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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June 24, 2015
Revised - November 28, 2016

Table of Contents

ABSTRACT

An 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. The criterion 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, a factor of 0.08 (ng/kg/day)/(ng/ml) was developed to relate PFNA intake to the estimated increase in PFNA in blood serum in humans. This corresponds to a blood serum:drinking water ratio of 200:1 for humans from ongoing drinking water exposure to PFNA. Using this information, a water concentration protective of chronic drinking water exposure of 13 ng/L was derived. As ground water criteria are rounded to one significant figure, the recommended interim specific ground water criterion for PFNA is 10 ng/L (0.01 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 ^{o}C
Boiling Point:	218 ^{o}C at 740 mm Hg
Vapor Pressure:	No data
Water Solubility	9.5 g/L at 25 ^{o}C
Log octanol/water partition coefficient:	Not applicable
Taste Threshold (water):	No data
Odor Threshold (water):	No data
Odor Threshold (air):	No data

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

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

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

Production and Use

The production, industrial uses, and environmental fate of PFNA were reviewed by Prevedouros et al. (2006). Production of PFNA began in 1975; it was made primarily in Japan. It is produced as the linear isomer of its ammonium salt (ammonium perfluorononanoate, APFN). It was estimated that 10% of the APFN that was produced was released to air and water at the production facility, resulting in global emissions of 70,000 to 200,000 kg 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%; perfluorooctanoic acid (PFOA; C8), 0.78%; perfluorodecanoic acid (C10), 0.37%; and perfluorododecanoic acid (C12), 0.1%.

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

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

It is estimated that 60% of the PFNA used in PVDF manufacturing worldwide was released to the environment, resulting in global emissions of 400,000 to 1,400,000 kg from 1975-2004 (Prevedouros et al., 2006). Data provided to NJDEP about PFC use at the PVDF manufacturing facility located in Thorofare (West Deptford), NJ indicate that 86.6% of the 125,069 kg of the Surflon S-111 PFC

mixture (primarily PFNA) used between 1991-2010 was released to the environment (air and water) (Roux Associates Inc., 2013). The environmental fate of PFNA is discussed below.

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

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

Evaluations by other government agencies

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

The European Chemical Agency (ECHA) Risk Assessment Committee finalized its classification and labeling opinion (CLH) for PFNA in September 2014 (ECHA, 2014). The ECHA classifications are related to hazard identification and qualitative weight of evidence for various endpoints and do not include dose-response, quantitative risk assessment, or criteria development. ECHA concluded that PFNA is a presumed human reproductive toxicant for damage to the unborn child, a suspected human reproductive toxicant for fertility effects, a suspected human carcinogen, causes specific target organ toxicity to liver, thymus, and spleen after prolonged or repeated exposure, and causes harm to the breast-fed child through effects on or via lactation. These conclusions are based on data on PFNA itself, as well as supporting information from PFOA, which is closely related. The background document for the ECHA classification of PFNA was prepared by the Swedish Environmental Agency (Swedish Environmental Agency, 2013).

ENVIRONMENTAL SOURCES, FATE, AND OCCURRENCE

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

PFCs including PFNA are found in environmental media, including wildlife, in worldwide locations including remote polar regions. In addition to release from industrial facilities where it is made or

used, an additional 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 [$CF_3(CF_2)_7CH_2CH_2OH$] may be 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 in fish (Butt et al., 2014). Formation of PFNA was not observed in experimental studies of the degradation of 8:2 fluorotelomer alcohol to PFOA and other fluorinated compounds (Wang et al., 2005; Wang et al., 2009).

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

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

Drinking Water

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

In addition to industrial releases, sources of PFCs found in ground water or surface water include: discharge from wastewater treatment plants that treat domestic and/or industrial waste; street- and 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, and possibly ion exchange treatment systems designed for this purpose (Rahman et al., 2014).

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

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

The highest PFNA concentration (96 ng/L) reported in the 2009 NJDEP drinking water study was at a public water supply well (Paulsboro Water Department) in southern NJ about 2 miles from the West Deptford, NJ facility that used and discharged PFNA from 1985 until 2010 (Post et al., 2013). In follow-up sampling of this well in 2013, PFNA was found at 140 ng/L in raw water and 150 ng/L in finished water (Post et al., 2013). This well is currently not in use, and installation of treatment to remove PFCs from this well is planned. PFNA levels in another recently constructed well of this public water supply were lower (< 20 ng/L) in September 2013 testing. PFNA data are not available from two other wells of this water system which were used only on a limited basis until May 2012 and are not currently in use. PFNA was also detected at up to 72 ng/L in wells of a second public water supply (NJ American Logan-Birch Creek) located about 10 miles from the industrial facility (Post et al., 2013). The presence of PFNA (80 ng/L) reported at a third site by Post et al. (2013), located in northern NJ, was not confirmed in follow-up sampling reported to NJDEP in 2013.

In further public water supply sampling reported to NJDEP through March 2014, PFNA was also found in a public water supply well in West Deptford, within the same township as the industrial facility, at up to 48 ng/L in 2013, and in wells of 5 other Gloucester County public water supplies at up to 50 ng/L. In results reported to NJDEP as of July 2014, PFNA was detected ($\geq$2.5 ng/L) at up to 1500 ng/L in 20 of 84 (24%) private wells that were tested in the vicinity of the West Deptford industrial facility.

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 ($\geq$ 20 ng/L) is much higher than the Reporting Levels in the NJDEP studies and other monitoring data reported to NJDEP and in the drinking water occurrence studies reported in the literature (generally $\leq$ 5 ng/L, reviewed by Post et al., 2013). In initial UCMR3 data

from 3483 public water supplies outside of New Jersey reported to USEPA through January 2015, PFNA (20 ng/L or above) was found in only six public water systems outside of New Jersey (USEPA, 2015a). As of January 22, 2015, PFNA was found in UCMR3 monitoring in three public water supplies sites in Gloucester County, NJ (Woodbury City Water Department, up to 56 ng/L; Monroe Township MUA, up to 28 ng/L; West Deptford Township Water Department, 30 ng/L) including one public water supply (Monroe Township MUA) which had not previously reported detections of PFNA to NJDEP. In all but two of the non-NJ public water supplies reporting PFNA in UCMR3, other PFCs were also present, while PFNA was the only PFC reported at the three Gloucester County, NJ, sites.

Ambient Surface Water

In 2007−09, PFNA was found in the Delaware River water at up to 976 ng/L starting near and downstream of the discharge location of the above-mentioned industrial facility; this is higher than the surface water concentrations elsewhere in the U.S. and worldwide in studies located in the literature. Elevated levels of 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).

Wildlife

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

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

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

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) conducted by the U.S. Centers for Disease Control and Prevention (CDC; Kato et al., 2011; CDC, 2015); PFCs are also ubiquitous in the serum of populations worldwide (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 NHANES data from 2011-12 (CDC, 2015), geometric mean serum levels were PFNA, 0.88 ng/ml; PFOA, 2.08 ng/ml; PFOS, 6.31 ng/ml; and PFHxS, 1.28 ng/ml. In another series

of studies of PFC serum levels in U.S. blood donors, the geometric mean from the most recent data (2006) was 0.97 ng/ml (Olsen et al., 2011).

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

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 (Lau, 2012; Post et al., 2013). Based on the infrequent occurrence and low concentrations of PFNA reported in drinking water throughout the U.S. (discussed above), the mean and median PFNA serum levels found in the U.S. 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. Humans may be exposed to PFNA formed to so some extent from telomer alcohols in environmental media (discussed above) and by metabolism of telomer alcohols in the human body (Henderson and Smith, 2007; Nilsson 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 (D'eon et al., 2009).

In contrast to other persistent and bioaccumulative organic compounds that are not water-soluble, ingestion of contaminated drinking water can be an important source of human exposure to PFCs. Elevated serum levels of PFOA, PFOS, and PFHxS have been found in communities with contaminated private and/or public water supplies (PWS). However, no scientific 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). Consistent with their higher daily water consumption rate (ml/kg/day), serum levels are generally higher in young children than in adults exposed to the same PFC concentration in drinking water (Emmett et al., 2006; Mondal et al., 2012).

As discussed above, PFNA exists in drinking water in its non-volatile anionic form. Therefore, inhalation 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. Similarly, evaluation of the potential for dermal absorption of PFNA during showering and bathing indicates that this route of exposure is insignificant compared to exposure through ingestion (NJDOH, 2014). The evaluation was based on skin permeability data for PFOA (Franko et al., 2012), a compound which is expected to have a slightly higher potential for dermally absorption than PFNA.

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 were 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.

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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 in rodents soon after oral administration (Tatum-Gibbs et al., 2011).

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

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

Distribution and Metabolism

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

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

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

Excretion

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

In pharmacokinetic studies of linear (*n*-) and branched (*iso*-) PFNA after a single-dose (male rats only; Benskin et al., 2009) and subchronic exposure (males and females dosed for12 week; De Silva et al., 2009), the linear form was excreted somewhat more slowly than the branched form. Half-lives were similar to those in the studies shown in Table 1. The half-lives in male rats were 41-48 days for linear PFNA and 21-32 days for branched PFNA, while the half-lives in females were 2.1 days for linear and 0.82 days for branched PFNA. In the male rats in these studies, the half-lives of PFNA were 3-5 times longer than for PFOA, based on comparison of groups treated with the same isomer for the same time period.

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

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]	5.0-7.5	8.2-30
Mouse	34.3[a]/68.4[b]	25.8[a]/68.9[b]	19[d]	17[d]	2.0-4.0	1.4-3.6

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

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. (2013a) estimated the human half-lives of a series of PFCs, including PFOA and PFNA, based on renal clearance estimates. In women less than 50 years old, modeled excretion through menstrual blood loss was also considered. The study included 86 adults (age 21-88 years), from the Chinese general population. The median serum PFNA concentration in the subjects was 0.37 ng/ml, which was several fold lower than the median of 1.5 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. The PFNA half-life estimates in males and older females for PFNA (n=50) ranging from 0.34 to 20 years, while for PFOA (n=66), the range was 0.059 to 14 years. In younger females, the range for PFNA (n=16) was 0.38 to 7.7 years and for PFOA (n=20) was 0.19 to 5.2 years.

Blood loss (e.g. through menstruation, blood donation, or venesection) is an excretion route for PFCs (Harada and Koizumi, 2009; Taylor et al., 2014; Lorber et al., 2015; MDH, 2013). The estimates of PFNA half-life in women under 50 years of age are based on modeling of this pathway

and are considered more uncertain than the estimates for men and older women. Although children were not included in this study, the increased excretion rate due to menstrual blood loss is not applicable to children. Similarly, the additional clearance through menstrual blood is not relevant to pregnant women. Other potential clearance pathways, such as fecal excretion, were not considered by Zhang et al. (2013a), but were believed by the researchers to be less significant than elimination through urine and menstrual blood.

