating domestic and/or industrial waste; street- and stormwater 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 PNFA 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, 2014). 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, or 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 PFCs in raw water from 31 NJ public water supplies (29 sampled by NJDEP in 2009, and 2 sampled by a water company using the same laboratory and method). In this study, PFNA was found in three ground water sources at concentrations (72-96 ng/L) above the highest raw or finished drinking water level (58 ng/L) reported elsewhere in the studies located in the literature. At these three sites, PFNA was the sole or predominant PFC detected, whereas PFNA was a minor component of a mixture of PFCs when it was detected in drinking water elsewhere.

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). PFNA levels in another well of this public water supply were lower (<20 ng/L) in September 2013 testing. 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.

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 Level in the laboratories used in NJDEP studies and other monitoring data reported to NJDEP and in the drinking water occurrence studies reported in the literature (generally $\leq$5 ng/L, reviewed by Post et al., 2013). In initial UCMR3 data from 1470 public water supplies through January 2014, PFNA (20 ng/L or above) was found in only 2 sites outside of New Jersey (USEPA, 2014) and in 2 sites in Gloucester County, NJ (Woodbury City Water Department, 56 ng/L, and Monroe Township (Gloucester County) MUA, 26 ng/L). In the two non-NJ public water supplies, other PFCs were also present, while PFNA was the only PFC reported at the Gloucester County, NJ, sites.

In other recent sampling reported to NJDEP, 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. Additional testing of public water supply systems in Gloucester County is underway.

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 which 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 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 reported in a literature search of studies elsewhere in the U.S. and worldwide. Elevated levels of perfluoroundecanoic acid (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). PFNA and PFUnDA were also elevated in fish from the same Delaware River locations in 2004−2007 (DRBC, 2009).

HUMAN EXPOSURE AND SERUM LEVELS

PFNA is one of four PFCs (PFOA, PFOS, PFNA, 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) monitoring conducted by the U.S. Centers for Disease Control and Prevention (CDC; Kato et al., 2011); PFCs are also ubiquitous in the serum of populations worldwide (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 published NHANES data from 2007-08 (Kato et al., 2011), geometric mean serum levels were PFNA, 1.49 ng/ml; PFOA, 4.13 ng/ml; PFOS, 13.2 ng/ml; and PFHxS, 1.96 ng/L. However, while serum levels of the other three PFCs have declined (PFOS) or remained relatively constant (PFOA and PFHxS) in the four NHANES studies conducted between 1999-2000 and 2007-08, the geometric mean serum level for PFNA has increased by about 2.5-fold, from 0.547 ng/ml to 1.49 ng/ml (Kato et al., 2011). Similarly, other studies of PFC serum levels in U.S. blood donors found an increase in geometric mean PFNA levels from 0.57 ng/ml in 2000-01 to 0.97 ng/ml in 2006 (Olsen et al., 2011). In both studies, PFNA serum levels were slightly higher in men than in women. This increasing trend for serum levels of PFNA in recent years was also observed in Swedish general population studies (Borg and Hakansson, 2012). 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.

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, of non-Hispanic white, non-Hispanic black, and Mexican-American ethnicity were generally similar in both age categories and both genders.

Sources of human exposure to PFCs in general 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. Based on the infrequent occurrence and low concentrations of PFNA reported in drinking water (discussed above), the mean and median PFNA serum levels found in the general population in NHANES are not likely to be influenced by drinking water exposures. Since PFNA bioaccumulates in fish, consumption of contaminated fish 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. However, humans may be exposed to PFNA formed from telomer alcohols in environmental media (discussed above) and by metabolism of telomer alcohols in the human body (Henderson and Smith, 2007; Nillson et al., 2010; reviewed by Butt et al., 2014). Telomer 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 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 and/or public water supplies (PWS). 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 (Post et al., 2012; discussed in detail below). As expected due to 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).

As discussed above, PFNA exists in drinking water in its non-volatile anionic form. Therefore, significant exposure is not expected from non-ingestion uses of drinking water such as showering, bathing, laundry, and dishwashing that are important exposure routes for volatile drinking water contaminants.

Tao et al. (2008a) 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 infrequently found (PFOS – one detection at 11.3

ng/L; PFHxS-two detections at up to 3.59 ng/L). Based on these data, commercially available infant formula products do not appear to be a major source of exposure to PFNA or other PFCs in the U.S. 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 only by 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 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.

Distribution and Metabolism

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

PFNA is 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 and 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 are primarily excreted in urine, with the rate of excretion generally decreasing with increasing carbon chain length (Lau, 2012). PFNA was excreted in the urine and feces of rats (Mertens et al., 2010). 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. These differences in excretion rates between genders in the rat are believed to result from gender differences in renal organic anion transporters (OATs). 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.

Table 1. 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]	5-7.5	8.2-30
Mouse	25.8-68.4[a]	34.3-68.9[a]	17[c]	19[c]	1.5-4	1.8-3.6

[a]Tatum-Gibbs et al. (2011). [b]Ohmori et al. (2003)

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 Post et al., 2012 and Lau et al., 2012). For PFNA, no such data are available.

Zhang et al. (2013) recently estimated the human half-lives of a series of PFCs, including PFOA and PFNA, based on renal clearance estimates from 86 adults (age 21-88 years), from the Chinese general population. The mean serum PFNA concentration in the subjects was 0.45 ng/L, which was several fold lower than the mean of 1.59 ng/ml in the 2007-08 NHANES (Kato et al., 2011). Renal clearance estimates for each PFC in each participant were based on paired urine and blood or serum measurements.

Because the half-lives for menstruating women are based on estimation through modeling of excretion of PFCs through menstrual blood loss in subjects less than 50 years of age, they 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 is not relevant to pregnant women. Other potential clearance pathways, such as fecal excretion, were not considered by Zhang et al. (2013), but were believed by the researchers to be less significant than elimination through urine and menstrual blood.

Zhang et al. (2013) reported PFNA half-life estimates in males and older females for PFNA (n=50) ranging from 0.34 to 20 years, and 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), 0.19 to 5.2 years. 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 2, 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 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 2. Estimated half-lives of PFNA and PFOA in Humans (years)

	PFNA		***PFOA***		***PFNA:PFOA $t_{1/2}$ Ratio***	
Based on serum levels	No information		2.3-10.1 years[a]/3.8[b]		----	----
Based on urinary excretion, with estimated menstrual clearance in females <50 years[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. (2013) – 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 (Lau et al., 2009: Wolf et al., 2010). Like other PFCs, PFNA is found in human umbilical cord blood (reviewed below), placenta, and amniotic fluid (Zhang et al., 2013), thereby demonstrating that maternal-fetal transfer also occurs in humans.

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 (Fromme et al., 2010; Zhang et al., 2013; Ode et al, 2013; Beesoon et al., 2011; Kim et al., 2010; Liu et al., 2011; Needham et al., 2011: Gutzkow et al., 2011; Monroy et al., 2008).

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 - 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, 2004). 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.

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 reporting maximum levels of >100 ng/L. In the only study conducted in the U.S. (Tao et al., 2008), 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.

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 3). 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 3: PFNA (ng/ml) in serum from 53 mother:infant pairs (Fromme et al., 2010)

	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

Relationship between drinking water concentration 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).

The relationship between drinking water concentration and serum concentration has been extensively evaluated for PFOA. As discussed in detail in Post et al. (2009a,b) and Post et al.

(2012), 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. 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 two toxicokinetic modeling efforts.

Although upper percentile exposure factors are typically used in risk assessment, 100:1 represents a central tendency estimate for the ratio in exposed populations and is 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 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 ratio is supported by data from several other studies. In 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 (MDH, 2009), if the expected post-exposure decline in serum levels is considered.

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 the USEPA (2004) estimated daily water intake of 17 ml/kg/day, application of this factor predicts a serum:drinking water ratio of 133:1.

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 in males and 8 µg/L in females, or a mean of 10.5 µg/L. Assuming a drinking water intake of 0.017 L/kg/day (USEPA, 2004), a dose of 0.0017 µg/kg/day would result from a water concentration of 0.1 µg/L. The ratio between a serum concentration of

10.5 µg/L and this water concentration of 0.1 µg/L is 105:1, identical to the median ratio reported by Emmett et al. (2006)

For persistent compounds, the relationship between daily intake (as expressed either as a dose (mg/kg/day) or a concentration in drinking water (ng/L)) and body burden (as measured by serum levels, in the case of PFCs) is proportional to the half-life (USEPA, 2003). Thus, the serum:drinking water ratio for persistent PFCs is expected to be proportional to their half-lives. The half-life data from rats and mice, and the preliminary human half-life data (presented above) support an estimated half-life of PFNA at least twice that of PFOA. These data, along with information discussed above indicating that the 100:1 ratio for PFOA likely underestimates the median ratio, support an estimated serum:drinking water ratio of 200:1 for PFNA. Based on assumed daily drinking water intake of 17 ml/kg/day (USEPA, 2004), this ratio corresponds to an increase of 1 ng/ml PFNA in blood serum per 0.085 (ng/kg/day) PFNA ingested or a factor of 0.085 (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 1.49 times the daily PFNA dose 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 New Jersey or elsewhere, is estimated to increase, PFNA serum levels, on average, by 30 µg/L (30 ng/mL, ppb) in serum. This represents about a 20-fold increase from the general population mean serum value of 1.5 ng/ml (Kato et al., 2011).

HEALTH EFFECTS

Human Studies

Information on effects of PFNA in humans includes a number of studies in the general population and one study of occupationally exposed workers. These studies evaluate a number of health endpoints and differ in regard to study populations, the categorization of the health-related parameters analyzed, the type of regression analysis, the confounders that were assessed, the median and range of PFNA serum levels, and other factors.

To our knowledge, there have been no studies of communities with elevated exposures to PFNA through contaminated drinking water or other environmental media. Such communities would be expected to have a higher range of serum PFNA values than the range found in the general population. However, it is relevant to note that extensive studies of health effects have been conducted in communities with drinking water contaminated with the closely related PFC, PFOA. These PFOA studies are discussed briefly at the end of this section.

General Population Studies

In cross-sectional studies of the general population (the NHANES study population in the U.S., and similar populations in other countries) that are discussed in this sections, associations have been reported between blood serum PFNA levels and increased cholesterol in adults, increased serum glucose and related parameters in adolescents, diabetes in the elderly, decreased response to rubella vaccine in children, increased thyroid hormone levels in children, and behavioral

effects in children. Some of these effects (metabolic effects and decreased immune response) are consistent with effects seen in the animal studies discussed below. Causality cannot be established from these epidemiologic studies because of their cross-sectional design in which exposure and outcome are assessed at the same point in time. Other cross-sectional studies in the general population discussed in this section did not find significant associations between PFNA and the health endpoints that were assessed.

In the general population studies of PFNA, associations were also assessed for other PFCs, with the specific suite of compounds evaluated differing among studies. A general issue with interpretation of these studies is that serum levels of PFCs are often correlated, making it difficult to assess the contribution of individual PFCs when several PFCs are associated with a given effect. However, some of the effects associated with PFNA exposure were not associated with exposure to other PFCs. This is the case even though serum PFNA concentrations were much lower than the serum concentrations of the other PFCs that were evaluated in most of the general population studies. For such effects, associations observed for PFNA are unlikely to be confounded by other co-occurring PFCs.

Serum lipids

Nelson et al. (2010) evaluated associations of PFNA, PFOA, PFOS, and PFHxS with total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, non-HDL (HDL subtracted from TC), low-density lipoprotein (LDL) cholesterol, body mass index (BMI), waist circumference (WC), and insulin resistance assessed as Homeostatic Model Assessment (HOMA) from the 2003-2004 NHANES survey population. All analyses excluded those older than 80 years of age, pregnant, breast-feeding, taking insulin, or undergoing dialysis. The median PFNA serum level in the subpopulation included in the analysis of PFC and cholesterol outcomes (20-80 year olds and not taking cholesterol-lowering drugs) was 1 ng/ml, the mean was 1.3 ng/L, and the range was 0.1-10.3 ng/ml. In the subjects included in the analysis for these parameters (n=416 or 860, depending on parameter), study subjects in the highest PFNA quartile had TC levels 13.9 mg/dL (95% CI, 1.9-25.9) higher than those in the lowest quartile, with an increasing linear trend across the quartiles (P for Trend 0.04). No meaningful associations were observed between PFNA and HDL. Results for non-HDL were similar to those for TC with the magnitude of effect slightly increased, and a relatively similar pattern was observed for LDL. PFNA was not associated with BMI, WC, or HOMA.

Although the serum levels of PFNA in Nelson et al. (2010) were lower than for the other PFCs (medians for PFOA, 3.9 ng/ml, PFOS, 21.0 ng/ml, and PFHxS, 1.8 ng/ml), the associations for PFNA were stronger and more consistent than for the other PFCs studied. 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). Increased cholesterol has also been associated with serum PFOA in numerous studies of the general population, communities with drinking water exposure, and workers (reviewed by Post et al., 2012).

The associations with TC, HDL, and LDL reported by Nelson et al. (2010) were not found in cross-sectional analysis of 891 pregnant Norwegian women (Starling et al., 2014). Additionally, no association was found with triglycerides and PFNA. However, the PFNA levels in the Norwegian population were lower than in 2003-04 NHANES; the median in the Norwegian

population was 0.39 ng/ml and the 95th percentile value was 0.81 ng/ml, which was below the median in the NHANES population of 1 ng/ml.

In summary, two human studies evaluated associations of PFNA n serum and serum lipids. One study found significant associations with total cholesterol, non-HDL, and LDL. The other study, which studies a population with lower PFNA serum levels, did not find associations with serum lipids.

Metabolic Effects & Diabetes

The associations of PFCs (PFNA, PFOA, PFOS, PFHxS) with glucose homeostasis and metabolic syndrome/metabolic syndrome components (WC, glucose, HDL, and triglycerides) in 474 adolescents (age 12-20 years) and 969 adults in the 1999-2000 and 2003-04 NHANES surveys was evaluated by Lin et al. (2009). The mean PFNA serum levels in the adolescents and adults were 0.70 ng/ml and 0.81 ng/ml, respectively, and were lower than for the other PFCs studied. Associations of PFNA with a number of parameters were found in adolescents, but not in adults. Of the PFCs studied, only PFNA was significantly associated with increased risk of clinically defined hyperglycemia (OR 3.16, CI 1.39-7.16). PFNA, but not other PFCs, was also significantly associated with lower prevalence of the metabolic syndrome and HDL below the clinically defined criterion. Additionally, only PFNA was associated with decreased blood insulin levels and decreased pancreatic beta-cell function, both with borderline statistical significance.

It should be noted that the other lipid parameters (TC, non-HDL cholesterol, and LDL cholesterol) evaluated by Nelson et al. (2010) were not evaluated by Lin et al. (2009). Additionally, the analysis of parameters including HDL cholesterol, glucose, and triglycerides was based on number of subjects with levels defined as indicative of metabolic syndrome, rather than the magnitude of change in the parameter that was associated with PFNA.

In a second study from this research group, the association of serum PFOA, PFOS, PFUA, and PFNA with parameters related to glucose metabolism was evaluated in 287 Taiwanese adolescents and young adults (age 12-30 years) (Lin et al., 2011). The median PFNA serum level was 1.68 ng/L, lower than for the other PFCs. Log transformed mean adiponectin, a hormone that modulates glucose regulation and other metabolic processes, was significantly increased across increasing percentile categories of PFNA (P for trend=0.010). This association remained after adjustment for age, gender, lifestyle, and physiological parameters. Other PFCs were not associated with adiponectin. PFNA was also associated with decreased insulin and HOMA-IR (homeostatic model assessment used to quantify insulin resistance based on insulin and glucose levels) when adjusted for age, gender, and lifestyle factors, but not when physiological parameters were also considered. PFNA concentration was not associated with levels of glucose, HDL, triglyceride, or CRP (serum high sensitivity (hs)-C-reactive protein). Lin et al. (2011) hypothesize that the increased adiponectin may be related to activation of the nuclear receptor peroxisome proliferator activated receptor-gamma which is strongly activated by PFNA, but not by PFOA or PFOS (discussed further in Mode of Action section, below).

Lind et al. (2014) studied associations of seven PFCs (PFOA, PFNA, perfluoroheptanoic acid (C7), perfluoroundecanoic acid (C11), PFOS, PFHxS, and perfluorooctane sulfonamide) with diabetes in 1016 elderly Swedish adults aged 70 years or older, of whom 114 had diabetes. The

median, 25th, and 75th percentile PFNA serum levels were 0.7, 0.5, and 1.0 ng/ml, respectively. As in other studies discussed above, serum levels of PFOA, PFOS, and PFHxS (medians of 3.3, 13.2, and 2.1 ng/ml, respectively) were higher than for PFNA. A significant non-linear relationship between serum concentration of PFNA and diabetes was found for PFNA, but not for the other PFCs. This association remained after adjustment for multiple risk factors for diabetes. The effect of PFNA was primarily seen at the higher serum levels (above 1 ng/ml).

Halldorsson et al. (2012) studied associations of prenatal PFC exposure (assessed by PFCs in maternal serum samples from gestational week 30) with risk of overweight/obesity (n=345) and biomarkers of adiposity (leptin, adiponectin, and insulin; n=252) in offspring at 20 years of age. The analysis focused on PFOA, with serum levels of three other PFCs presented for each quartile of serum PFOA. Maternal serum PFOA (median, 3.7 ng/L; range, 0.1-19.8 ng/ml) was significantly associated with BMI waist circumference, and the clinical parameters associated with adiposity among female offspring. PFNA serum levels were much lower and in a narrower range than for PFOA, with mean of 0.3 ng/ml and interquartile range of 0.2 ng/ml. These levels are also lower than the PFNA serum levels in the three other studies of metabolic parameters discussed above. PFNA was significantly associated with BMI in female offspring at 20 years in univariate analysis. However, PFNA was correlated with PFOA and was not associated with increased BMI after adjustment for PFOA.

In summary, three studies found associations of PFNA with diabetes and/or related clinical parameters. A fourth study which evaluated associations of prenatal exposure to PFNA with obesity and associated clinical parameters in 20 year old female offspring did not find the association with increased adiponectin that was reported in one of the other studies. However, this negative study differed from the other studies in several ways, including the fact that it evaluated effects of prenatal exposure on outcomes at age 20 years and because the range of PFNA serum levels was lower than in the other studies.

Immune System

In a study of Norwegian children (n=56), Granum et al. (2013) found that maternal serum levels (collected at time of delivery) of PFNA, PFOA, PFOS, and PFHxS were associated with decreased anti-vaccine antibody levels for the rubella vaccine at age 3 years. The mean, median, and range of serum PFNA in 99 subjects were 0.3 ng/ml, 0.3 ng/ml, <0.05–0.9 ng/ml, respectively. From these subjects, a subgroup of 52 with vaccine response data at age 3 years was evaluated. The association was stronger for PFNA than for the other three PFCs, for which median serum levels were PFOA – 1.1 ng/ml, PFOS – 5.6 ng/ml, and PFHxS – 0.3 ng/ml. No association between PFCs and response to three other vaccines was found. The number of episodes of the common cold in these children was associated with maternal PFNA and PFOA, while the number of episodes of gastroenteritis was associated with PFOA and PFHxS. No significant associations were found between concentrations of PFCs and reported eczema and itchiness, wheeze, otitis media, and doctor-diagnosed atopic eczema or asthma. 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.

Granum et al. (2013) discuss that other studies have found that PFCs in maternal blood and breast milk are highly correlated. Thus, effects due to post-natal exposure through breast milk cannot be excluded. Finally, the authors discuss the possibility of chance findings of statistically

significant associations because of the multiple comparisons made in a small study population, but note that their serological and clinical findings are consistent and are supported by both other human studies and animal data.

Associations of serum levels of PFNA and eight other PFCs with asthma and immunological markers were evaluated in a case-control study of 231 asthmatic and 225 non-asthmatic Taiwanese children (Dong et al., 2013). Mean, median, and ranges for PFNA in serum in non-asthmatic children were 0.9 ng/ml, 0.8 ng/ml, and 0.26-2.5 ng/ml, respectively, and were 1.1 ng/ml, 1.0 ng/ml, and 0.28-3.6 ng/ml, respectively, in asthmatic children. Associations with asthma were found for PFNA and for all exceprt two of the other PFCs (pefluorohexanoic acid (C6) and perfluorotetradecanoic acid (C13)). There was no association of PFNA and most other PFCs with immunological biomarkers (absolute eosinophil count, immunoglobulin E (IgE), and eosinophilic cationic protein) among non-asthmatic children, but PFNA was strongly associated with increases in these parameters in asthmatic children (p for trend by quartile: <0.001-0.003). Several other PFCs were also associated with one or more of these biomarkers in asthmatic children, and the authors note that associations with individual PFCs may be biased due to correlations with other PFCs.

Wang et al. (2011) evaluated the effects of prenatal exposure to PFNA, PFOA, PFOA, and PFHxS (assessed by umbilical cord blood serum concentration) on atopic dermatitis and immunoglobulin E (IgE) levels in cord blood and at age 2 years in 244 Taiwanese children of whom 43 (17.6%) had developed atopic dermatitis. The median and range for cord blood serum PFNA were 2.30 ng/ml and 0.38 – 63.87 ng/ml, and the medians for the other PFCs were PFOA, 1.71 ng/ml; PFOS, 5.50 ng/ml; and PFHxS, 0.035 ng/ml. Cord blood PFOA and PFOS, but not PFNA or PFHxS, were associated with cord blood IgE levels in boys. No statistically significant associations between serum PFNA or other PFCs and IgE at age 2 years or atopic dermatitis were found.

Thyroid

The association between serum PFCs (PFOA, PFOS, and PFNA) and thyroid function in 10,725 children (age 1-17 years) from Ohio and West Virginia communities with exposure to PFOA from contaminated drinking water was evaluated by Lopez-Espinosa et al. (2012). Serum levels of PFOA were elevated in these subjects compared to the general population, with a median of 29.3 ng/ml. Medians for PFOS and PFNA were 20.0 and 1.5 ng/ml, respectively, and were similar to levels found in the general population. Serum levels of PFNA were negligibly correlated with PFOA (r=0.09) and moderately correlated with PFOS serum levels (r=0.41). PFNA and PFOS, but not PFOA, were both associated with a small increase in total thyroxine (TT4). The authors concluded that PFNA had a stronger effect, as the percentage increase in total thyroxine was the same with a shift from 1.2 to 2.0 ng/ml PFNA or 15 to 28 ng/L PFOS. Serum PFCs were not associated with thyroid stimulating hormone (TSH) levels or the incidence of thyroid disease in this study, and triiodothyronine (T3) and free thyroxine (FT4) were not measured.