Median and geometric mean values represent estimates of the 50th percentile value and are less affected by outliers than mean values. As shown in Table 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 decline in serum levels	No information		2.3-10.1[a] /3.8[b] years		----	----
Based on urinary excretion, with estimated menstrual clearance in females <50 years of age[c]	*all males and females >50 years:*	*females 21-50 years:*	*all males and females >50 years:*	*females 21-50 years:*	*all males and females >50 years:*	*females 21-50 years:*
Mean	4.3	2.5	2.6	2.1	1.65	1.19
Geometric Mean	3.2	1.7	1.2	1.5	2.67	1.13
Median	3.5	1.5	1.7	1.8	2.06	0.83

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

Fetal exposure - Maternal and cord blood serum levels

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

In human studies, PFNA levels in fetal cord blood serum generally correlate with maternal serum levels. In nine studies in which both maternal and cord blood PFNA levels were measured, the mean cord blood serum:maternal serum (or plasma) ratios ranged from about 0.3

to about 1, with a median value of about 0.5 (Beesoon et al., 2011; Fromme et al., 2010; Gutzkow et al., 2011; Kim et al., 2011a; Liu et al., 2011; Monroy et al., 2008 Needham et al., 2011; Ode et al, 2013; Zhang et al., 2013b).

Infant Exposure – Distribution to Human Breast Milk

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

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

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

[a] Fromme et al., 2010

Relationship between 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. As discussed below, this ratio is supported by data from several studies of populations whose public water supplies or private wells were contaminated with a wide range of PFOA concentrations (60 ng/L to 13,300 ng/L), as well as two toxicokinetic modeling efforts.

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

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

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

daily water intake recommended by USEPA (2011) of 16 ml/kg/day, application of this factor predicts a serum:drinking water ratio of 126:1.

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

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

The half-life data from rats and mice (Table 1), and the limited human half-life data (Table 2) presented above support an estimated half-life of PFNA at least twice that of PFOA. These data, along with information (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 an assumed daily drinking water intake of 16 ml/kg/day (USEPA, 2011), this ratio corresponds to an increase of 1 ng/ml PFNA in blood serum per 0.08 ng/kg/day ingested PFNA, or a factor of 0.08 ng/kg/day/(ng/ml). For comparison, the estimated daily dose of PFOA estimated to result in a 1 ng/ml increase in serum level (0.127 ng/kg/day/(ng/ml); Clewell (2006, 2009) is associated with a serum:drinking water ratio of 133:1. This estimated daily dose is 1.59 times the daily PFNA dose (0.08 ng/kg/day) estimated to increase the serum level by 1 ng/ml.

Based on the above toxicokinetic considerations, ongoing exposure to drinking water with 150 ng/L PFNA (the highest concentration reported in public drinking water in New Jersey or elsewhere) is estimated to increase PFNA serum levels, on average, by 30 ng/ml (µg/L; ppb) in serum. This represents about a 34-fold increase from the general population geometric mean serum value of 0.88 ng/ml (CDC, 2015).

HEALTH EFFECTS

Human Studies

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, reviewed below, 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 performed, 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

Studies of the general population (the NHANES study population in the U.S., and similar populations in other countries), most of which were cross-sectional, have evaluated the association of PFNA with a number of health endpoints. Some of these studies reported significant associations, while other studies did not find significant associations between PFNA and the health endpoints that were assessed. Causality cannot be established from cross-sectional studies because exposure and outcome are assessed at the same point in time. Thus, they do not establish whether exposure occurred before, after, or during the onset of the outcome being assessed. Some of the health endpoints associated with PFNA relate to biological endpoints (glucose metabolism and immune response) that are also targets for PFNA in the animal toxicology studies discussed below.

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 may be 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.

Cancer

Associations between prostate cancer and PFCs (PFHxS, PFOS, PFOA, PFNA, PFDA, and PFUnDA) were evaluated in a case-control study which included 201 cases of prostate cancer and 186 population based controls (Hardell et al., 2014). Cases included men admitted to a hospital for treatment for prostate cancer from 2007-2011 and participating before receiving treatment. Controls were matched on age and geographical area and had no previous cancer diagnosis. Cases had a median PFNA serum concentration of 0.61 ng/ml and controls had a median concentration of 0.57 ng/ml; these values were not statistically significantly different (p-value=0.24). Unconditional logistic regression, adjusted for age, BMI, and year of sampling, was used to calculate odds ratios (ORs) and 95% confidence intervals using the median and 75th percentile as cut-off values, ORs for PFNA were not associated with higher risks. Cases were also grouped into risk groups (low, intermediate, or high) using Gleason score and Prostate-Specific Antigen (PSA), and no patterns were found. There was some evidence of an interaction between PFNA levels and reporting a first-degree relative with prostate cancer; however this was not statistically significant.

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/ml, 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, below the median in the NHANES population of 1 ng/ml.

The association of carotid intima-associated thickness (CIMT), a marker of subclinical atherosclerosis, and PFCs was evaluated in a cross-sectional study of 664 individuals with abnormal urinalysis results (246 with elevated blood pressure and 398 with normal blood pressure) aged 12-30 years, who had been originally recruited from a population-based mass urine screening in Taiwan (Lin et al., 2013a). Screening occurred from 1992-2000, and interview and follow-up were conducted from 2006-2008. The median serum concentrations of the PFCs were: PFOA (3.49 ng/ml), PFOS (8.65 ng/ml), PFNA (0.38 ng/ml), and PFUA (6.59 ng/ml). Linear regression adjusting for age, gender, smoking status, alcohol consumption, and BMI analyzed the association of cardiovascular risk factors (systolic blood pressure (SBP), BMI, LDL, homeostatic model assessment for insulin resistance (HOMA-IR) by categories of PFCs. The only positive finding in this analysis was that the mean level of log-HOMA-IR decreased significantly with increasing levels of PFNA (p-value=0.009). Linear regression models adjusted for age gender, smoking status, SBP, BMI, LDL, CRP, TG, and HOMA-IR in the fully-adjusted model found mean CIMT decreased insignificantly with increasing levels of PFNA.

The association 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.

In summary, one study of NHANES participants found positive association of PFNA with total cholesterol. The same study found no association with HDL, while one study found a non-statistically significant positive association, and a different NHANES analysis found a statistically significant inverse relationship. A cross-sectional study did not find significant associations with PFNA and markers of atherosclerosis.

Metabolic Effects & Diabetes

The associations of serum PFOA, PFOS, PFUnDA (perfluoroundecanoic acid, C11), and PFNA with parameters related to glucose metabolism were evaluated in 287 Taiwanese adolescents and young adults (age 12-30 years) recruited from a hypertension cohort (Lin et al., 2011). The median PFNA serum level was 1.68 ng/ml, 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 (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, but no association was found 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.

Lin et al. (2009) studied the associations of PFC exposure with hyperglycemia, blood insulin levels, and beta-cell function along with additional serum lipid parameters that are discussed above. PFNA was significantly associated with clinically defined hyperglycemia and lower prevalence of the metabolic syndrome, and with decreased blood insulin levels and decreased pancreatic beta-cell

function with borderline statistical significance among adolescents.

In the Nelson et al. (2010) NHANES analysis, also discussed above, PFNA was not associated with BMI, waist circumference, or HOMA.

In summary, two cross-sectional studies found decreased blood insulin levels, and pancreatic beta-cell function or HOMA, and a third cross-sectional study found increased diabetes among the elderly. Other findings from these studies included decreased metabolic syndrome and increased adiponectin. A fourth cross-sectional study did not find an association with HOMA or BMI.
A prospective birth cohort study which evaluated associations of prenatal exposure to PFNA with obesity and associated clinical parameters in 20 year old female offspring found that PFNA was associated with increased BMI, but this association did not remain after adjustment for PFOA. This study 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 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 prospective birth-cohort 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., 2014) 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 postnatal 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 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 except two of the other PFCs (pefluorohexanoic acid (C6) and perfluorotetradecanoic acid (C13)). PFNA and most other PFCs were not associated with immunological biomarkers (absolute eosinophil count, immunoglobulin E (IgE), and eosinophilic cationic protein) in 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.

Humblet et al. (2014) evaluated associations between self-reported lifetime asthma, recent wheezing, and current asthma among adolescents (aged 12-19 years) and PFCs (PFOA, PFOS, PFNA, and PFHxS) from combined 1999-2000 and 2003-2008 NHANES. Multivariate models adjusting for survey cycle, age, sex, race/ethnicity, poverty income ratio, smoking, health insurance, found PFOA statistically significantly associated with higher odds of ever having asthma. PFNA serum concentration among participants with ever having asthma were borderline statistically significantly (p-value=0.053) higher than among those never having asthma.

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 follow up 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.

In summary, PFNA was statistically significantly associated with asthma among children in a case-control study and positively, but not statistically significantly, associated in a cross-sectional study, while a prospective birth cohort found no association with asthma among children aged 3 years. One prospective birth cohort found decreased vaccine antibody response for rubella vaccine, but not other vaccines and found no associations with PFNA and eczema, itchiness, wheeze, otitis media. Another prospective birth cohort found no association with IgE or atopic dermatitis.

Liver

Associations between liver enzymes (ALT, log-transformed GGT, total bilirubin) and PFOA, PFOS, PFNA, and PFHxS were evaluated from combined 1999-2000 and 2003-2004 NHANES (Lin et al., 2010). Individuals who had fasted less than 6 hours, were hepatitis B or C virus carriers, or had missing data were excluded from analysis for a final study population of 2,216 individuals. Median PFC serum concentration were PFHxS (1.80 ng/ml), PFOA (4.20 ng/ml), PFNA (0.70 ng/ml), and PFOS (23.50 ng/ml). Total bilirubin increased across quartiles of PFNA (p-value=0.014), while there was not a statistically significant increase for ALT or log-GGT. After natural log transformation of each PFC, linear regression coefficients were calculated for two models, one adjusting for age, gender, race/ethnicity and the second additionally adjusting for smoking status, alcohol consumption status, education level, BMI, HOMA-IR (homeostasis model assessment of insulin resistance), metabolic syndrome, and iron saturation status for each PFC individually and also with all four PFCs entered into the models. When PFNA was analyzed separately, it was associated with total bilirubin in the age, gender, and race/ethnicity adjusted model (p-value=0.005) and with borderline statistical significant when further adjusted for other covariates (p-value=0.053). PFNA remained statistically significantly associated with total bilirubin when all three PFCs were included in the composite analysis (p-value=0.004).