PFNA and thyroid function (measured as TSH and free thyroxine, FT4) was also studied in 551 adolescents and young adults (age 12-30 years) in Taiwan (Lin et al., 2013). The group consisted of 221 participants with elevated blood pressure during childhood and 310 participants with normal BP during childhood, and were a subset of a larger cohort that had abnormal

urinalysis findings in childhood. Of the 551 subjects, 41 were hypertensive. Geometric mean serum levels for the PFCs that were evaluated were PFNA (1.01 ng/ml), PFOA (2.67 ng/ml), PFOS (7.78 ng/ml) and C11 (5.81 ng/ml). A statistically significant increase in FT4, described as small and subclinical, was associated with serum PFNA across exposure categories (P for trend =0.012) and when considered as a continuous exposure metric. No associations of FT4 were found for the other three PFCs. TSH was not associated with PFNA or the other PFCs.

No association of PFNA with thyroid parameters was found in an analysis of 672 men and 508 women (>20 years) from combined 2007-08 and 2009-10 NHANES (Wen et al., 2013). Thyroid parameters evaluated were TT4, FT4, T3, TSH, and thyroglobulin. In this study the PFCs evaluated and corresponding geometric mean serum levels were PFNA (1.54 ng/ml), PFOA (4.15 ng/ml), PFOS (14.2 ng/ml), and PFHxS (2.00 ng/ml). After weighting for sampling design, no associations were found for PFNA. Small associations described as subclinical were found for PFOA and increased T3 in women, and for PFHxS with increased TT4 and T3 and decreased FT4 in men.

No statistically significant associations were found between serum levels of six PFCs including PFNA and TSH or FT4 in a small study of 31 New York anglers (Bloom et al., 2010). PFNA was detected in serum from 84% of subjects with a geometric mean and range of 0.79 ng/ml and 0.35-2.08 ng/ml.

The effects of PFCs in blood serum on TSH levels were studied in 903 pregnant Norwegian women (Wang et al., 2013). Blood samples were taken between week 17 and 18 of gestation and analyzed for thirteen PFCs and TSH. Only the seven PFCs detected in more than 69% of the samples were evaluated for relationship with TSH. The median, interquartile range, and maximum PFNA levels were 0.39 ng/ml, 0.28-0.51 ng/ml, and 3.01 ng/ml, respectively. PFOS levels in the serum (media 12.81 ng/ml; maximum 104.18 ng/ml) were much higher than for the other PFCs evaluated and PFOS was the only PFC that was associated with a small increase in TSH.

A small study evaluated associations between eight PFCs in maternal serum during pregnancy and umbilical cord blood serum with fetal (umbilical cord blood) thyroid hormones (TSH, T3, and T4) in 34 Korean mother:infant pairs (Kim et al., 2011). Median and interquartile ranges for PFNA were 0.44 ng/ml (0.23-0.62 ng/ml) in maternal serum and 0.45 ng/ml (0.23-0.66 ng/ml) in cord blood serum. No associations were found between fetal thyroid hormones and maternal or cord blood concentrations for PFNA. Only a few statistically associations for other PFCs were noted, including for TSH and PFOA in maternal serum. The only associations that were significant after adjusting for major covariates were decreased T3 and maternal PFOS and decreased T3 and T4 with maternal perfluorotridecanoic acid (C13).

In summary, small increases in free or total thyroxine were associated with PFNA in two studies of children and adolescents/young adults. Three studies did not find associations of PFNA with thyroid-related parameters in adults, and no associations with fetal thyroid hormones were found in a study of PFNA in maternal serum and umbilical cord blood serum PFNA.

Kidney Function

Serum PFCs (PFNA, PFOA, PFOS, and PFHxS) were associated with estimated glomerular filtration rate (eGFR), a measure of kidney function, in 9,660 children ≥ 1 to <18 years of age from the C8 Health Project conducted in Ohio and West Virginia communities with drinking water contaminated by PFOA (Watkins et al., 2013). The participants had elevated levels of serum PFOA due to drinking water exposure, while serum levels of the other PFCs were similar to those found in the general population. Median serum levels were: PFNA-1.5 ng/ml; PFOA-28.3 ng/ml; PFOS-20.0 ng/ml; PFHxS-5.2 ng/ml. Decreased eGFR was significantly associated with serum levels of all four PFCs, but was not associated with predicted PFOA serum concentrations based on estimated historical exposure modeling. Based on these results, the authors concluded that there is a possibility that the increases in serum PFOA may, at least in part, be a consequence and not a cause of decreased kidney function. Furthermore, although it was not possible to predict serum concentrations for the other PFCs, including PFNA, from historical exposure modeling, the authors conclude that the associations of these PFCs with decreased kidney function may also, at least in part, be a result of reverse causality.

Birth Outcomes

Chen et al. (2012) investigated associations between cord blood plasma levels of PFNA and other PFCs with birth outcomes (gestational age, birth weight, birth length, head circumference, ponderal index, preterm birth, low birth weight, and small for gestational age) in 429 Taiwanese infants. The geometric mean PFNA level in the cord blood plasma was 2.36 ng/ml, higher than in most of the other general population studies that were reviewed. After adjustment for covariates in linear regression, PFNA was positively associated with birth length and negatively associated with ponderal index, and PFOS was negatively associated with gestational age, birth weight, and head circumference. Additionally, the odds ratio of preterm birth, low birth weight, and small for gestational age increased with PFOS (geometric mean 5.94 ng/ml) exposure while no association of birth weight with PFNA, PFOA, or PFUA was found. While an adverse dose-dependent relationship with PFOS and birth outcomes was observed, convincing evidence of associations with other PFCs was not observed.

A study of 101 Canadian mother:infant pairs evaluated exposure to PFCs and associations with birth weight. PFNA and other PFCs were analyzed in maternal serum and umbilical cord blood. PFNA was detected in almost all of the maternal serum samples, both during the second trimester and at delivery, but in only 26% of the umbilical cord blood samples. Serum and cord blood PFNA was lower than for the other PFCs, with median values of 0.73 ng/ml in the second trimester, 0.69 ng/ml at delivery, and 0.72 ng/ml in cord blood. No association was found between PFNA, PFOA, PFOS, or PFHxS in maternal serum at delivery and birth weight (Monroy et al., 2008).

It is relevant to note that a recent systematic review was conducted of the numerous studies evaluating associations of serum PFOA with fetal growth, as indicated by birth weight, and other parameters (Woodruff, 2013). While some studies found associations and others did not, Woodruff (2013) concluded that the overall body of data from human studies indicates that decreased fetal growth is associated with PFOA exposure.

Reproductive

Prenatal exposure to PFCs, including PFNA, was not associated with daughters' age at menarche in a study of 448 girls born in 1991-1992 in Avon, UK (Christensen et al., 2011). Of the 448 girls, 218 who reported menarche before age 11.5 years were cases and 230 who reported menarche at 11.5 years of age or later were controls. Maternal serum samples taken during pregnancy were analyzed for 8 PFCs including PFNA. PFNA was detected in 99.8% of samples at >0.1 ng/ml with a median of 0.6 ng/ml and an interquartile range of 0.5-0.8 ng/ml. No associations between early menarche (defined as <11.5 years of age) and maternal serum levels of any individual PFC, including PFNA, or total PFCs were observed.

Serum levels of PFNA, as well as other PFCs (PFOA, PFOS, and PFHxS) were associated with earlier age at menopause in a study of 2732 women from NHANES 1999-2000, 2003-04, 2005-06, 2007-08, and 2009-10 (Taylor et al., 2013). Of the 2732 participants, 1800 were pre-menopausal, 501 had experienced menopause, and 431 had experienced hysterectomy. As in other studies based on NHANES, serum levels of PFNA were lower than for the other three PFCs evaluated. PFC serum levels were lower in pre-menopausal women than in those who had experienced menopause or hysterectomy. For PFNA, medians were 0.9 ng/ml in pre-menopausal women; 1.2 ng/ml in post-menopausal women; and 1.3 ng/ml in those with hysterectomy. After adjusting for relevant confounding factors, higher levels of PFNA and the other PFCs were associated with earlier age at menopause. Associations were strongest between serum levels of PFNA and PFHxS and the rate of menopause. Positive dose-response associations for all four PFCs and hysterectomy were also observed. Correlations among the four PFCs made it difficult to assess the effects of the individual compounds. Because higher PFCs were associated with both natural menopause and hysterectomy, the authors conclude that the accumulation of PFCs may be due to the absence of menstruation as an excretion pathway (reverse causality).

The effects of PFCs on semen quality were studied in 105 young Danish men 18-25 years of age (Joensen et al., 2009). Eight serum PFCs including PFNA were measured in serum samples. Of these eight PFCs, analysis of associations with semen quality parameters was conducted only for PFOA, PFOS, and PFHxS. The high combined PFOA-PFOS category was associated with reduced numbers of normal sperm, and non-significant effects on other semen parameters and reproductive hormones. However, analysis of associations of these parameters with PFNA (median, 0.8 ng/ml) and the other PFCs present at >1 ng/ml was not conducted.

In summary, three studies evaluated different reproductive endpoints and PFNA. A study on age of menarche found no association with PFNA, a study of age at menopause found associations which may result from reverse causality, and a study of semen quality and PFCs analyzed PFNA in serum but did not evaluate associations of PFNA with semen quality endpoints.

Neurobehavioral

Associations of serum PFCs (PFNA, PFOA, PFOS, and PFHxS) and attention deficit/hyperactivity disorder (ADHD) were evaluated in 571 children 12-15 years of age who participated in NHANES in 1999-2000 or 2003-2004 (Hoffman et al., 2010). Of the 571 subjects, 48 had ADHD based on parental report of medical diagnosis. Serum levels of PFNA were much lower than for the other PFCs, with a median of 0.6 ng/ml and a range of <0.1-5.9 ng/ml, as compared to medians for PFOA, PFOS, and PFHxS of 4.4 ng/ml, 22.6 ng/ml, and 2.2 ng/ml respectively. All four PFCs were associated with increased risk of ADHD, but this increase was not significant for PFNA. The odds ratio and 95% confidence interval of ADHD

for a 1 ng/ml increase in serum PFNA were 1.32 (0.86-2.02), and the odds ratio and confidence interval based on an increase in serum equal to the interquartile range was 1.15 (0.93-1.42)

The association of serum PFCs with impaired response inhibition was evaluated in 83 children, 9-11 years of age, from Oswego County, NY (Gump et al., 2011). Of eleven PFCs measured in their serum, the six PFCs, including PFNA, that were detected in at least 87.5% of the serum samples were included in the analysis. The mean, median, and range for serum PFNA were 0.82 ng/ml, 0.72 ng/ml, and 0.10-4.14 ng/ml. Performance was assessed through a 20 minute differential reinforcement of low rates of responding (DRL) task which requires children to learn that they need to wait for 20 seconds before responding in order to earn a reward. Results are evaluated by inter-response times (IRTs), with longer delays in response (longer IRTs) indicating better performance. PFNA, as well as PFOS, PFHxS, PFOSA, and perfluorodecanoic acid (PFNA, C10), but not PFOA, were all associated with significantly poorer performance on the task. Poor performance on this task is considered to be a measure of greater impulsivity, a defining feature of ADHD. It was not possible to determine whether the effects were due to a particular PFC or PFCs in general because the PFCs were correlated with each other.

Inverse associations between serum PFCs (PFNA, PFOA, PFOS, PFHxS) and memory impairment were observed in 21,024 adults $\leq$50 years of age from the C8 Health Project conducted Ohio and West Virginia communities with drinking water contaminated by PFOA (Gallo et al., 2012). Of the 21,024 subjects, 4462 (21.2%) reported short term memory loss. In the study population, serum PFNA was lower than serum levels of the other PFCs, with third quintile (median) range of 1.3-1.4 ng/ml, compared to 20.5-27.1 ng/ml for PFOS, 27.1-53.8 ng/ml for PFOA, and 2.7-3.6 ng/ml for PFHxS. For all PFCs, associations of a similar magnitude (odds ratios: 0.93 to 0.97) for a doubling of PFC serum concentration with decreased risk of memory impairment were found. These associations were highly significant for PFOA and PFOS, but of borderline statistical significance for the PFNA (OR, 0.96; confidence interval, 0.92-1.02) and PFHxS. The authors hypothesized that PFCs may prevent memory impairment through anti-inflammatory effects mediated through PPAR activation, but noted that confounding or reverse causality could also account for these findings.

In summary, PFNA in children was associated with a non-significant increase in ADHD, and a significant decrease in performance of a task assessing response inhibition. PFNA was also associated with a decreased risk of memory impairment in older adults, with borderline statistical significance.

Although neurobehavioral effects of PFNA have not been studied in animals, it is relevant to note that gestational or neonatal exposure to the closely related PFC, PFOA, causes persistent neurobehavioral effects, particularly increased activity, in mice (Johansson et al., 2008; Onischenko et al., 2010). Similar effects also were observed in neonatal mice exposed to PFOS (Johannson et al., 2008) and PFHxS (Viberg et al., 2013)

Occupational Exposure Study (Mundt et al., 2007)

Mundt et al. (2007) evaluated clinical chemistry parameters in workers ($\geq$85% men at each time point studied) at a U.S. facility where Surflon S-111 was used in polymer production. A total of 630 active and former employees from 1989-2003 were eligible for inclusion in the study. After

exclusions for insufficient records, negligible period of employment (< 1 month), and missing information on gender the analysis included 592 individuals (518 men and 74 women).

Workers were assigned to no exposure, low exposure, and high exposure groups based on job description. It is stated that, "Before the start of the study, the company had obtained blood samples from a subset of current employees who worked in various areas of the plant, to ascertain whether PFNA levels could be detected in the blood. These limited biomonitoring results were used to validate the exposure categories generated based on the occupational history, but were insufficient to be used in any analyses." However, data on PFC levels in serum and results of the exposure validation effort are not presented.

Three types of analyses were conducted including a cross-sectional analysis to evaluate differences in average values of 32 clinical parameters at the five time points (1976, 1989, 1995, 1998, and 2001), additional annual cross-sectional analyses of mean laboratory values by exposure groups, and longitudinal analysis accounting for multiple measurements in the same individual. Disease incidence was not evaluated in this study.

Pairwise comparisons (high exposure v. low exposure, low v. none, high v. none) of adjusted means of each of the 32 clinical parameters were made across exposure groups. In this analysis, the number of subjects classified as having high, low, or no exposure varied at each time point. Few statistically significant differences between exposure groups were observed and those that were, were not consistent across time periods or between men and women. Adjusted means for liver and blood enzymes are presented, but data for other parameters including electrolytes, BUN, creatinine, thyroid hormones, and uric acid are not shown.

Of the 518 men included in the overall study, a smaller number (n=163-323) was evaluated at each of the five time points in the main cross-sectional analysis. At each time point, most of the subjects (64%-80%) were classified as having low exposure, with fewer classified as highly exposed (9-11%) or not exposed (11-28%). Significant differences were reported in 1976 for high exposed compared to low exposure men and in 2001 for high exposed men compared to non-exposed men for the liver enzyme ALT, in 1998 for high exposed men compared to non-exposed men for alkaline phosphatase, and in 1976 and 1989 for differences in all exposure categories for total cholesterol. Other non-significant results in men include increased alkaline phosphatase with exposure category at all time points (except 1976, for which the data are in a different range (26.1-29.1 IU/L) than for the other time points (72.1-101.5 IU/L)), highest total cholesterol and LDL cholesterol in the high exposure category at all time points, and highest triglycerides in the high exposure category at all time points except 2001.

The number of women evaluated at each time point ranged from 35 to 52, and women were classified only as exposed or not exposed. The percentage of women classified as exposed at the four time points at which women were assessed ranged from 29-42%. No significant findings were reported in women.

It is not possible to evaluate the data for the extended cross-sectional analysis because these data are not presented. It is stated that in this analysis, adjusted annual means for liver enzymes and blood lipids were graphed separately for men and women for all years (1976-2003) for which data were available. It is stated that laboratory results were not plotted for a given year if data

for less than five individuals were available for that parameter from that year, but the numbers of subjects included in these analyses is not provided. 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. Dips and peaks in the data for some parameters were attributed to changes in the laboratories used to analyze the blood samples.

In the longitudinal study, factors considered included annual measures of liver enzymes and blood lipids, age at entry into the cohort, annual measures of BMI, exposure (none or any) in the month before blood sample was taken, and a weighted cumulative intensity score up to the month before blood was taken. The cumulative intensity score was based on proportion of the year spent in each exposure category. Additionally an indicator variable of powdered versus liquid surfactant containing PFCs (with exposure from the liquid form assumed to be lower than from the powdered form) was investigated.

Results for men are presented as the change in the clinical parameter with a 1 unit change in cumulative exposure intensity score, and change in the clinical parameter associated with current exposure by operating condition (powder and non-powder). It is stated that no significant increase or decrease was observed based on unit increase in exposure intensity for the seven parameters analyzed (total cholesterol, GGT, AST, ALT, alkaline phosphatase, bilirubin, and triglycerides), while the effect of powder versus liquid form of the surfactant for some clinical laboratory values was the opposite of what was hypothesized. Results are presented only for cholesterol and triglycerides. The analysis was not conducted for women because there were too few data.

Limitations of this study include the fact that data are not presented for some of the findings that are discussed, the small percentage of subjects in high and no exposure groups compared to low exposure groups, limited data for women, and lack of serum PFC data.

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), and 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 PFCs with some clinical parameters, including cholesterol, appear to exhibit a steep dose-response curve in the lower exposure range found in the general population and communities with drinking water exposure, with a plateau at higher exposures, such as those found with occupational exposure. 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.

Studies of Communities with Drinking Water Exposure to PFOA

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, including two types of cancer (Barry et al., 2013; Vieira et al., 2013), as well as other effects that have not been evaluated in humans for PFNA. 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/)

Animal Toxicology

Acute Exposure Studies

No studies which determined the acute oral LD_{50} of pure PFNA were located. However, Mertens et al. (2010) state that the acute LD_{50} (unpublished data, calculated as 65 mg/kg) for the Surflon S-111 mixture of PFCs consisting primarily of PFNA (see below) was 2.9-fold lower than the acute LD_{50} 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 0, 67, 590, 610, 910, 1600, or 4600 mg/m^3 ammonium perfluorononanoate (the ammonium salt of PFNA) as a dust was 820 mg/m^3, and the lowest dose that caused death was 590 mg/m^3. Animals were observed for 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 Post et al., 2012; Lau et al., 2007), severe body weight loss occurred in surviving rats of all but the lowest dose group.

Liver enlargement is a well-established effect of PFCs, including PFNA (Lau, 2012). In another part of the Kinney et al. (1989) study, male rats (10 per group) were exposed to 67 or 590 mg/m^3 PFNA for four hours, sacrificed at 5 or 12 days post exposure, and assessed for relative liver weight and gross liver appearance. The ratio of liver weight to body weight was increased at both dose levels and both time points, and gross lesions were observed in livers from some rats in the high dose group at both time points.

Rockwell et al. (2013) studied immune system effects in male (n=5) and female (n=4) C57Bl/6 mice 14 days after a single high dose of 0.1 mM/kg (46.4 mg/kg) PFNA. 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. 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. Although this study indicates the potential for PFNA to cause immune toxicity, the dose used was high enough to caused overt toxicity, as demonstrated by the severe weight loss seen in treated animals.

Short Term Repeated Dose Studies (21 days or less)

21-day mouse study (Kennedy, 1987)

Male and female mice (5 per gender per dose group) were fed diets containing 0, 3, 10, 30, 300, or 3000 ppm PFNA for 21 days. Mice given higher doses (300 or 3000 ppm) in their diets died during the 21 day study, and weight loss and weakness were observed in the 30 ppm group. Liver weights (absolute and relative to body weight) were increased in all doses in a dose-related fashion in male and female mice given 3, 10, and 30 ppm (Kennedy, 1987). The increased liver weight was similar in males and females given the same dose. 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.

Based on assumed food consumption of 1.5 g/10 g body weight/ day (University of Wisconsin, 2014), the doses are estimated as 0.45 mg/kg/day at 3 ppm, 1.5 mg/kg/day at 10 ppm, and 4.5 mg/kg/day at 30 ppm. Thus, the LOAEL in this study was 3 ppm (estimated as 0.45 mg/kg/day) and no NOAEL was identified.

Serum levels of PFNA were not measured in this study.

Carbohydrate and lipid metabolism in 14-day mouse study (Fang et al., 2012a)

Fang et al. (2012a) studied the effects of PFNA on carbohydrate metabolism in male rats (6 per group) given 0, 0.2, 1, or 5 mg/kg/day PFNA for 14 days.

PFNA caused a dose-related increase in serum glucose that was seen at all doses and was significant at 1 and 5 mg/kg/day, as well as a dose-related decrease in both serum HDL (significant at all doses $\geq$0.2 mg/kg/day) and a decrease in the HDL/LDL ratio (significant at 1 and 5 mg/kg/day). The authors note that a decreased 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 (Mertens et al., 2008; see below) in which the other parameters mentioned above were not assessed.

Investigation of the potential mode of action for these metabolic effects showed that at all doses of PFNA (0.2 mg/kg/day and above), there were significantly reduced hepatic levels of four proteins that are part of the insulin signaling pathway (Fang et al., 2012a). 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 can be explained by the inhibition of the insulin signaling pathway by PFNA.

Serum PFNA levels were not measured in this study.

This study found potentially important effects on carbohydrate metabolism at doses of 0.2 mg/kg/day and above, with no NOAEL identified. The findings in this study are of particular interest because associations of PFNA with glucose levels or diabetes have been seen in several human epidemiology studies (discussed in detail below). PFNA has been associated with hyperglycemia in adolescents (Lin et al., 2009) and diabetes in the elderly (Lind et al., 2013) (discussed below).

Immune system effects in 14- day mouse study (Fang et al., 2008)
PFCs other than PFNA, including PFOA and PFOS, cause toxicity to the immune system in animals (reviewed by DeWitt et al., 2009, 2012; Lau, 2012), and PFCs have been associated with decreased vaccine response and other immune system related effects in humans (Dong et al., 2013; Granum et al., 2013, discussed above).