Associations between PFCs and liver function biomarkers (ALT, GGT, asparate aminotransferase (AST), alkaline phosphate (ALP), and total bilirubin) and uric acid were evaluated in an NHANES analysis 2007-2010 (Gleason et al., 2015). Median PFC serum concentrations were PFHxS (3.7 ng/mL), PFOS (11.3 ng/mL), PFOA (3.7 ng/mL), and PFNA (1.4 ng/mL). All liver function

biomarkers and PFCs were natural log-transformed for linear regression. Confounders and covariates which were considered included age, gender, race/ethnicity, BMI, poverty, smoking, and alcohol consumption. PFNA was statistically significantly associated with linear increases in ALT (p-value<0.001) and had an increasing trend associated with increasing quartiles of PFNA (p-value=0.04). There was evidence of a linear association with PFNA and GGT (p-value<0.01) and uric acid (p-value<0.001) but the trend was not statistically significant. PFNA was not associated with the other clinical biomarkers examined in this analysis.

In summary, PFNA was statistically significantly associated with total bilirubin in one of two general population cross-sectional studies. PFNA was associated with ALT, with evidence of an association with uric acid and GGT, in one of these studies but not the other. ALP and AST were assessed in only one of the two studies, and PFNA was not found to be associated with either parameter.

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/ml 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., 2013b). The group consisted of 221 participants with elevated blood pressure (BP) during childhood and 310 participants with normal BP during childhood, and was a subset of a larger cohort with abnormal urinalysis findings in childhood. Of the 551 subjects, 41 were hypertensive. Geometric mean serum levels for for PFCs 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 at delivery with fetal (umbilical cord blood) thyroid hormones (TSH, T3, and T4) in 34 Korean mother:infant pairs (Kim et al., 2011b). 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).

Associations between thyroid profile parameters (FT3, FT4, TGN, TSH, TT3, and TT4) and PFCs were evaluated from 2007-2008 NHANES (Jain, 2013). After exclusions (pregnancy, evidence of thyroid condition, and missing data) the final unweighted sample size included 1733 participants. Serum concentrations of PFCs for this study population were not provided. PFCs and thyroid parameters were log-transformed and linear regression models were adjusted for age, gender, race/ethnicity, smoking status, iodine status, C-reactive protein, BMI, fasting time, caloric intake. PFNA was not found to be statistically significantly associated with any of the six thyroid function parameters.

In summary, small increases in free or total thyroxine were associated with PFNA in two studies of children and adolescents/young adults. Four 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; and 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 taken at second trimester and delivery 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 two recent systematic reviews evaluated the numerous studies of associations of serum PFOA and fetal growth, as indicated by birth weight and other parameters (Johnson et al., 2014; Bach et al. 2015). Both reviews found that PFOA was associated with decreased average birth weight in most studies, and Johnson et al. (2014) concluded that the overall body of data from human studies is sufficient to conclude that decreased fetal growth is associated with PFOA exposure.

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 selected from female offspring of a prospective cohort study members, 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., 2014). 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, but not for PFNA (median, 0.8 ng/ml) and the other PFCs present at <1 ng/ml. 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.

A case-cohort study of nulliparous pregnant women (466 cases, 510 non-cases) selected from a prospective pregnancy cohort (Norwegian Mother and Child Birth Cohort (MoBa)), evaluated the associations between PFC concentrations and diagnosis of preeclampsia (Starling et al, 2014). A proportional hazards model with adjustment for maternal age at delivery, pre-pregnancy BMI, maternal educational level, and smoking at mid-pregnancy was used to estimate effect of increasing PFC concentrations (quartiles, and log transformed continuous) on preeclampsia. Other covariates which were considered include: plasma creatinine, cystatin C, and HDL cholesterol. Median PFC serum concentrations were as follows: PFOS (12.9 ng/ml), PFOA (2.9 ng/ml), PFNA (0.54 ng/ml), and PFHxS (0.69 ng/ml). Findings do not support an increased risk of preeclampsia with increasing PFNA among nulliparous pregnant women.

In summary, four 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, a study of semen quality and PFCs analyzed PFNA in serum but did not evaluate associations of PFNA with semen quality endpoints, and a study of preeclampsia found no association with PFNA.

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 ≤50 years of age from the C8 Health Project conducted Ohio and West Virginia communities with drinking water contaminated by PFOA (Gallo et al., 2013). 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.

Associations between cognitive ability in older adults (aged 60-85 years old) and PFCs (PFOA, PFOS, PFNA, and PFHxS) were evaluated from combined 1999-2000 and 2003-2008 NHANES. Cognitive ability was measured by the main outcome, self-reported difficulty due to remembering or periods of confusion, and secondarily the outcomes self-reported difficulty with activities of daily-living due to senility and performance on the Digit-Symbol Substitution Task (DSST) was investigated (Power et al., 2013). Final sample size was 1,766 individuals with the following geometric mean PFC serum concentrations: PFOS (22.6 ng/ml), PFOA (4.08 ng/ml), PFNA (1.01 ng/ml), and PFHxS (2.05 ng/ml). Multivariate logistic regression models were adjusted for age, age-squared, race/ethnicity, gender, NHANES cycle, education, poverty-income ratio, food security, health insurance, social support, physical activity, smoking, and alcohol consumption and diabetes was assessed as an effect modifier. Estimates based on a doubling of natural log transformed PFNA concentration show a protective association with difficulty remembering or periods of confusion (OR=0.91, 95% CI 0.79-1.04), and with senility (OR=0.92, 95% CI 0.59-1.44); neither were statistically significant. Protective effects of PFNA on cognitive ability may be concentrated in diabetics (OR=0.77, 95% CI 0.59-1.00) as compared to non-diabetics (OR=0.98, 95% CI 0.84-1.14); these associations are not statistically significant.

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 and a statistically non-significant protective effect for difficulty remembering and senility.

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 (Johansson 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 (≥85% male 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. no exposure, high v. no exposure) 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. When statistically significant differences were observed, they were not consistent across time periods or between men and women. Adjusted mean values 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 exposed men and in 2001 for high exposed men compared to non-exposed men for the liver enzyme ALT, in 1998 for highly 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. Women were classified only as exposed or not exposed, and 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 associated with a 1 unit change in cumulative exposure intensity score, and change in the clinical parameter associated with current exposure by operating condition (powder or 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, some of which have not been evaluated for PFNA in humans, including two types of cancer (Barry et al., 2013; Vieira et al., 2013). For other health endpoints, no associations were found in the C8 Health Project population. C8 Science Panel reports and citations for peer- reviewed publications presenting the results of these studies are found at the C8 Science Panel website (http://www.c8sciencepanel.org/).

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 (Kennedy, 1987). All mice in the two highest dose groups (300 or 3000 ppm) 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. 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), hepatic levels of four proteins that are part of the insulin signaling pathway were significantly reduced (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.

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). In some studies, 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); Grandjean et al., 2012).

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 also been associated with immune system effects in studies of the human general population.

Serum levels were not measured in this study.

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.

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. (2008) were not evaluated in theses longer duration studies.

Male reproductive system in 14 day rat study (Feng et al., 2009)

Feng et al. (2009) studied effects of PFNA on the testes 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 toxicity 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, a modulator of testicular function) were significantly reduced by 1, 3, or 5 mg/kg/day PFNA. A change in the level of inhibin B is considered to be a biomarker of testicular toxicity.

These studies showed potentially important effects of PFNA 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 below.

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

Unpublished study of Surflon S-111 (Wolterbeek et al., 2004)

An unpublished study of Surflon S-111 (described below) in male rats (Wolterbeek, 2004) is discussed in Stump et al. (2008) and Mertens et al. (2010). In this study, serum phospholipids, cholesterol, and calcium were decreased in male rats in all dose groups (0.1, 0.5, and 2 mg/kg/day) after 14 days of exposure. It was also mentioned that in this study, all males dosed with 2 mg/kg/day for 23 days were euthanized on day 23 due to severe clinical findings (no details provided) and severe body weight loss (Wolterbeek, 2004).

Longer term Studies of Surflon S-111

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. 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.

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; and 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 male and female animals, and on liver, duodenum, and glandular and non-glandular stomach from males in all dose groups and the two recovery groups (control and high dose).

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 given the same dose 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 given the same dose 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 about 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 were:

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 remained 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 decreased, and bilirubin, BUN, chloride (data not shown), and alkaline phosphatase were increased. 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 group 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-111 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 that the greater toxicity of Surflon S-111 in male rats than female rats in the unpublished reports (WIL Research Laboratories, 2006) of the subchronic study (Mertens et al., 2010) is consistent with the higher serum PFNA levels in male as compared to 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 in Mode of Action section, 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 again 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 were dosed by gavage starting at age 6 weeks, for at least 70 days prior to mating, throughout mating, gestation, and lactation, and until 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, until 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 were 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. The incidence of necrosis in the control, low, mid, and high dose groups was 0/30, 2/30, 5/30, and 5/30 in the F_0 males and 0/30, 3/30, 4/29, and 8/30 in the F_1 males. 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 on all F_0 dose groups and only in the control and high dose F_1 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.

Renal 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 the F_0 males. Renal inflammation (1/30 mid dose and 1/30 low dose), brown pigment (2/30 high dose), or capsular fibrosis (1/30 high dose) was seen in a few F_0 males.

In females, histopathological examination of the kidney was performed on all F_0 dose groups and only in the control and high dose F_1 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, or pup weight through weaning in F_1 or F_2 pups. Age at vaginal opening and 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 generations. 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 that, with continued exposure, effects occur at doses below the LOAEL from the subchronic study.

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 (Das et al., 2015; 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 an 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.

Das et al. (2015)

Study Design

PFNA (97% pure, stated by supplier to be primarily linear) was given to timed-pregnant CD-1 mice (19-27 per dose group) by oral gavage on GD 1-16 or GD 1-17 (see below) at doses of 1, 3, 5 or 10 mg/kg/day; controls received water (Das et al., 2014). This study was reported earlier as an abstract and poster presentation (Lau et al., 2009). The design of this study was similar as in the analogous study of PFOA (1, 3, 5, 10, 20, and 40 mg/kg/day) from the same laboratory (Lau et al., 2006).

The highest dose, 10 mg/kg/day, caused decreased body weight starting at GD 8. All mice in this group were sacrificed on GD 13 and all pregnant mice in this group had full litter resorptions. At GD 17, mice from each surviving dose group were subdivided in two groups. Some dams (8-10 pregnant per group; dosed until GD 16) were sacrificed for maternal and fetal (skeletal and visceral) examinations. Mice sacrificed on GD 17 that had no live or dead fetuses were considered to be non-pregnant. Livers and serum from pregnant and non-pregnant adult females and some fetuses were weighed and preserved

for PFNA and PCR gene expression (fetal liver only) analysis.