Fang et al. (2008) evaluated immune system effects in male Balb/C mice (6 per group) given 0, 1, 3, or 5 mg/kg/day PFNA by gavage for 14 days. In the 3 and 5 mg/kg/day dose groups, body weight loss occurred over the 14 day period, while body weight was not affected at 1 mg/kg/day.

Absolute and relative thymus weights were decreased at 3 and 5 mg/kg/day. The percentages of T-cell subsets and percentages of thymocytes in different phases of the cell cycle were also altered at these doses, suggesting effects on thymocyte maturation and differentiation, and impaired cell cycle progression. Apoptosis was increased at 5 mg/kg/day.

Absolute spleen weight was decreased at 3 and 5 mg/kg/day, and relative spleen weight at 5 mg/kg/day. Percentages of several types of immune cells innate to the spleen were decreased at all doses (1 mg/kg/day and above), and the authors concluded that PFNA treatment caused damage to these cells. Proportions of splenocytes in different phases of the cell cycle were affected at all doses (1 mg/kg/day and above), and apoptosis was increased at 5 mg/kg/day. Interleukin-4 was reduced in the spleen at all doses, and interleukin-gamma was reduced at the highest dose.

Adrenocorticotrophic hormone (ACTH) in the serum was increased at 5 mg/kg/day, and cortisol at 3 and 5 mg/kg/day, suggesting that the hypothalamic-pituitary axis may be involved in PFNA's effects on the immune system.

This study shows potentially important effects on the immune system in male mice at doses as low as 1 mg/kg/day, with no NOAEL identified. These effects are notable because PFNA has been associated with immune system effects in studies of the human general population.

Serum levels were not measured in this study.

Rockwell et al. (2013) also reported immune system toxicity in mice given a single high dose of PFNA that also caused severe weight loss (discussed above). The longer duration rat studies (Mertens et al., 2010; Stump et al., 2008, see below) did not report effects on thymus or spleen weight. The other parameters measured by Fang et al. (2012) were not evaluated in theses longer duration studies.

A second study by this research group (Fang et al., 2010) suggests that the PFNA-induced apoptosis in the spleen may involve induction of oxidative stress and activation of a cell death-signaling pathway involving the mitochondria.

Male reproductive system in 14 day rat study (Feng et al., 2009)
Feng et al. (2009) studied effects of PFNA on the testis of male rats. Serum hormone levels were measured in rats (6 per group) dosed with 0, 1, 3, or 5 mg/kg/day PFNA for 14 days. Estradiol

was increased by 104% at 5 mg/kg/day, while testosterone was increased by 88% at 1 mg/kg/day and decreased by 85% at 5 mg/kg/day. Histological studies showed changes in the seminiferous tubule of the testes only in the 5 mg/kg/day group. A specific stain for DNA fragmentation indicative of germ cell death showed damage to spermatocytes and spermatagonia in the 3 and 5 mg/kg/day groups. Other parts of this study investigated potential mechanisms of testicular toxicity by PFNA, and indicated that PFNA causes apoptosis mediated by the death receptor pathway in the testes of male rats.

An additional study by this research group (Feng et al., 2010) found that PFNA caused to testicular Sertoli cells in rats (6 per group) given 0, 1, 3, and 5 mg/kg/day PFNA for 14 days. Among other *in vivo* and *in vitro* effects related to Sertoli cell function, serum levels of inhibin B, a glycoprotein produced in Sertoli cells that is important in regulating the production of FSH which modulates testicular function, was significantly reduced by 1, 3, or 5 mg/kg/day PFNA. Inhibin B is considered to be a biomarker of testicular toxicity.

These studies showed potentially important effects on the male reproductive system, with some effects seen at the lowest dose, 1 mg/kg/day, after two weeks of dosing. Possible effects on the male reproductive system were also suggested by the findings of Mertens et al. (2008) discussed above.

Serum PFNA levels were not measured by Feng et al. (2009) or Feng et al. (2010).

Longer term Studies

No longer term studies (i.e. >21 days of dosing) of pure PFNA were located. The two available studies, an oral subchronic rat study (Mertens et al., 2010) and a 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. 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. 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 presented below.

Subchronic (90 day) oral Surflon S-111 rat study (Mertens et al., 2010)

In the subchronic study (Mertens et al., 2010), groups of 10 rats per gender per dose group were administered Surflon S-111 (0.025, 0.125, or 0.6 mg/kg/day) dissolved in water daily by gavage for 90 days; controls were dosed with water.

Based on the assumed percentages of PFCs in Surflon S-111 given above, the daily doses of PFNA in the low, medium, and high dose groups 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).

The study included functional observational battery and locomotor activity assessments at week 12 and in the recovery animals at week 21; ophthalmic examinations before treatment, near the

end of treatment, and near the end of recovery; hematology, clinical chemistry, and urinalysis evaluations. At necropsy, complete macroscopic examination was performed and 10 organs were weighed. Histopathological examination was performed on all tissues from control and high dose animals, on liver, duodenum, and glandular and non-glandular stomach from all low- and mid-dose males, and all recovery group (control and high dose) males.

In the control and high dose groups, 5 additional animals were kept for 60 additional days following the 90 days of dosing, as a recovery period. Toxicokinetic studies were performed on separate groups of 5 rats per gender per dose. Peroxisome proliferation in the liver was assessed after 10 days of dosing in additional groups of 5 rats per gender per dose level.

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 has been requested from the study sponsors but has not been provided.

In part because of the gender differences in toxicokinetics for some, but not all, of the constituent PFCs, the serum data are complex and a detailed discussion is beyond the scope of this document. A few key points relevant to the interpretation of Mertens et al. (2010) are:

-Consistent with the gender differences in excretion rates in rats discussed above, serum levels of PFNA in males were about 5-fold higher than in females after two weeks of dosing, and this difference appears to have become even greater as dosing continued.

-In contrast, a striking gender difference is not seen for serum levels of C11 and C13, with serum levels of these PFCs in females somewhat higher than in males at many data points. PFNA was the predominant PFC in serum by at least several-fold in males in all dose groups, while serum levels of PFNA and C11 were generally similar in females.

-Serum levels of PFNA, C11, and C13 had not reached steady state at the end of the 90 day dosing period; serum concentrations of these PFCs were still increasing.

-At the end of the 90 day study, serum levels in the mid dose (0.125 mg/kg/day) males were 20,000 ng/ml for PFNA and about 1500 ng/ml for C11. In the high dose (0.6 mg/kg/day) females, serum PFNA levels were similar to those in the mid dose (0.125 mg/kg/day) males (about 20,000 ng/ml), but C11 levels in the high dose (0.6 mg/kg/day) females were about 15,000 ng/ml, about 10-fold higher than in the mid dose (0.125) mg/kg/day males.

The major findings related to toxicological effects in this study are:
Clinical signs: Two of 10 males in the high dose (0.6 mg/kg/day) group exhibited clinical signs, stated to be associated with decreased body weight and food consumption, beginning in week 10 of the study.

Body weight: Statistically significant weight loss or decreased weight gain in high dose (0.6 mg/kg/day) males occurred beginning in weeks 2 to 3, with weight decreased to 24% below controls at day 90. Body weight in the high dose (0.6 mg/kg/day) male recovery group was 12.5% below controls after the 60 day recovery period.

Hematology: A number of hematological parameters were affected in the high dose (0.6 mg/kg/day) males at the end of the 13 week dosing and/or after the recovery period at week 21 of the study. 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) males, serum protein and globulin were increased and bilirubin, BUN, chloride, and alkaline phosphatase were decreased. In the mid dose (0.125 mg/kg/day) males and high dose (0.6 mg/kg/day) females, only increased alkaline phosphatase and decreased globulin were observed. The effects on these clinical chemistry parameters were more pronounced in males than females. There were no effects on a number of other clinical chemistry parameters including albumin, total protein, creatinine, several liver enzymes, glucose, total cholesterol, calcium, phosphorus, potassium, and sodium; the data for these parameters were not provided.

Hepatic Effects: Liver weight (absolute, and relative to body weight and to brain weight) was increased in a dose related fashion, with significant increases in the mid- and high dose males, and the high dose females. The liver weight/body weight ratio was similar in mid dose (0.125 mg/kg/day) males and high dose (0.6 mg/kg/day) females. At the end of the 60 day recovery period, liver weight parameters remained increased in high dose males but not in high dose females,.

Hepatic beta-oxidation, a marker of peroxisome proliferation, was significantly increased at 90 days in mid dose and high dose males, and in high dose females. The magnitude of the increase was similar in mid dose (0.125 mg/kg/day) males (2-fold) and high dose (0.6 mg/kg/day) females (1.5–fold). Beta-oxidation remained elevated in high dose (0.6 mg/kg/day) males at the end of the 60 day recovery period.

These data show comparable hepatic effects in high dose (0.6 mg/kg/day) females and mid dose (0.125 mg/kg/day) males, but no effects in mid dose (0.125 mg/kg/day) females. As discussed above, serum levels of PFNA were similar (about 15,000 ng/ml) in mid dose males and high dose females. However, C11 levels were about 10-fold higher in the high dose females (about 20,000 ng/L) than in the mid dose S-111 males (about 1500 ng/ml). In contrast, hepatic effects did not occur in mid dose (0.125 mg/kg/day) females with serum C11 of about 2000 ng/ml. If C11 were a major contributor to the hepatic effects, 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 the increased liver weight and beta-oxidation are primarily due to PFNA, not C11 (assuming that males and females are equally susceptible to hepatic effects of these PFCs).

Histopathological changes were seen in livers in mid dose (0.125 mg/kg/day) males and high dose (0.6 mg/kg/day) males. Hepatocellular hypertrophy and eosinophilic foci were observed in some or all rats in both of these dose groups, with a higher incidence at the higher dose. Acute inflammation, degeneration, and necrosis occurred in some high dose (0.6 mg/kg/day) males. No histopathological changes were observed in control or low dose (0.025 mg/kg/day) males.

Most of these hepatic effects were also seen in males after the 60 day recovery period, with hypertrophy persisting in all recovery males. Serum data were not presented for this time point. Since 60 days represents two half-lives for PFNA in male rats (see above), it is expected that serum levels would have decreased to about 25% of the serum level when dosing ended at 13 weeks.

In females, histopathological evaluation was performed on only the control and high dose (0.6 mg/kg/day) animals. Data for females were not shown, and it was reported that no histological effects occurred in treated females.

Gastrointestinal effects: Inflammation, ulceration, erosion, and hyperplasia were observed 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.

As above, histopathological evaluation was performed on only the control and high dose (0.6 mg/kg/day) females. Data for females were not shown, and it was reported that no histological effects occurred in treated females.

Additional parameters: There were no treatment-related macroscopic changes and treatment-related histopathological effects were limited to the liver and the gastrointestinal tissues discussed above. Data were not shown for the other organs examined microscopically in the control and high dose groups.

No treatment-related effects were observed in the functional observational battery and locomotor activity assessments, the ophthalmic examinations, or urinalysis. Data were not shown for these parameters.

Summary
The LOAEL in this study is 0.125 mg/kg/day Surflon S-111 (0.09 mg/kg/day PFNA) in males and 0.6 mg/kg/day Surflon S-111 (0.44 mg/kg/day PFNA) in females. Effects at these doses included changes in clinical chemistry parameters, increased liver weight, and increased rate of beta-oxidation in males and females, and histopathological changes in the livers in males. The study authors state that the results of this study indicate that Surflon S-111 is more potent than PFOA.