The other group of dams (dosed until GD 17) were allowed to give birth (11-17 litters per dose group), and postnatal survival, weight gain, and development (eye opening, vaginal opening, and preputial separation) of the offspring were monitored. The pups were weaned on PND 24, and the dams were sacrificed on PND 28. Four males and four female pups per litter were weighed biweekly after weaning beginning on PND 25, until PND 287. In dams sacrificed on PND 28 and in one male and one female pup per litter on PND 1, 10, 24, 42, and 70, livers were weighed, and serum and liver samples were taken for PFNA analysis (dams and offspring) and PCR gene expression analysis (offspring only).

PFNA levels in serum and liver

PFNA levels increased with dose in serum and liver from GD 17 pregnant and non-pregnant adult females, GD17 fetuses (livers only, serum not analyzed), dams at post-weaning on PND 28, and offspring followed from PND 1 to PND 70. On GD 17, PFNA levels increased with dose in both serum and liver from pregnant and non-pregnant adult females, and in fetal livers. Serum levels were generally about twice as high in non-pregnant than in pregnant adult female mice, with the lower levels in the pregnant mice presumably due to transfer to the fetal compartment (Das et al., 2015),

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

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

Serum levels in offspring soon after birth (PND 1) were somewhat higher than maternal serum levels at a similar time point (GD 17). In offspring, PFNA levels in serum and liver decreased over time, with a slower decline in liver than in serum. PFNA persisted at low levels in liver and serum of treated offspring until 43 weeks (10 months), the last time point assessed. In male offspring at age 10 months, PFNA in serum was about 1% of PND 1 levels, while in liver, levels were about 2-4% of PND 1 levels. Liver and serum levels in female offspring were lower than in males at this time point (Numerical data obtained from C. Lau).

Effects on maternal weight gain and pregnancy outcome

As discussed above, PFNA at 10 mg/kg/day caused severe maternal weight loss and full liter resorptions in all pregnant mice. Maternal body weight gain through GD 17 was not affected at 1, 3, and 5 mg/kg/day. In the dams sacrificed on GD 17, PFNA at up to 5 mg/kg/day did not produce significant increases in full litter resorptions and did not alter the number of implants, number of percent live fetuses, prenatal liver loss, or fetal weight. No increases in skeletal or visceral abnormalities were observed in fetuses from treated groups.

Effects on offspring mortality and development

In the offspring of the dams which gave birth at term, postnatal mortality was not affected at 1 and 3 mg/kg/day. Pup survival was severely affected at 5 mg/kg/day, a dose which did not affect maternal

weight gain, cause overt maternal toxicity, or impact pregnancy outcome. Less than 20% of the 5 mg/kg/day pups survived to PND 21, compared to greater than 80% of the controls.

The neonatal mortality in the 5 mg/kg/day offspring was gradual, with a sharp increase in pup deaths throughout the first 10 days of life. The pups were weak and failed to thrive, although lack of maternal care was not observed. Das et al. (2015) contrast these findings to those in similar studies PFOA and PFOS. These other PFCs caused neonatal mortality in mice in the first two days of life at higher doses (10-20 mg/kg/day), but mortality did not continue to occur at later time points. They suggest that the neonatal deaths in the 5 mg/kg/day PFNA pups may be due to effects on intermediary metabolism (i.e. the utilization of nutrients to produce energy needed for growth through glycolysis and other metabolic pathways), since PFNA is a more potent than PFOA or PFOS as an activator of PPAR-alpha, a receptor which is involved with regulation of intermediary metabolism. The relative potency of PFNA for PPAR-alpha activation as compared to other PFCs is further discussed in the Mode of Action section below.

Body weights of pups on PND 1-24 were decreased by PFNA in a dose-related fashion at all doses, with significance at 3 and 5 mg/kg/day. At weaning, body weight of the offspring in the 3 mg/kg/day and 5 mg/kg/day groups was about 27% and 50% lower than the controls, respectively. These body weight decrements persisted in male offspring, with no catch-up, until PND 287 (9 months of age), a time at which PFNA was essentially eliminated from the serum. These persistent delays in growth from PFNA are in contrast to the findings in a PFOA study of similar design in CD-1 mice in the same laboratory (Lau et al., 2006). In the PFOA study, body weights of pups from mothers dosed with 3 or 5 mg/kg/day during gestation were 25-30% lower than controls at weaning, but recovered and reached control levels by 6.5 weeks in males and 13 weeks in females.

Dose-dependent delays in markers of postnatal development occurred in mouse pups exposed to PFNA. The endpoints assessed were day of eye opening, day of vaginal opening, and day of preputial separation, with significant delays in all three endpoints in the 3 and 5 mg/kg/day PFNA groups. The magnitude of these delays at 3 mg/kg/day PFNA were similar to those at the highest dose (20 mg/kg/day) in the analogous PFOA study (Lau et al., 2006), while 5 mg/kg/day PFNA caused much greater delays than 30 mg/kg/day PFOA (Table 4).

Table 4. Comparison of developmental delays in CD-1 mice from PFNA (Das et al., 2015) and PFOA (Lau et al., 2006)

	PFNA		**PFOA**
Endpoint	*3 mg/kg/day*	*5 mg/kg/day*	*20 mg/kg/day*
Eye opening	2 days	5 days	~3 days
Vaginal opening	3 days	7 days	~3 days
Preputial Separation	2 days	5 days	~1 day

Effects on liver weight in adults, fetuses, and offspring

Absolute and relative liver weights were significantly increased in a dose-related fashion at all doses in pregnant and non-pregnant females on GD 17. Maternal serum levels and liver weight were assessed at the same time point (GD 17) one day after the last dose was administered. Dose-related increases in absolute and relative liver weight persisted at all doses in dams on PND 28, four days after weaning.

Absolute and relative fetal liver weights were significantly increased at all doses of PFNA, except that the increase in absolute liver weight was not significant at 5 mg/kg/day. The magnitude of the increases in liver weight was similar in all dose groups.

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.

Gene expression in fetal and offspring liver

Real time PCR analysis was used to study the expression of genes of interest in livers from fetuses (GD 17) and offspring on PND 1, 24, 42, and 70. PFNA clearly caused expression of genes associated with PPAR-alpha activation in fetal and pup liver. Gene changes associated with PPAR-alpha persisted until PND 42, although the effects were weaker after PND 24.

Summary

In this study, several dose-dependent maternal and developmental effects were seen at PFNA 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 developmental exposure persisted well into adulthood (PND 287), long after PFNA had been eliminated from the serum. Das et al. (2015) conclude that the adverse developmental effects caused by PFNA were more severe and persistent than from PFOA, likely related to PFNA's greater intrinsic potency and longer persistence in the body.

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 in 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 the last dose at all doses in non-pregnant WT and PPAR-alpha KO females, with significance in all groups except 0.83 mg/kg/day 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. However, it is important to note that, in mice that had given birth, PFNA serum levels in the KO mice were much lower than in the WT mice for reason(s) that were not determined. The NOAELs and LOAELs for increased liver weight based on serum levels are consistent in the WT and KO mice, suggesting that the differences in response is likely due to kinetic differences unrelated to PPAR alpha status.

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 Das et al. (2015) 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 specific low-dose developmental effects seen in mice given 0.01

mg/kg/day of PFOA, including delayed mammary gland development and histopathological effects on female reproductive system (reviewed in Post et al., 2012; Tucker et al., 2014), has not been evaluated.

Chronic effects/carcinogenicity

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

MODE OF ACTION

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

Perfluorinated carboxylic acids such as PFNA structurally resemble free fatty acids, and thus may act similarly to a free fatty acid in activating nuclear receptors such as PPAR-alpha, CAR (constitutive androstane receptor) and PXR (pregnane X receptor), binding to transporters and carrier proteins, and interacting with membranes (Butenhoff, 2009). However, PFNA and other PFCs are non-reactive and thus are not substrates for biochemical reactions involving fatty acids.

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

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), PFUnDA (C11) and PFDoA (C12) were less potent activators of both the human and mouse PPAR-alpha receptors than PFNA, with particularly low activity for activation of the human receptor, 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.

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's authors discuss results that indicate that primary hepatocytes are problematic as a model for *in vitro* effects, since many genes that are well known to be upregulated by PFCs *in vivo* were not affected in these *in vitro* cell assays.

In *in vivo* studies in rodents, PFNA activates PPAR-alpha, as well as other nuclear receptors such as CAR and PXR (Rosen et al., 2010; Das et al., 2015). These receptors are found in many tissues, and activation of these receptors affects the expression of genes involved in many pathways related to carcinogenicity, hepatotoxicity, metabolic functions, developmental toxicity, immunotoxicity, and other effects (Lau, 2012). 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). In *in vivo* studies of 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., 2010). Similarly, gene expression profiles in livers from PFNA-treated fetuses and pups also showed activation of PPAR-alpha, as well as CAR and PXR (Das et al., 2015).

The hepatic hypertrophy and hepatic lipid accumulations caused by PFNA in rodents has PPAR-alpha dependent and PPAR-alpha independent components (Rosen et al., 2010: Wolf et al., 2010), as is also the case for PFOA (reviewed in Post et al., 2012; Filgo et al., 2015; Quist et al., 2015). 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 mice that had given birth, the magnitude of liver weight increases in WT and KO mice were similar when the two strains were compared on the basis of PFNA serum levels (Wolf et al., 2010). Finally, a study presented in poster/abstract form reported that hepatic triglyceride levels and lipid accumulation were increased in both SV129 wild type (WT) and PPAR-alpha null (knockout, KO) mice given 10 mg/kg/day PFNA for 7 days (Das et al., 2013).

PPAR-alpha, as well as PPAR-beta and PPAR-gamma, are expressed in many fetal and adult tissues in rodents and humans, and their patterns of expression vary with developmental age (Abbott et al., 2010). Based on their physiological roles, they are expected to have important roles in reproduction and development in these species (Abbott, 2009). In regard to human relevance of PPPAR-alpha mediated effects of PFNA, Wolf et al. (2010) state: "Relevance of the PPAR-alpha mechanism to humans has been criticized primarily based on the lower number of these receptors in the liver of human versus mouse. However, PPARα is implicated here in the developmental effects of PFNA as well, and the etiology of PPARα in other tissues of the embryo, fetus and neonate of the human and the mouse that are involved in gross development has not been fully determined. Therefore, the possibility of relevance of PPARα to a human response to PFNA cannot be dismissed." Additionally, the USEPA Science Advisory Board (2006) concluded that available data are insufficient to dismiss the relevance of the PPAR-alpha MOA in children.