The NOAELs for males and females are 0.025 mg/kg/day Surflon S-111 (0.019 mg/kg/day PFNA) and 0.125 mg/kg/day (0.09 mg/kg/day PFNA), respectively. The PFNA serum levels at the LOAELS are roughly estimated from the graphs provided as similar in both genders: about 16,000 ng/ml in females and 18,000 ng/ml in males. The serum levels at the NOAELs are also similar based, on very rough estimates from the graphs provided of about 4,000 ng/ml in males and 2,000 ng/ml in females.

Evaluation of the serum levels of PFNA at the NOAELs and LOAELs in males and females suggests that the effects of Surflon S-111 observed in this study are, at least primarily, due to PFNA, rather than C11 or the other PFCs present in even lower concentrations. Effects common to both genders occurred at lower administered doses in males than females, and some effects (liver histopathology) occurred in males but not in females. Serum levels of PFNA in males

were much higher (about 8-fold at week 13) than in females given the same administered dose, while serum levels of C11 were similar, and generally somewhat higher in females, in males and females given the same administered dose.

Furthermore, a recent repeated dose and reproductive/developmental study of C11 administered orally to rats for 42 days (males) and 41-46 days (females) (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 in males and females, based on the occurrence of centrilobular hypertrophy of hepatocytes. As discussed above, the doses of C11 in the 0.025 mg/kg/day, 0.125 mg/kg/day and 0.6 mg/kg/day Surflon S-11 groups in Mertens et al. (2010) are estimated as 0.005, 0.025, and 0.12 mg/kg/day. Although serum levels were not measured by Takahashi et al. (2014) and the duration of exposure was about half of that in Mertens et al. (2010), 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) identified by Takahashi et al. (2014). Mundt et al. (2007) also discuss the greater toxicity of Surflon S-111 in male rats compared to female rats in the unpublished reports of the subchronic study of Mertens et al. (2010) as consistent with the higher serum PFNA levels in male as female rats. Additionally, 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 below).

Two generation oral Surflon S-111 rat study (Stump et al., 2008)

The two-generation rat study of Surflon S-111 (Stump et al., 2008) evaluated developmental effect as well as general toxicology endpoints. As in the subchronic study (Mertens et al., 2010), the doses of Surflon S-111 in this study were 0.025 mg/kg/day, 0.125 mg/kg/day, and 0.6 mg/kg/day. Based on the assumed percentages of PFCs in Surflon S-111 given above, the daily doses of PFNA are estimated as 0.019 mg/kg/day, 0.09 mg/kg/day, and 0.44 mg/kg/day.

Male and female rats (30 per dose group) of the F_0 generation dosed by gavage starting at age 6 weeks, for at least 70 days prior to mating, throughout mating, gestation, and lactation, and up to euthanasia. The total duration of exposure is not stated, but graphical data indicate the dosing period was 18 weeks.

For the F_1 generation (30 per sex per group), animals were also dosed for at least 70 days prior to mating, throughout mating, gestation, and lactation, through euthanasia. The age at which dosing began and the duration of dosing are not explicitly stated. From the data presented, it appears that dosing began at 4 or 6 weeks and continued for 21 weeks. Thus, exposure duration in this study was longer than in the 90 day (13 week) subchronic study discussed above.

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.

Clinical observations/survival: One high dose (0.6 mg/kg/day) F_1 male was euthanized *in extremis* after 14 weeks of dosing.

Body weights: Body weights of both F_0 and F_1 high dose (0.6 mg/kg/day) males were decreased starting at week 7-8 of dosing. This decrease in body weight was not attributed to decreased food consumption.

Reproductive parameters: The fertility index was significantly decreased only in the low dose (0.025 mg/kg/day) F_0 males and females. No effects were seen on other reproductive parameters in F_0 or F_1 animals.

Spermatogenic endpoints: Sperm motility and progressive motility was significantly decreased in high dose (0.6 mg/kg/day) 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 high dose F_0 and F_1 males and significantly decreased left epididymis sperm concentration in high dose F_0 males.

Potentially relevant to these findings, Feng et al. (2009, 2010) found testicular toxicity in male rats given PFNA doses (1, 3, and 5 mg/kg/day) for 14 days. These PFNA doses were higher than those in the two-generation study (estimated as 0.019, 0.09, and 0.44 mg/kg/day)

Hepatic effects (adult):
Liver weights (absolute and relative to body weight) were increased in mid (0.125 mg/kg/day) and high (0.6 mg/kg/day) dose males and high dose (0.6 mg/kg/day) females in both the F_0 and F_1 generations.

In males, histopathological examination of the liver was conducted in all dose groups. In F_0 and F_1 males, hepatocellular hypertrophy occurred at high frequency in all treated groups including the low dose (0.025 mg/kg/day) groups, with dose-related increases in frequency and severity. In the control, low, mid, and high dose groups, the incidence of this effect was 0/30, 21/30, 30/30, and 29/30 in F_0 males and 0/30, 23/30, 29/30, and 30/30 in F_1 males, respectively. Other histological changes seen in all dosed groups of F_0 and F_1 males, with severity and/or incidence increasing with dose, included subacute inflammation, clear cell foci, hepatocellular necrosis, and vacuolation. Of the effects mentioned above, minimal inflammation was seen in only one control F_0 male, with dose-related increase in frequency and severity of this endpoint in the treated groups.

In females, histopathological examination of the liver was performed only in the control and high dose groups. Hepatocellular hypertrophy occurred in 5 of 30 high dose F_0 (0.6 mg/kg/day) females, but was not found in F_1 females.

Kidney Effects (adult):
Kidney weight (absolute and relative to body weight) was increased in the mid (0.15 mg/kg/day) and high (0.6 mg/kg/day) dose groups of F_0 and F_1 males and in the high dose (0.6 mg/kg/day) group of F_0 females.

In males, histopathological examination of the kidneys was conducted in all dose groups. Microscopic studies showed renal tubule cell hypertrophy in 5/30 mid dose (0.125 mg/kg/day) and 28/30 high dose (0.6 mg/kg/day) F_0 males, and 30/ 30 high dose (0.6 mg/kg/day) F_1 males, but not in control or low-dose males. Severity for this effect increased with dose in these F_0

males. Renal inflammation (1 mid dose and 1 low dose), brown pigment (2 high dose), or capsular fibrosis (1 high dose) was seen in a few F_0 males.

In females, histopathological examination of the kidney was performed only in the control and high dose groups. Renal tubule cell hypertrophy was observed in 8 of 30 high dose (0.6 mg/kg/day) F_0 females.

Litter data:
No effects were seen on parameters such as number of pups born, live litter size, postnatal survival, pup weight through weaning in F_1 or F_2 pups. Age at vaginal opening or preputial separation were not affected in F_1 pups and were not assessed in F_2 pups.

Relative liver weights on PND 21 were significantly increased in mid and high dose F_1 males and females, and in high dose F_2 males and females.

Summary
In this study, the LOAEL was 0.025 mg/kg/day Surflon S-111 (0.019 mg/kg/day PFNA), based on histopathological changes in the liver of males at this dose. A NOAEL was not identified. These effects were seen in males dosed with 0.025 mg/kg/day Surflon S-111 (0.019 mg/kg/day PFNA) or higher in both the F_0 and F_1 generations. The incidence and severity of these effects increased with dose in both the F_0 and F_1. It is notable that histopathological changes in the liver, including necrosis, occurred at 0.025 mg/kg/day Surflon S-111 (0.019 mg/kg/day PFNA) in males, a dose at which liver weight was not increased. This suggests that histopathological changes in the liver are a more sensitive endpoint for PFNA than increased liver weight.

In the subchronic study (Mertens et al., 2010), rats were dosed for 13 weeks, as compared to 18 or 21 weeks in the two-generation study. Histopathological changes of the liver were seen in males in the subchronic study at 0.125 Surflon S-111 (0.09 mg/kg/day PFNA) and 0.6 mg/kg/day Surflon S-111 (0.44 mg/kg/day PFNA), and these changes persisted after a 60 day recovery period. However, no histopathological effects in the liver were seen at 0.025 mg/kg/day Surflon S-111 (0.019 mg/kg/day PFNA) in the subchronic study.

Additionally, no histopathological effects were reported in females in the subchronic study, while in the longer duration two-generation study, hepatocellular hypertrophy occurred in high dose females, and some other effects occurred infrequently. Furthermore, the kidney effects observed in both males and females in the two-generation study did not occur in the subchronic study. These results suggest that the subchronic study was not of sufficient duration to detect some of the effects produced by longer exposures to Surflon S-111/PFNA, and those effects occur at doses below the LOAEL from the subchronic study with continued exposure.

In this study, delayed development of pups (reduced body weight gain, delayed age of markers of sexual development) was not observed in F_1 pups (and was not assessed in F2 pups), while such effects were prominent in mice (Lau et al., 2009; Wolf et al., 2010; discussed below). 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). These results from developmental studies of PFNA in rats and mice are consistent with results for the related compound, PFOA, in the rat versus the mouse. As is the case for PFOA, the rat may not

be the most appropriate model for assessment of developmental effects of PFNA because it is eliminated much more rapidly in female rats than in female mice. Thus, the developing rat fetus receives a much lower dose than the developing mouse fetus at the same maternally administered dose.

As above, the doses of C11 in the 0.025 mg/kg/day, 0.125 mg/kg/day and 0.6 mg/kg/day Surflon S-11 groups in Mertens et al. (2010) are estimated as 0.005, 0.025, and 0.12 mg/kg/day. For reproductive/developmental toxicity of C11 in rats, the NOAEL and LOAEL 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 (Takahashi et al., 2014). Although serum levels are not available for these studies, making direct comparisons uncertain, it is notable that 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).

Developmental Studies in Mice

Two studies of developmental effects of pure PFNA in mice have been conducted.

Lau et al. (2009)

PFNA was given to timed-pregnant CD-1 mice (20-25 per dose group) by oral gavage daily throughout pregnancy at doses of 1, 3, 5 or 10 mg/kg; controls received water (Lau et al., 2009). The protocol for this study was the same as in the analogous study of PFOA from the same laboratory (Lau et al., 2006). Because a preliminary study showed that 10 mg/kg PFNA led to severe maternal toxicity that included mortality, this dose was dropped from subsequent studies. Maternal weights were monitored. At GD 17, mice from each dose group were subdivided: some dams were sacrificed for maternal and fetal examinations while the rest were allowed to give birth. In this latter group, postnatal survival, growth, and development of the offspring were monitored.

Serum PFNA levels were measured in dams at GD 17 and in pups at PND 1, 10, 24, and 42. Serum levels of PFNA in dams at GD 17 were similar to those in neonatal (PND 1) pups. By day 24, pup serum levels decreased several fold from PND 1 levels, and PFNA was almost totally eliminated from the serum by PND 42. Exposure to pups resulted from both *in utero* exposure to the PFNA administered to the mothers throughout pregnancy and from PFNA that was transferred to the breast milk after maternal dosing had ended.

With the exception of 10 mg/kg/day group, PFNA did not produce significant changes in maternal weight gains; the small body weight increases seen at 10 mg/kg/day were stated to be likely due to increased liver weight.

PFNA treatment did not produce significant increases in full-litter resorptions and did not alter the number of implants, live fetuses, or fetal weight. Neonatal mortality was greatly increased at 5 mg/kg/day, but was not affected at the lower doses. Neonatal survival was decreased at 5 mg/kg/day. Survival rates at birth, PND 10, and PND 21 in the 5 mg/kg/day pups were 75%, 28%, and 17%, respectively, as compared to 87%, 85%, and 84% in controls at these time points.

Body weights of pups were decreased in a dose-related fashion at all doses. It is notable that these decreases in growth persisted until PND 287 (9 months of age), long after PFNA was

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

Dose-dependent delays in postnatal development of mouse pups exposed to PFNA were observed at all doses, including for delays in day of eye opening, day of vaginal opening, and day of preputial separation. These three endpoints were significantly delayed in the 3 and 5 mg/kg/day PFNA groups. The magnitude of these delays at the highest dose of PFNA (5 mg/kg/day) was much greater than at the highest dose (20 mg/kg/day) in the analogous PFOA study (Lau et al., 2006). Comparative data for the delays at 5 mg/kg/day PFNA and 20 mg/kg/day PFOA are: Eye opening: PFOA, ~3 days; PFNA, 5 days. Vaginal opening: PFOA, ~3 days; PFNA, 7 days. Preputial separation: PFOA, ~1 day; PFNA, 6 days.

Liver weights were significantly increased in a dose-related fashion at all doses in pregnant females on GD 17. Maternal serum levels and liver weight were assessed at the same time point (GD 17).

In the pups, relative liver weights were increased in a dose-related fashion on PND 1 through PND 70. These increases were significant at all doses on PND 1 and 24, and at 5 mg/kg/day on PND 70. It is notable that pup liver weight remained elevated after PFNA was no longer present at significant levels in serum.

In this study, maternal and pup effects were seen at doses of 1 mg/kg/day and above. Thus, the LOAEL was 1 mg/kg/day, and no NOAEL was identified. It is notable that decreased growth from prenatal and early-life exposure persisted well into adulthood (PND 287), long after PFNA had been eliminated.

It should be noted that histopathological evaluation of liver was not performed in this study. As discussed above, histopathological effects, including necrosis, occurred in the liver at Surflon S-111/PFNA doses and serum levels lower than those causing increased liver weight or other effects in the two-generation rat study. These histopathological effects were the most sensitive endpoints for toxicity of Surflon S-111/PFNA (Stump et al., 2008).

Wolf et al. (2010)

A second study of developmental effects of PFNA was conducted in peroxisome proliferator-activated receptor-alpha (PPAR-alpha) wild type (WT) and knockout (PPAR-alpha KO) mice (Wolf et al., 2010). The authors state that, based on previous studies in CD-1 mice (Lau et al, 2006, 2009), PFNA appears to be more potent as a developmental toxicant than PFOA (Wolf et al., 2010). Since PPAR-alpha is known to mediate some of the effects of PFCs including the developmental toxicity of PFOA (see below), the study was undertaken to elucidate the role of PPAR-alpha in developmental toxicity of PFNA in mice. Pregnant females (9-18 per group) were dosed with PFNA by gavage from GD 1-18 at 0, 0.83, 1.1, 1.5, or 2 mg/kg/day. All animals were sacrificed on PND 21 or 42 days post-coitus for non-pregnant females. Serum PFNA was measured in all adult females and 2 pups per litter 23 days after the last dose.

Maternal weight gain, number of uterine implants, and number of live plus dead pups per litter were not affected by PFNA. Percent litter loss was increased from 14.3% in controls to 35.3% in the high dose group in WT mice, but this change was not significant.

Pregnancy rate was significantly ($p<0.001$) reduced by all doses of PFNA in the PPAR-alpha KO mice compared to untreated PPAR-alpha KO mice, but was not affected by PFNA in WT mice. These data suggest that PFNA affects implantation in the absence of functioning PPAR-alpha.

The number of live pups at birth was decreased at all PFNA doses in WT mice; this effect was significant at 1.1 and 2.0 mg/kg/day. Pup survival from birth to weaning was reduced in a dose-related fashion in all treated WT groups and this decrease was significant at the two highest doses. Most pup deaths occurred within the first few post-natal days. WT pup survival at PND 21 in the two highest dose groups were 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.

Pup weight at birth was not affected by PFNA treatment. 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. Similarly, eye opening, a marker of postnatal development, was significantly delayed in 2 mg/kg/day WT pups but not at lower doses in WT pups or any dose in KO pups.

Absolute and relative liver weights were increased by PFNA in both WT and PPAR-alpha KO mice. Relative liver weights were increased 23 days after last dose at all doses in non-pregnant WT and PPAR-alpha KO females, with significance in all groups except 0.83 mg/kg PPAR-alpha KO. In mice who had given birth, relative liver weight at PND 21 was significantly increased at all doses in WT, but was not increased in PPAPR-alpha KO at any dose. In pups at weaning (PND 21), relative liver weight was increased at all doses in WT mice but only at the highest dose in PPAR-alpha KO mice.

In this study, pregnancy rate was significantly affected by PFNA in the PPAR-alpha KO mice but not the WT mice, while developmental parameters (number of live pups per litter, pup survival, day of eye opening, weight gain) were adversely affected by PFNA only in the WT mice. The developmental effects of PFNA seen in this study thus appear to be dependent on PPAR-alpha. Based on higher serum levels in PPAR-alpha KO pups than WT pups, the authors conclude that that lack of effects in PPAR-alpha KO pups is not attributable to lower PFNA levels in their serum.

As mentioned above, serum data in adult females and pups were measured 23 days after the last maternal dose. The serum levels measured at this time point in the adult females are considerably lower than at the end of the dosing period due to excretion of PFNA and, additionally, transfer to breast milk in the adult females who delivered and nursed live pups.

Wolf et al. (2010) conclude that the relevance of PPAR alpha to human developmental effects cannot be dismissed. PPAR-alpha and other PPAR isoforms are expressed in many fetal and adult tissues in rodents and humans (Abbott et al., 2010). Based on its physiological roles, PPAR-alpha is expected to have important roles in reproduction and development in these species (Abbott, 2009). Wolf et al. (2010) state that the effects of PFNA on liver weight in the

WT and PPAR-alpha KO mice are consistent with other data demonstrating both PPAR-alpha dependent and independent effects of PFNA and other PFCs on the liver (discussed further below).

As in the Lau et al. (2009) study discussed above, histopathological examination of the liver was not conducted by Wolf et al. (2010). Thus, histopathological changes such as those observed by Stump et al. (2008) and Mertens et al. (2010) were not assessed in this study.

Additional considerations

The potential for PFNA to cause the same specific low-dose developmental effects seen in mice given 0.01 mg/kg/day of PFOA (delayed mammary gland development and histopathological effects on female reproductive system, reviewed in Post et al., 2012) has not been evaluated.

Chronic effects/carcinogenicity

Chronic studies, including studies designed to detect carcinogenicity have not been conducted for PFNA. PFOA and PFOS, the only two PFCs for which chronic studies have been conducted, caused tumors in rats (Sibinski, 1987; Biegel et al., 2001; Thomford et al., 2002).

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, binding to transporters and carrier proteins, and interacting with membranes (Butenhoff, 2009). However, PFNA is non-reactive and thus is not a substrate for biochemical reactions involving fatty acids.

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 uM and 400 uM, 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 uM PFNA.

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, a nuclear receptor that is believed to mediate many of the effects of PFCs. 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 tested additional PFCs (Wolf et al., 2012), PFUnA (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, indicating 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.

In rodents, PFNA activates PPAR-alpha, as well as other nuclear receptors such as CAR (constitutive androstane receptor) and PXR (pregnane X receptor) (Kudo et al., 2000, 2006; Rosen et al., 2009). 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). The hepatic hypertrophy caused by PFNA in rodents has PPAR-alpha dependent and PPAR-alpha independent components (Rosen et al., 2009: Wolf et al., 2010), as is also the case for PFOA (reviewed in Post et al., 2012). In rats and mice, PFNA and other PFCs induce hepatic peroxisomal beta-oxidation, an effect that is associated with PPAR-alpha activity (Kudo et al., 2000, 2006). Furthermore, 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 wild type (WT) and PPAR-alpha KO mice dosed with PFNA (1 or 3 mg/kg/day) or PFOA (3 mg/kg/day) for 7 days, 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. Genes associated with the constitutive androstane receptor (CAR) were activated by PFNA in both WT and PPAR-alpha KO mice (Rosen et al., 2009).

Lin et al. (2011) hypothesize that the increased levels of the hormone adiponectin may be related to activation of the nuclear receptor peroxisome proliferator activated receptor-gamma which is strongly activated by PFNA (Fang et al., 2008; Fang et al., 2010). Relevant to this hypothesis, 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 may be activated by PFNA (Rosen et al., 2009).

Gene activation profiles of a series of 12 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 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 upregulated by PFCs *in vivo* were not affected in these in vitro cell assays.

Estrogenic activity may also be involved in the mode of action of PFNA and other PFCs. Studies in rainbow trout, which have long been used as a model for human liver carcinogenesis because they are 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). PFNA was more potent than PFOA as a promoter of liver

tumor development in rainbow trout after initiation with aflatoxin B_1 (AFB). 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*.

Many 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). Studies by Fang et al. (2012a,b,c) suggest that both Kupffer cells (hepatic macrophages) and hepatocytes contribute to the liver toxicity of PFNA. More research is needed to determine if the modes of action mentioned above and/or others yet to be investigated are responsible for PFNA's toxicity.

DEVELOPMENT OF INTERIM SPECIFIC GROUND WATER CRITERION

General Approach Used

The interim specific ground water criterion is intended to be protective for chronic (lifetime exposure) through drinking water. It is based on the general approach used to develop the New Jersey health-based drinking water guidance for PFOA described in NJDEP (2007) and Post et al. (2009a). This general approach is also applicable to other persistent PFCs found in drinking water.

Because the half-life of 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 animals. 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 by at least 100:1 with several studies indicating ratios significantly greater than 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 children. The half-life of PFNA is several times longer than that of PFOA in rats and mice, and limited human data indicates that its human half-life is also longer than that of PFOA (Tables 1 and 2). These data on the relative half-lives of PFOA and PFNA support an estimated serum:drinking water ratio of 200:1 for PFNA and indicate that this estimated ratio is not an overly conservative estimate. It should be noted that 200:1 represents a central tendency estimate, rather than an upper percentile value, for the PFNA ratio.

As discussed above, a factor of 0.085 (ng/kg/day)/(ng/ml) relating PFNA intake and increase in serum level corresponds to a 200:1 serum:drinking water ratio for PFNA, assuming the mean daily water consumption value recommended by USEPA (2004) of 17 ml/kg/day. For comparison, the 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 1.49 times the daily PFNA dose estimated to increase the serum level by 1 ng/ml.

Key and Supporting Studies and Endpoints

The quantitative basis for the interim ground water standard is increased liver weight in pregnant mice in a developmental study (Lau et al., 2009) conducted by USEPA in which pregnant mice were dosed with 0, 1, 3, or 5 mg/kg/day PFNA throughout gestation. Increased liver weight is a well-established effect of PFNA and other PFCs in experimental animals.

The Lau et al. (2009) study was selected because it is the only study appropriate for use as the basis for risk assessment that provides the serum PFNA data needed for interspecies extraplation. The only other toxicology studies in which serum PFNA data were reported are the developmental toxicity study in Wild Type and PPAR-alpha 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 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 as the basis for risk assessment.