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., 2010).

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 effects on both Kupffer cells (hepatic macrophages) and hepatocytes contribute to the liver toxicity of PFNA. Additional information relevant to the mode of action of PFNA, including studies in zebrafish and other studies, are discussed in NJDWQI (2015). 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 that is 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 long-chain PFCs such as PFNA is much longer in humans (several years) than in rats and mice, a given administered dose (mg/kg/day) results in a much greater internal dose (as indicated by serum level) in humans than in these animal species. Therefore, comparisons between effect levels in animal studies and human exposures were made on the basis of serum levels rather than administered dose.

As discussed above, ongoing exposure to PFOA in drinking water increases PFOA serum levels, on average, in a serum:drinking water ratio of at least 100:1 with several studies indicating ratios substantially above 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 indicate 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 is intended to represent a central tendency estimate, rather than an upper percentile value, for the PFNA ratio.

As discussed above, a factor of 0.08 (ng/kg/day)/(ng/ml) relating PFNA intake and increase in serum PFNA level corresponds to a 200:1 serum:drinking water ratio for PFNA, assuming the mean daily water consumption value recommended by USEPA (2011) of 16 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; Clewell, 2009) is 1.59 times the daily PFNA dose estimated to increase the serum level by 1 ng/ml.

Key and Supporting Studies and Endpoints

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

The quantitative basis for the interim ground water standard is increased liver weight in pregnant mice in a developmental study conducted by USEPA in which mice were dosed with 0, 1, 3, or 5 mg/kg/day PFNA throughout gestation (Das et al., 2015). Increased liver weight is a well-established effect of PFNA and other PFCs in experimental animals. Das et al. (2015) was selected as the only study appropriate for use as the basis for quantitative risk assessment because it provides the numerical serum PFNA data, including statistical parameters, needed for benchmark dose (BMD) modeling. (These numerical data corresponding to the same data presented graphically in Das et al (2015) were provided by C. Lau.) PFNA serum levels at the time point when liver weight was measured are also available in a study of male mice exposed to 0, 0.2, 1, or 5 mg/kg/day PFNA for 14 days (Wang et al., 2015). When compared on the basis of PFNA serum levels, the NOAELs and LOAELs for increased liver weight are consistent in male mice (Wang et al., 2015) and pregnant female mice (Das et al., 2015), and the dose-response curves for increased relative liver weight are similar in the two studies.

The only other toxicology studies in which serum PFNA data were reported are the developmental toxicity study in Wild Type and PPAR-alpha knockout 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 Das et al. (2015), 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.

Dose-response curves for endpoints of developmental toxicity assessed in the offspring in Das et al. (2015) are similar as for maternal liver weight. These endpoints were not used as the basis for quantitative dose response modeling of maternal serum levels because of greater uncertainty about the relationship of maternal serum levels on GD17 to these effects that were assessed 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 male offspring persisted until at least 287 days of age, long after serum PFNA levels had declined to background (control) levels.

PFNA caused similar effects at similar or lower doses in a study in male mice (Wang et al., 2015) and a second developmental study in another strain of mice (Wolf et al., 2010). PFNA also caused several other types of toxicity in other studies at similar or lower doses and/or estimated serum levels than those ($\geq$1 mg/kg/day) causing increased maternal liver weight in Das et al. (2015). These studies are discussed in detail above, and include: increased liver weight at 0.45 mg/kg/day in mice dosed for 21 days (Kennedy, 1987); increased serum glucose and other effects at 1 mg/kg/day, and related biochemical effects at 0.2 mg/kg/day, in mice dosed for 14 days (Fang et al., 2012a); immunotoxicity at 1 mg/kg/day in mice dosed for 14 days (Fang et al., 2008); and damage to testicular Sertoli cells and other related effects at 1 mg/kg/day in rats dosed for 14 days. 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 Das et al. (2015), 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 2.6.0.86 (USEPA, 2015b) was used to perform BMD modeling of the data on liver weight on GD 17 in pregnant mice exposed to PFNA on GD 1-17 (Das et al., 2015).

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

BMD and the BMDL serum levels were determined for a 10% increase from the mean liver weight in the pregnant control mice using EPA BMD software (version 2.6.0.86).All models for continuous

data included in the software were run. Results of the BMD modeling are shown in Table 5, and the complete output of the BMDS software for each model are presented in Appendix 1.

The Hill model and the Exponential model 5 gave almost identical AIC statistics, and these were the lowest AIC values of all the models run. Both of these models also show an excellent visual fit to the data (Appendix 1). As there is no basis for choosing between two models that yield comparable values, have equally good statistics and fit, and are equally biologically plausible, the average of the BMDLs from these two models was used as the Point of Departure. The BMDLs for the Exponential model 5 and the Hill model are 4.43 µg/ml and 5.43 µg/ml, respectively. The average of these values is 4.93 µg/ml which rounds to 4.9 µg/ml (4,900 ng/ml).

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

Table 5: Benchmark Dose Modeling for 10% Increase in Liver Weight in Pregnant Mice from Das et al. (2015)

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

Derivation of health-based water concentration

Application of uncertainty factors

The choice of uncertainty factors is consistent with current USEPA IRIS guidance (USEPA, 2012c) and previous risk assessments developed by NJDEP and the New Jersey Drinking Water Quality Institute.

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

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

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

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

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

10 – Duration of exposure

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

3 – Incomplete database

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

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

Relative Source Contribution factor

A Relative Source Contribution (RSC) factor that accounts for non-drinking water sources including food, soil, air, water, and consumer products is used in the development of health-based drinking water concentrations based on non-carcinogenic effects. This approach is used by NJDEP for health-based ground water criteria, by the New Jersey Drinking Water Quality Institute (DWQI) for Health-based MCLs, by USEPA for Maximum Contaminant Level Goals, and by other states in development of similar health-based drinking water values. An RSC is intended to prevent total exposure from all sources from exceeding the RfD (USEPA, 2000). When sufficient chemical-specific information on

non-drinking water exposures are not available, a default RSC of 0.2 is used, meaning that 20% of the RfD may come from drinking water (USEPA, 2000). When sufficient chemical-specific exposure data are available, a chemical-specific RSC may be derived, with floor and ceiling RSC values of 20% and 80%, respectively (USEPA, 2000).

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

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

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

Because the most recent fish data from 2010 and 2012 do not suggest elevated exposures from recreationally caught fish in communities where PFNA is present in drinking water, the 95th percentile PFNA serum level of 2.54 ng/mL from 2011-12 NHANES (CDC, 2015), which rounds to 2.5 ng/ml, is assumed to represent a reasonable and protective estimate of total non-drinking water exposure. It was therefore considered appropriate to use this 95th percentile serum value to develop a chemical-specific RSC for PFNA. The RSC for PFNA is developed using the "subtraction" approach described by USEPA (2000), but on the basis of serum levels rather than administered dose.

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

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

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

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

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

The factor relating PFNA intake (ng/kg/day) and increase in serum level (ng/ml) of 0.08 (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 2.5 ng/ml (2500 ng/L) as follows.

$$\frac{0.08 \text{ ng/kg/day}}{\text{ng/ml}} \times 2.5 \text{ ng/ml} = 0.2 \text{ ng/kg/day}$$

The average daily water consumption value for drinking water consumers of all ages recommended by USEPA (2011) is 16 ml/kg/day (0.016 L/kg/day). Based on this value, the drinking water concentration that will result in exposure to 0.2 ng/kg/day is:

$$\frac{0.2 \text{ ng/kg/day}}{0.016 \text{ L/kg/day}} = 12.5 \text{ ng/L which rounds to } 13 \text{ ng/L}$$

Using the chemical specific RSC of 0.5 and default drinking water consumption and body weight assumptions for derivation of interim specific ground water criteria for a non- carcinogen, the Reference Dose that support the derivation of a criterion of 13 ng/L is 0.74 ng/kg/day, as follows:

$$\frac{13 \text{ ng/L} \times 2 \text{ L/day}}{70 \text{ kg} \times 0.5} = 0.74 \text{ ng/kg/day}$$

And:

$$\frac{0.74 \text{ ng/kg/day} \times 70 \text{ kg} \times 0.5}{2 \text{ L/day}} = 13 \text{ ng/L}$$

Where: 13 ng/L = Interim specific ground water criterion
70 kg = Average adult body weight
2 L/day = Assumed daily water consumption
0.5 = 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 10 ng/L (0.01 µg/L).**

DISCUSSION OF UNCERTAINTIES

- Ongoing exposure to PFNA at 10 ng/L (0.01 µg/L) in drinking water is estimated to contribute an additional 2 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 2.9 ng/ml, 3.2-fold higher than the geometric mean serum level of about 0.9 ng/ml in the adult general population (which is assumed to result from exposure sources other than drinking water). In infants and children, serum levels from ongoing exposure to 10 ng/L PFNA in drinking water would likely be greater than in adults, due to their greater water consumption on a body weight basis.

Although the epidemiological data on PFNA is limited, several epidemiological studies have found associations of PFNA serum levels in the general population with potentially important health endpoints; some of these affect the same biological systems that are targets for PFNA toxicity in animal studies. Causality cannot be established for these effects because of the cross-sectional design of the studies and because, in some studies, the associations cannot be definitively attributed to PFNA because of correlations with other PFCs. However, these data contribute to the overall body of evidence about the potential hazard of PFNA. Thus, there is uncertainty about the extent of protection provided by a criterion that will result in serum PFNA levels that are several-fold above the general population range.

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

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

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

- Histopathological changes in the liver, including necrosis, occurred in F_0 and F_1 males in the two-generation rat study (Stump et al., 2008) at a dose (Surflon S-111, 0.025 mg/kg/day; PFNA, 0.019 mg/kg/day) below the doses that caused increased liver weight in the same study. Thus, histopathological changes, including necrosis, were a more sensitive endpoint than increased liver weight in this rat study. No NOAEL was identified for these histopathological effects in liver in the two generation rat study, and the LOAEL in male rats was 0.025 mg/kg/day Surflon S-111 (0.019 mg/kg/day PFNA). It is important to note that the histopathological changes in the liver reported by Fang et al. (2012a), Stump et al. (2008), and Mertens et al. (2010) are not of the same nature as are associated with PPAR-alpha activation (peroxisome proliferation and increased smooth endoplasmic reticulum), suggesting that PFNA causes liver toxicity independent of PPAR-alpha mediated effects.

  In part because numerical serum level data are not available, this study and endpoint cannot be used for quantitative risk assessment. Available graphical information from this study and the accompanying study (Mertens et al., 2010) suggests that the serum PFNA level in males at 0.025 mg/kg/day was well below the serum PFNA level at the lowest dose (1 mg/kg/day) in the Das et al., (2014) mouse developmental study used as the basis for quantitative risk assessment. However, histopathological changes in the maternal and pup liver were not evaluated by Das et al. (2015), and it is not known if these effects occurred in this study. Quantitative risk assessment based on liver histopathology could result in a significantly lower BMDL than the one based on Das et al. (2015).

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

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

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

- There is uncertainty about the factor relating drinking water intake and the increase in serum level of 0.08 (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 scientific studies of serum levels in communities exposed to PFNA in drinking water are not available. Although upper percentile exposure assumptions are typically used in risk assessment, these values are intended to represent central tendency estimates, rather than upper percentile values.

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

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

CITATIONS

Abbott, B.D. (2009). Review of the expression of peroxisome proliferator-activated receptors alpha (PPAR alpha), beta (PPAR beta), and gamma (PPAR gamma) in rodent and human development. Reprod. Toxicol. 27, 246–257.

Abbott, B.D., Wood, C.R., Watkins, A.M., Das, K.P., Lau, C.S. (2010). Peroxisome proliferator-activated receptors alpha, beta, and gamma mRNA and protein expression in human fetal tissues. PPAR Res. pii: 690907, 26. Epub 2010 Jul.

ALS Environmental. Pefluorononanoic acid. <http://www.caslab.com/Perfluorononanoic-Acid.php5> Accessed February 6, 2014.

ATSDR. (2009). Agency for Toxics Substances and Disease Registry. Toxicological Profile for Perfluoroalkyls. Draft for Public Comment. May 2009. <http://www.atsdr.cdc.gov/toxprofiles/tp200.pdf>

Bach, C.C., Bech, B.H., Brix, N., Nohr, E.A., Bonde, J.P., Henriksen, T.B. (2015). Perfluoroalkyl and polyfluoroalkyl substances and human fetal growth: A systematic review. Crit. Rev. Toxicol. 45, 53-67.

Barry, V., Winquist, A., Steenland, K. (2013). Perfluorooctanoic acid (PFOA) exposures and incident cancers among adults living near a chemical plant. Environ. Health Perspect. 121, 1313-8.

Beesoon, S., Webster, G.M., Shoeib, M., Harner, T., Benskin, J.P., Martin, J.W. (2011). Isomer profiles of perfluorochemicals in matched maternal, cord, and house dust samples: manufacturing sources and transplacental transfer. Environ. Health Perspect. 119, 1659-64.

Benninghoff, A.D., Bisson,W.H., Koch, D.C., Ehresman, D.J., Kolluri, S.K., Williams, D.E. (2011). Estrogen-like activity of perfluoroalkyl acids in vivo and interaction with human and rainbow trout estrogen receptors in vitro. Toxicol. Sci. 120, 42–58.

Benninghoff , A.D., Orner, G.A., Buchner, C.H., Hendricks, J.D., Duffy, A.M., Williams, D.E. (2012). Promotion of hepatocarcinogenesis by perfluoroalkyl acids in rainbow trout. Toxicol. Sci. 125, 69-78.

Benskin, J.P., De Silva, A.O., Martin, L.J., Arsenault, G., McCrindle, R., Riddell, N., Mabury, S.A., Martin, J.W. (2009). Disposition of perfluorinated acid isomers in Sprague-Dawley rats; part 1: single dose. Environ Toxicol Chem. 28:542-54.

Biegel, L.B., Hurtt, M.E., Frame, S.R., O'Connor, J.C., Cook, J.C. (2001) Mechanisms of extrahepatic tumor induction by peroxisome proliferators in male CD rats. Toxicol. Sci. 60, 44–55.

Bloom, M.S., Kannan, K., Spliethoff, H.M., Tao, L., Aldous, K.M., Vena, J.E. (2010). Exploratory assessment of perfluorinated compounds and human thyroid function. Physiol. Behav. 99, 240-5.

Buck, R.C., Franklin., J., Berger, U., Conder, J.M., Cousins, I.T., de Voogt, P., Jensen,

A.A., Kannan, K., Mabury, S.A., van Leeuwen, S.P. (2011). Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. Integr. Environ. Assess. Manag. 7, 513–541.

Butenhoff, J.L. (2009). Mechanistic and pharmacokinetic determinants of perfluoroalkyl toxicity. PowerPoint Presentation to USEPA, USEPA, Washington DC.

Butt, C.M., Berger, U., Bossi, R., Tomy, G.T. (2010). Review: levels and trends of poly and perfluorinated compounds in the arctic environment. Sci. Total Environ. 408, 2936–2965.

Butt, C.M., Muir, D.C., Mabury, S.A. (2014). Biotransformation pathways of fluorotelomer-based polyfluoroalkyl substances: a review. Environ. Toxicol Chem. 33:243-67.
C8 Science Panel website. C8 Science Panel reports and publications. http://www.c8sciencepanel.org/ Accessed February 6, 2014.

CDC (2015). Centers for Disease Control and Prevention. NHANES 2011-2012. http://wwwn.cdc.gov/nchs/nhanes/search/nhanes11_12.aspx Accessed January 22, 2015.

Chen, M.H., Ha, E.H., Wen, T.W., Su, Y.N., Lien, G.W., Chen, C.Y., Chen, P.C., Hsieh, W.S. (2012). Perfluorinated compounds in umbilical cord blood and adverse birth outcomes. PLoS. One 7, e42474.

Christensen, K.Y., Maisonet, M., Rubin, C., Holmes, A., Calafat, A.M., Kato, K., Flanders, W.D., Heron, J., McGeehin, M.A., Marcus, M. (2011). Exposure to polyfluoroalkyl chemicals during pregnancy is not associated with offspring age at menarche in a contemporary British cohort. Environ. Int. 37, 129-35.

Clewell, H.J. (2006). Application of pharmacokinetic modeling to estimate PFOA exposures associated with measured blood concentrations in human populations. Society for Risk Analysis 2006 Annual Meeting (PowerPoint Presentation).

Clewell, H.J. (2009). Pharmacokinetic modeling of PFOA and PFOS, USEPA, Washington DC. PowerPoint Presentation to USEPA.

Conder, J.M., Hoke, R.A., De Wolf, W., Russell, M.H., Buck, R.C. (2008). Are PFCAs bioaccumulative? A critical review and comparison with regulatory criteria and persistent lipophilic compounds. Environ. Sci. Technol. 42: 995–1003.

Das, K.P., Wood, C.R., Rosen, M.B., Lau, C., Abbott, B.D. (2013). Short-term exposure to perfluoroalkyl acids causes increase of hepatic lipid and triglyceride in conjunction with liver hypertrophy. The Toxicologist, Abstract #1981.

Das, K.P., Grey, B.E., Rosen, M.B., Wood, C.R., Tatum-Gibbs, K.R., Zehr, R.D., Strynar, M.J., Lindstrom, A.B., Lau, C. (2015). Developmental toxicity of perfluorononanoic acid in mice. Reproductive Toxicology 51:133-144.

Davis, K.L., Aucoin, M.D., Larsen, B.S., Kaiser, M.A., Hartten, A.S.. (2007). Transport of ammonium perfluorooctanoate in environmental media near a fluoropolymer manufacturing

facility. Chemosphere. 67: 2011-2019.

D'eon, J.C., Crozier, P.W., Furdui, V.I., Reiner, E.J., Libelo, E.L., Mabury, S.A. (2009). Observation of a commercial fluorinated material, the polyfluoroalkyl phosphoric acid diesters, in human sera, wastewater treatment plant sludge, and paper fibers. Environ. Sci. Technol. 43: 4589–4594.

De Silva, A.O., Benskin, J.P., Martin, L.J., Arsenault, G., McCrindle, R., Riddell, N., Martin, J.W., Mabury, S.A. (2009). Disposition of perfluorinated acid isomers in Sprague-Dawley rats; part 2: subchronic dose. Environ Toxicol Chem. 28:555-67.

Dewitt, J.C., Shnyra, A., Badr, M.Z., Loveless, S.E., Hoban, D., Frame, S.R., Cunard, R., Anderson, S.E., Meade, B.J., Peden-Adams, M.M., Luebke, R.W., Luster, M.I. (2009). Immunotoxicity of perfluorooctanoic acid and perfluorooctane sulfonate and the role of peroxisome proliferator-activated receptor alpha. Crit. Rev. Toxicol. 39, 76–94.

Dewitt, J.C., Peden-Adams, M.M., Keller, J.M., Germolec, D.R., 2012. Immunotoxicity of perfluorinated compounds: recent developments. Toxicol. Pathol. 40, 300–311.

Dong, G.H., Tung, K.Y., Tsai C.H., Liu, M.M., Wang, D., Liu, W., Jin, Y.H., Hsieh, W.S., Lee, Y.L., Chen, P.C. (2013). Serum polyfluoroalkyl concentrations, asthma outcomes, and immunological markers in a case-control study of Taiwanese children. Environ. Health Perspect. 121, 507-13, 513e1-8.

DRBC (2009). Delaware River Basin Commission. Contaminants of Emerging Concern in the Tidal Delaware River Pilot Monitoring Survey 2007−2009. Delaware River Basin Source Water Collaborative Webinar. Source Water Protection and Contaminants of Emerging Concern. September 25, 2012. <http://www.state.nj.us/drbc/library/documents/DRBSWC-MacGillivray-2012.pdf>

DRBC (2012). Delaware River Basin Commission. Contaminants of emerging concern in the tidal Delaware River. Pilot monitoring survey, 2007−2009, July 2012. Revised August 2013. <http://www.state.nj.us/drbc/ library/documents/contaminants-of-emerging-concernAug2013rev.pdf>

ECHA (2014). European Chemical Agency. Minutes of the 30th Meeting of the ECHA Committee for Risk Assessment. September 2014. http://echa.europa.eu/documents/10162/13579/rac_30_minutes_en.pdf

Ellis, D.A., Martin, J.W., De Silva, A.O., Mabury, S.A., Hurley, M.D., Sulbaek Andersen, M.P., Wallington, T.J. (2004). Degradation of fluorotelomer alcohols: a likely atmospheric source of perfluorinated carboxylic acids. Environ. Sci. Technol. 38, 3316-21.

Emmett, E.A., Shofer, F.S., Zhang, H., Freeman, D., Desai, C., Shaw, L.M. (2006). Community exposure to perfluorooctanoate: Relationships between serum concentrations and exposure sources. J. Occ. Environ. Med. 48, 759–770.