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 has been requested from the sponsors of these studies but has not been provided.

Of the numerous effects observed in Lau et al. (2009), increased maternal liver weight was selected as the critical endpoint for 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 identified.

Modeling of endpoints of developmental toxicity assessed in the offspring in Lau et al. (2009) based on maternal serum levels measured on GD17 gave similar results to those obtained 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 how the maternal serum levels on GD17 relate to these effects in offspring at later time-points.

Serum levels of PFNA measured in the pups were not used in dose-response modeling of the pup developmental endpoints because they were measured after maternal dosing ended and/or at different time points than when endpoints were assessed in the pups. Thus, serum levels in the pups may not be indicative of the serum levels which caused the observed effects. It is notable that decreased body weights in offspring in all dose groups persisted until at least 287 days of age, a time point at which essentially all PFNA had been excreted.

PFNA caused similar effects at similar or lower doses 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 Lau et al. (2009). 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., 2012); 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. Histopathological effects in the liver, including necrosis, occurred in male mice dosed with a PFC mixture containing primarily PFNA 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 Lau et al. (2009), provide further qualitative support to the dose and serum level chosen as the 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 2.40 (USEPA, 2014b) was used to perform BMD modeling of the data on liver weight on GD 17 in pregnant mice (20-25 per dose group) exposed to PFNA on GD 1-17 (Lau et al., 2009).

The data that are presented graphically in Lau et al. (2009) were obtained in numerical form from the investigator (Lau, 2013). Average serum levels in the pregnant mice on GD 17 in the 0, 1, 3, and 5 mg/kg/day groups were 15 ng/ml, 13,700 ng/ml, 21,800 ng/ml, and 79,500 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. BMD and the BMDL serum levels were determined for a 10% increase from the mean liver weight in the pregnant control mice. Of the models that gave an acceptable fit to the data, the BMDL was derived from the Hill model based on the overall fit of that model based on the statistical criteria incorporated in the BMDS software. The BMDL for this model, 5,200 ng/ml, was selected as the POD.

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 concentration during the dosing period, and the average exposure over this period was lower. 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 a lower exposure experienced at an earlier time during the dosing period.

For comparison, BMD and BMDL values were also derived for pup endpoints from Lau et al. (2009), based on maternal serum level on GD 17. BMD and BMDL values for these other endpoints were similar to that obtained for maternal liver weight. The BMD and BMDL values and supporting data for maternal liver weight and the additional endpoints are presented in Appendix 1.

Derivation of health-based water concentration

Uncertainty factors (UFs) are applied to the POD serum level of 5.2 ug/ml (5200 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 but expressed in terms of internal, rather than administered, dose.

The total uncertainty factor applied to the POD serum level is 300, and includes the following uncertainty factors:

3 – Interspecies (mouse-human), 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 serum levels rather than administered dose.

10 – Intraspecies variability (sensitive human populations)

10 – Duration of exposure/ data gaps (combined).

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 indicate that PFNA may cause additional and/or more severe effects as exposure duration increases.

Gaps in the toxicology database for PFNA include the lack of developmental neurobehavioral studies, the absence of chronic/carcinogenicity studies, as well as the lack of studies of specific developmental effects such as mammary gland development affected by low doses of PFOA.

The target human serum level is: $\frac{5{,}200 \text{ ng/ml}}{300} = 17 \text{ ng/ml}.$

The default value for the Relative Source Contribution (RSC) factor to account for non-drinking water sources of exposure, 20%, is used when sources of exposure to a contaminant are not fully characterized, as is the case with PFNA. It should be noted that the ground water criterion for PFNA will be applied in locations where industrial releases of PFNA have contaminated ground water and other environmental media. At these sites, PFNA exposure from non-drinking water sources, such as soil, house dust, recreationally caught fish, and locally grown crops, may be greater than in the general population.

An RSC of 0.2 is applied to the target human serum level of 17 ng/ml to derive the target human serum level from drinking water exposure only: 17 ng/ml x 0.2 = 3.4 ng/ml (3400 ng/L .

The factor relating PFNA intake (ng/kg/day) and increase in serum level (ng/ml) of 0.085 (ng/kg/day)/(ng/ml) is used to derive the daily PFNA intake from drinking water (ng/kg/day) which will result in an increase in the serum level of 3.4 ng/ml (3400 ng/L) as follows.

$$\frac{0.085\ \text{ng/kg/day}}{\text{ng/ml}} \times 3.4\ \text{ng/ml} = 0.29\ \text{ng/kg/day}$$

Based on the average daily water consumption value recommended by USEPA (2004) of 0.017 L/kg/day, the drinking water concentration that would result in exposure to 0.29 ng/kg/day is:.

$$\frac{0.29\ \text{ng/kg/day}}{0.017\ \text{L/kg/day}} = 17\ \text{ng/L}$$

Using default assumptions for derivation of interim specific ground water criteria for a non-carcinogen, the Reference Dose that support the derivation of a criterion of 17 ng/L is 2.43 ng/kg/day, as follows:

$$\frac{17\ \text{ng/L} \times 2\ \text{L/day}}{70\ \text{kg} \times 0.2} = 2.43\ \text{ng/kg/day}$$

And:

$$\frac{2.43\ \text{ng/kg/day} \times 70\ \text{kg} \times 0.2}{2\ \text{L/day}} = 17\ \text{ng/L}$$

Where: 17 ng/L = Interim specific ground water criterion
70 kg = Average adult body weight
2 L/day = Assumed daily water consumption
0.2 = Relative Source Contribution factor.

As interim ground water criteria are rounded to one significant figure, **the recommended Interim Specific Ground Water Criterion for PFNA is 20 ng/L (0.02 µg/L).**

DISCUSSION OF UNCERTAINTIES

- Ongoing exposure to PFNA at 20 ng/L (0.02 µg/L) in drinking water is estimated to contribute an additional 4 ng/ml, on average, to the PFNA concentration in blood serum already present in the general population. Thus, the average serum level in communities with drinking water at this concentration is estimated at about 5.5 ng/ml, 3.7-fold higher than the average serum level of about 1.5 ng/ml in the adult general population (which is assumed not to result from drinking water exposure). A serum level of 5.5 ng/ml is well above the 95th percentile PFNA serum level (4.0 ng/ml) in the U.S. general population in the most recent (2007-08) published NHANES (Kato et al., 2011). In infants and children, serum levels from ongoing exposure to 20 ng/L PFNA in drinking water would possibly be greater than in adults, due to their greater water consumption on a body weight basis.

  Several epidemiological studies have found associations of PFNA serum levels in the general population with potentially important health endpoints, some of which are consistent with effects seen in animal studies. Although 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, these data contribute to the weight of evidence about the potential hazard of PFNA. Thus, there is uncertainty about the extent of protection provided by a criterion that will result in serum PFNA levels several-fold above the general population range.

- Communities with elevated exposures to PFNA from drinking water or other environmental media have not been studied. 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 PFOA in studies of populations with drinking water exposure, including cancer, have not been evaluated in humans exposed to PFNA.

- 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) lower than the doses that caused increased liver weight in the same study. Thus, histopathological changes, including necrosis, were a more sensitive endpoint than liver weight in the 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).

  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 Lau et al. (2009) mouse developmental study used as the basis for quantitative risk assessment. However, histopathological changes in the maternal and pup liver were not evaluated by Lau et al. (2009), 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 Lau et al. (2009).

- 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). 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 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 the factor relating drinking water intake and increase in serum level of 0.085 (ng/kg/day)/(ng/L) that corresponds to a serum:drinking water ratio of 200:1 and PFNA. Although these values are reasonable estimates supported by the available toxicokinetic data from animals and humans, human information on the half-life of PFNA is limited and serum levels have not been measured in communities exposed to PFNA in drinking water. 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. Based on the available information, it is considered appropriate to assume that effects of PFNA observed in experimental animals are relevant to humans for the purposes of risk assessment.

- The USEPA study used as the quantitative basis for the derivation of the interim specific criterion (Lau et al., 2009) was presented at the Annual Meeting of the Society of Toxicology in 2009 and is expected to be published in a peer reviewed journal in the near future. The PFNA serum level and maternal liver weight data used to develop the interim ground water criterion were presented graphically by Lau et al. (2009) and were obtained in numerical form from the investigator (Lau, 2013). They are final data and are not expected to change in the published version of this study.

- Available information discussed in the draft Technical Support Document indicates that the target organs and modes of action are 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.

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APPENDIX 1. Benchmark Dose calculation data for maternal and pup endpoints from Lau et al. (2009)[a]

(USEPA BMDS software version 2.4.0)

Endpoint	*Model*	*BMR*	*Akaike information criterion (AIC) statistic*	*BMD (maternal serum PFNA (ng/ml))*	*BMDL (maternal serum PFNA (ng/ml))*
Maternal liver weight at GD 17	Hill	+10%	-14.67	8,300	**5,200**[b]
	Exponential (BMDS model 5)	+10%	-10.58	6,300	3,200
Pup relative liver weight at PND 1	Hill	+10%	-35.45	11,000	8,100
	Exponential (BMDS model 5)	+10%	-35.45	8,800	5,900
	Polynomial (2-deg.)	+10%	-35.44	3,400	3,000 [b]
	(Average of Hill and Exponential models)				**(7,000)**[c]
Pup relative liver weight at PND 24	Hill	+10%	7.17	8,600	5,500
	Exponential (BMDS model 5)	+10%	7.17	7,900	4,900
	(Average of Hill and Exponential models)				**(5,200)**[c]
Pup body weight at PND 1	Hill	-10%	-171.54	19,400	11,100
	Exponential (BMDS model 4)	-10%	-171.54	19,900	11,500
	Polynomial (2-deg.)	-10%	-173.43	19,500	13,100
	(Average of Hill and Exponential models)				**(11,300)**[c]
Pup Body weight at PND 24	Hill	-10%	130.90	14,600	6,800
	Exponential (BMDS model 2,3 –(identical))	-10%	129.48	12,400	**10,100**[c]
	Exponential (BMDS model 4)	-10%	130.71	9,200	5,800
	Exponential (BMDS model 5)	-10%	130.90	14,700	6,900
	Polynomial (2-deg.)	-10%	130.55	9,300	6,300
	Power (function not restricted to ≥1)	-10%	131.34	17,300	8,000
Vaginal Opening (day)	Hill	+1 SD	83.89	15,670	10,700
	Exponential (BMDS model 5)	+1 SD	83.89	15,800	10,600
	(Average of Hill and Exponential models)				**(10,700)**[c]
Preputial Separation (day)	Hill	+1 SD	47.33	13,400	10,100
	Exponential (BMDS model 5)	+1 SD	47.33	13,700	9,900
	(Average of Hill and Exponential models)				**(10,000)**[c]
Eye opening (day)	Exponential (BMDS model 4)	+1 SD	28.23	4,100	2,600
	Exponential (BMDS model 5)	+1 SD	27.66	8,100	**3,700**
	Polynomial (2 deg.)	+1 SD	27.95	4,200	2,800

a. Results are shown for all models providing an acceptable fit to the dose-response data for each endpoint.

b. Although this model gives a good fit to the data, the nature of the fit does not appear to reflect a realistic dose-response relationship.

c. The BMDL value shown in **bold** for each endpoint represents the most appropriate value based on consideration of the lowest AIC value and the biological reasonableness of the modeled dose-response.