Ericson, I., Domingo, J. L., Nadal, M., Bigas, E., Llebaria, X., van Bavel, B., Lindstro̧m, G. (2009). Levels of perfluorinated chemicals in municipal drinking water from Catalonia, Spain: Public health implications. Arch. Environ. Contam. Toxicol. 57, 631−638.

Eriksen, K.T., Raaschou-Nielsen, O., Sørensen, M., Roursgaard, M., Loft, S., Møller, P. (2010). Genotoxic potential of the perfluorinated chemicals PFOA, PFOS, PFBS, PFNA and PFHxA in human HepG2 cells. Mutat. Res. 700, 39-43.

Fairley, K.J., Purdy, R., Kearns, S., Anderson, S.E., Meade, B.J. (2007). Exposure to the immunosuppressant, perfluorooctanoic acid, enhances the murine IgE and airway hyperreactivity response to ovalbumin. Toxicol. Sci. 97, 375–383.

Fang, X., Zhang, L., Feng, Y., Zhao, Y., Dai, J. (2008). Immunotoxic effects of perfluorononanoic acid on BALB/c mice. Toxicol. Sci. 105, 312−321.

Fang, X., Feng, Y., Wang, J., Dai, J. (2010). Perfluorononanoic acid-induced apoptosis in rat spleen involves oxidative stress and the activation of caspase-independent death pathway. Toxicology 267, 54-9.

Fang, X., Gao, G., Xue, H., Zhang, X., Wang, H. (2012a). Exposure of perfluorononanoic acid suppresses the hepatic insulin signal pathway and increases serum glucose in rats. Toxicology 294, 109-15.

Fang, X., Gao, G., Xue, H., Zhang, X., Wang, H. (2012b). In vitro and in vivo studies of the toxic effects of perfluorononanoic acid on rat hepatocytes and Kupffer cells. Environ. Toxicol. Pharmacol. 34, 484-94.

Fang, X., Zou, S., Zhao, Y., Cui, R., Zhang, W., Hu, J., Dai, J. (2012c) Kupffer cells suppress perfluorononanoic acid-induced hepatic peroxisome proliferator-activated receptor α expression by releasing cytokines. Arch. Toxicol. 86, 1515-25.

Fasano, W.J., Kennedy, G.L., Szostek, B., Farrar, D.G., Ward, R.J., Haroun, L., Hinderliter, P.M. (2005). Penetration of ammonium perfluorooctanoate through rat and human skin in vitro. Drug Chem. Toxicol. 28, 79-90.

Feng, Y., Shi, Z., Fang, X., Xu, M., Dai, J. (2009). Perfluorononanoic acid induces apoptosis involving the Fas death receptor signaling pathway in rat testis. Toxicol. Lett. 190, 224-30.

Feng, Y., Fang, X., Shi, Z., Xu, M., Dai, J. (2010). Effects of PFNA exposure on expression of junction-associated molecules and secretory function in rat Sertoli cells. Reprod. Toxicol. 30, 429-37.

Filgo, A.J., Quist, E.M., Hoenerhoff, M.J., Brix, A.E., Kissling, G.E., Fenton, S.E. (2015). Perfluorooctanoic acid (PFOA)-induced liver lesions in two strains of mice following developmental exposures: PPARα is not required. Toxicol. Pathol. 43:558-68.

Franko J, Meade BJ, Frasch HF, Barbero AM, Anderson SE. (2012). Dermal penetration potential of perfluorooctanoic acid (PFOA) in human and mouse skin. J Toxicol Environ Health A. 75:50-62.

Frisbee, S.J., Brooks Jr., A.P., Maher, A., Flensborg, P., Arnold, S., Fletcher, T., Steenland, K., Shankar, A., Knox, S.S., Pollard, C., Halverson, J.A., Vieira, V.M., Jin, C., Leyden, K.M.,

Ducatman, A.M. (2009). The C8 Health Project: design, methods, and participants. Environ. Health Perspect. 117, 1873–1882.

Fromme, H., Mosch, C., Morovitz, M., Alba-Alejandre, I., Boehmer, S., Kiranoglu, M., Faber, F., Hannibal, I., Genzel-Borovicze!ny, O., Koletzko, B., Volkel, W. (2010). Pre- and postnatal exposure to perfluorinated compounds (PFCs). Environ. Sci. Technol. 44, 7123–7129. and Supporting Information.

Fujii Y., Yan, J., Harada, K.H., Hitomi, T., Yang, H., Wang, P., Koizumi, A. (2012). Levels and profiles of long-chain perfluorinated carboxylic acids in human breast milk and infant formulas in East Asia. Chemosphere 86, 315-21.

Gallo, V., Leonardi, G., Brayne, C., Armstrong, B., Fletcher, T. (2013). Serum perfluoroalkyl acids concentrations and memory impairment in a large cross-sectional study. BMJ Open. 3.

Gleason, J.A., Post, G.B., Fagliano, J.A. (2015). Associations of perfluorinated chemical serum concentrations and biomarkers of liver function and uric acid in the US population (NHANES), 2007-2010. Environ Res. 136:8-14.

Goss, K.U. (2008). The pKa values of PFOA and other highly fluorinated carboxylic acids. Environ. Sci. Technol. 42 , 456–458.

Grandjean, P., Andersen, E.W., Budtz-Jørgensen, E., Nielsen, F., Mølbak, K., Weihe, P., Heilmann, C. (2012). Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. JAMA. 307: 391-7.

Granum, B., Haug, L.S., Namork, E., Stølevik, S.B., Thomsen, C., Aaberge, I.S., van Loveren, H., Løvik, M., Nygaard, U.C. (2013). Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood. J. Immunotoxicol. 10, 373-9.

Gump, B.B., Wu, Q., Dumas, A.K., Kannan, K. (2011). Perfluorochemical (PFC) exposure in children: associations with impaired response inhibition. Environ. Sci. Technol. 45, 8151-9.

Gützkow, K.B., Haug, L.S., Thomsen, C., Sabaredzovic, A., Becher, G., Brunborg, G. (2012). Placental transfer of perfluorinated compounds is selective--a Norwegian Mother and Child sub-cohort study. Int. J. Hyg. Environ. Health 215, 216-9.

Halldorsson, T.I., Rytter, D., Haug, L.S., Bech, B.H., Danielsen, I., Becher, G., Henriksen, T.B., Olsen, S.F. (2012). Prenatal exposure to perfluorooctanoate and risk of overweight at 20 years of age: a prospective cohort study. Environ. Health Perspect. 120, 668-73.

Han, X., Nabb, D.L., Russell, M.H., Kennedy, G.L., Rickard, R.W. (2012). Renal elimination of perfluorocarboxylates (PFCAs). Chem. Res. Toxicol. 25, 35–46.

Harada, K., Inoue, K., Morikawa, A., Yoshinaga, T., Saito, N., Koizumi, A. (2005). Renal clearance of perfluorooctane sulfonate and perfluorooctanoate in humans and their species-specific excretion. Environ. Res. 99, 253–261.

Harada, K.H. and Koizumi, A. (2009). Environmental and biological monitoring of persistent fluorinated compounds in Japan and their toxicities. Environ Health Prev Med. 14:7-19.

Hardell, E., A. Karrman, B. van Bavel, J. Bao, M. Carlberg and L. Hardell (2014). "Case-control study on perfluorinated alkyl acids (PFAAs) and the risk of prostate cancer." Environ Int 63: 35-39.

Henderson, W.M., Smith, M.A. (2007). Perfluorooctanoic acid and perfluorononanoic acid in fetal and neonatal mice following in utero exposure to 8-2 fluorotelomer alcohol. Toxicol. Sci. 95, 452-61.

Hoffman, K., Webster, T.F., Weisskopf, M.G., Weinberg, J., Vieira, V.M. (2010). Exposure to polyfluoroalkyl chemicals and attention deficit/hyperactivity disorder in U.S. children aged 12–15 years. Environ. Health Perspect. 118, 1762–1767.

Hoffman, K., Webster, T.F., Bartell, S.M., Weisskopf, M.G., Fletcher, T., Vieira, V.M. (2011). Private drinking water wells as a source of exposure to PFOA in communities surrounding a fluoropolymer production facility. Environ. Health Perspect. 119, 92–97.

Hölzer, J., Midasch, O., Rauchfuss, K., Kraft, M., Reupert, R., Angerer, J., Kleeschulte, P., Marschall, N., Wilhelm, M. (2008). Biomonitoring of perfluorinated compounds in children and adults exposed to perfluorooctanoate-contaminated drinking water. Environ. Health Perspect. 116, 651–657.

Houde, M., Wells, R.S., Fair, P.A., Bossart, G.D., Hohn, A.A., Rowles, T.K., Sweeney, J.C., Solomon, K.R., Muir, D.C. (2005). Polyfluoroalkyl compounds in free-ranging bottlenose dolphins (Tursiops truncatus) from the Gulf of Mexico and the Atlantic Ocean. Environ. Sci. Technol. 39:6591-6598.

Houde, M., De Silva, A.O., Muir, D.C., Letcher, R.J. (2011). Monitoring of perfluorinated compounds in aquatic biota: an updated review. Environ. Sci. Technol. 45, 7962–7973.

Humblet, O., L. G. Diaz-Ramirez, J. R. Balmes, S. M. Pinney and R. A. Hiatt. (2014). Perfluoroalkyl chemicals and asthma among children 12-19 years of age: NHANES (1999-2008). Environ Health Perspect. 122, 1129-1133.

Integral Consulting Inc. (2013). Perfluorinated Compounds Work Plan. West Deptford, New Jersey, Plant Prepared for Solvay Specialty Polymers USA, LLC. November 15, 2013. Posted at http://www.delawareriverkeeper.org/resources/Reports/Solvay%20integral_2013_perfluorinated_compounds_work_plan_11_15_2013.pdf

Jain, R. B. (2013). Association between thyroid profile and perfluoroalkyl acids: data from NHANES 2007-2008. Environ Res 126: 51-59.

Joensen, U.N., Bossi, R., Leffers, H., Jensen, A.A., Skakkebaek, N.E., Jørgensen, N. (2009). Do perfluoroalkyl compounds impair human semen quality? Environ. Health Perspect. 117, 923–927.

Johansson, N., Fredriksson, A., Eriksson, P. (2008). Neonatal exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) causes neurobehavioural defects in adult mice.

Neurotoxicology 29, 160–169.

Johnson, J. D., Gibson, S. J., and Ober, R. E. (1979). Extent and route of excretion and tissue distribution of total cabon-14 in rats after a single i.v. dose of FC-95-14C. Riker Laboratories, Inc., St Paul, MN, US EPA Administrative Record, 8EHQ-1180-00374. Cited in Lau et al. (2007).

Johnson PI, Sutton P, Atchley DS, Koustas E, Lam J, Sen S, Robinson KA, Axelrad DA, Woodruff TJ. (2014). The navigation guide-evidence-based medicine meets environmental health: systematic review of human evidence for PFOA effects on fetal growth.

Kato, K., Calafat, A.M., Wong, L.Y., Wanigatunga, A.A., Caudill, S.P., Needham, L.L. (2009). Polyfluoroalkyl compounds in pooled sera from children participating in the National Health and Nutrition Examination Survey 2001–2002. Environ. Sci. Technol. 43, 2641–2647.

Kato, K., Wong, L.Y., Jia, L.T., Kuklenyik, Z., Calafat, A.M. (2011). Trends in exposure to polyfluoroalkyl chemicals in the U.S. population: 1999–2008. Environ. Sci. Technol. 45, 8037–8045.

Kennedy, G.L. Jr. (1985). Dermal toxicity of ammonium perfluorooctanoate. Toxicol Appl Pharmacol. 81:348-355

Kennedy, G.L., Jr. (1987). Increase in mouse liver weight following feeding of ammonium perfluorooctanoate and related fluorochemicals. Toxicol. Lett. 39, 295-300.

Kim, S.K., Lee, K.T., Kang, C.S., Tao, L., Kannan, K., Kim, K.R., Kim, C.K., Lee, J.S., Park, P.S., Yoo, Y.W., Ha, J.Y., Shin, Y.S., Lee, J.H. (2011a). Distribution of perfluorochemicals between sera and milk from the same mothers and implications for prenatal and postnatal exposures. Environ. Pollut. 159, 169–174.

Kim, S., Choi, K., Ji, K., Seo, J., Kho, Y., Park, J., Kim, S., Park, S., Hwang, I., Jeon, J., Yang, H., Giesy, J.P. (2011b). Trans-placental transfer of thirteen perfluorinated compounds and relations with fetal thyroid hormones. Environ. Sci. Technol. 45, 7465-72.

Kinney, L.A., Chromey, N.C., Kennedy, G.L., Jr. (1989). Acute inhalation toxicity of ammonium perfluorononanoate. Food Chem. Toxicol. 27, 465-8.

Kudo, N., Bandai, N., Suzuki, E., Katakura, M., Kawashima, Y. (2000). Induction by perfluorinated fatty acids with different carbon chain length of peroxisomal beta-oxidation in the liver of rats. Chem. Biol. Interact. 124, 119-32.

Kudo, N., Suzuki, E., Katakura, M., Ohmori, K., Noshiro, R., Kawashima, Y. (2001). Comparison of the elimination between perfluorinated fatty acids with different carbon chain length in rats. Chem Biol Interact. 134: 203-16.

Kudo, N., Suzuki-Nakajima, E., Mitsumoto, A., Kawashima, Y. (2006). Responses of the liver to perfluorinated fatty acids with different carbon chain length in male and female mice:in relation to induction of hepatomegaly, peroxisomal beta-oxidation and microsomal 1- acylglycerophosphocholine acyltransferase. Biol. Pharm. Bull. 29, 1952-7.

Lau, C., Strynar, M. J., Lindstrom, A. B., Hanson, R. G., Thibodeaux, J. R., and Barton, H. A. (2005). Pharmacokinetic evaluation of perfluorooctanoic acid in the mouse. Toxicologist 84, 252 (cited in Lau et al., 2007).

Lau, C., Thibodeaux, J.R., Hanson, R.G., Narotsky, M.G., Rogers, J.M., Lindstrom, A.B., Strynar, M.J. (2006). Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. Toxicol. Sci. 90, 510–518.

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., Seed, J. (2007). Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicol. Sci. 99, 366–394.

Lau, C., Das, K.P., Tatum, K., Zehr, D., Wood, C.R., Rosen, M. B. (2009). Developmental toxicology of perfluorononanoic acid in the mouse. The Toxicologist 108, 417.

Lau, C. (2012). Perfluorinated compounds. EXS 101, 47−86.

Lin, C.Y., Chen, P.C., Lin, Y.C., Lin, L.Y. (2009). Association among serum perfluoroalkyl chemicals, glucose homeostasis, and metabolic syndrome in adolescents and adults. Diabetes Care. 32, 702-7.

Lin, C. Y., L. Y. Lin, C. K. Chiang, W. J. Wang, Y. N. Su, K. Y. Hung and P. C. Chen (2010). Investigation of the associations between low-dose serum perfluorinated chemicals and liver enzymes in US adults. Am J Gastroenterol 105(6): 1354-1363.

Lin, C.Y., Wen, L.L., Lin, L.Y., Wen, T.W., Lien, G.W., Chen, C.Y., Hsu, S.H., Chien, K.L., Sung, F.C., Chen, P.C., Su, T.C. (2011). Associations between levels of serum perfluorinated chemicals and adiponectin in a young hypertension cohort in Taiwan. Environ. Sci. Technol. 45, 10691-8.

Lin, C. Y., L. Y. Lin, T. W. Wen, G. W. Lien, K. L. Chien, S. H. Hsu, C. C. Liao, F. C. Sung, P. C. Chen and T. C. Su (2013a). Association between levels of serum perfluorooctane sulfate and carotid artery intima-media thickness in adolescents and young adults. Int. J. Cardiol. 168, 3309-16.

Lin, C.Y., Wen, L.L., Lin, L.Y., Wen, T.W., Lien, G.W., Hsu, S.H., Chien, K.L., Liao, C.C., Sung, F.C., Chen, P.C., Su, T.C. (2013b). The associations between serum perfluorinated chemicals and thyroid function in adolescents and young adults. J. Hazard. Mater. 244-245, 637-44.

Lind, L., Zethelius, B., Salihovic, S., van Bavel, B., Lind, P.M. (2014). Circulating levels of perfluoroalkyl substances and prevalent diabetes in the elderly. Diabetologia 57, 473-9.

Lindstrom, A.B., Strynar, M.J., Libelo, E.L. (2011a). Polyfluorinated compounds: Past, present, and future. Environ. Sci. Technol. 45, 7954–7961.

Liu, J., Li, J., Liu, Y., Chan, H.M., Zhao, Y., Cai, Z., Wu, Y. (2011). Comparison on gestation and lactation exposure of perfluorinated compounds for newborns. Environ. Int. 37, 1206–1212.

Looker, C., Luster, M.I., Calafat, A.M., Johnson, V.J., Burleson, G.R., Burleson, F.G., Fletcher, T. (2014). Influenza vaccine response in adults exposed to perfluorooctanoate and perfluorooctanesulfonate. Toxicol. Sci. 138, 76-88.

Lopez-Espinosa, M.J., Mondal, D., Armstrong, B., Bloom, M.S., Fletcher, T. (2012). Thyroid function and perfluoroalkyl acids in children living near a chemical plant. Environ. Health Perspect. 120, 1036-41.

Lorber, M., Eaglesham, G.E., Hobson, P., Toms, L.M., Mueller, J.F., Thompson, J.S. (2015). The effect of ongoing blood loss on human serum concentrations of perfluorinated acids. Chemosphere 118:170-177.

MDH. (2009). Minnesota Department of Health. East Metro Perfluorochemical Biomonitoring Pilot Project. July 21, 2009. <http://www.health.state.mn.us/divs/eh/tracking/finalpfcrpt.pdf>

MDH (2013). Minnesota Department of Health. Report to the Community. May 2013. http://www.health.state.mn.us/tracking/biomonitoring/projects/CommunityReport-May2013.pdf

Mertens, J.J., Sved, D.W., Marit, G.B., Myers, N.R., Stetson, P.L., Murphy, S.R., Schmit, B., Shinohara, M., Farr, C.H. (2010). Subchronic toxicity of S-111-S-WB in Sprague Dawley rats. Int. J. Toxicol. 29, 358−371.

Mondal, D., Lopez-Espinosa, M.J., Armstrong, B., Stein, C.R., Fletcher, T. (2012). Relationships of perfluorooctanoate and perfluorooctane sulfonate serum concentrations between mother-child pairs in a population with perfluorooctanoate exposure from drinking water. Environ. Health Perspect. 120, 752-7.

Monroy, R., Morrison, K., Teo, K., Atkinson, S., Kubwabo, C., Stewart, B., Foster, W.G. (2008). Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples. Environ. Res. 108, 56-62.

Mundt, D. J., K. A. Mundt, R. S. Luippold, M. D. Schmidt and C. H. Farr (2007). Clinical epidemiological study of employees exposed to surfactant blend containing perfluorononanoic acid. Occup Environ Med 64(9): 589-594.

Needham, L.L., Grandjean, P., Heinzow, B., Jørgensen, P.J., Nielsen, F., Patterson, D.G., Jr, Sjödin, A., Turner, W.E., Weihe, P. (2011). Partition of environmental chemicals between maternal and fetal blood and tissues. Environ. Sci. Technol. 45, 1121-6.

Nelson, J.W., Hatch, E.E., Webster, T.F. (2010). Exposure to polyfluoroalkyl chemicals and cholesterol, body weight, and insulin resistance in the general U.S. population Environ. Health Perspect. 118, 197-202.

Nilsson, H., Kärrman, A.,Westberg, H., Rotander, A., van Bavel, B., Lindström, G. (2010). A time trend study of significantly elevated perfluorocarboxylate levels in humans after using fluorinated ski wax. Environ. Sci. Technol. 44, 2150–2155.

NJDEP (2007). Guidance for PFOA in Drinking Water at Pennsgrove Water Supply Company. <http://www.nj.gov/dep/watersupply/pdf/pfoa_dwguidance.pdf>

NJDOH (2014). New Jersey Department of Health. ATSDR Technical Assistance Form. NJDOH response to NJDEP request for evaluation of showering/bathing exposure to PFNA.

NJDWQI (2015). New Jersey Drinking Water Quality Institute. Health-Based Maximum Contaminant Level Support Document: Perfluorononanoic Acid (PFNA). June 2015.

Ode, A., Rylander, L., Lindh, C.H., Källén, K., Jönsson, B.A., Gustafsson, P., Olofsson, P., Ivarsson, S.A., Rignell-Hydbom, A. (2013). Determinants of maternal and fetal exposure and temporal trends of perfluorinated compounds. Environ. Sci. Pollut. Res. Int. 20, 7970-8.

Ohmori, K., Kudo, N., Katayama, K., Kawashima, Y. (2003). Comparison of the toxicokinetics between perfluorocarboxylic acids with different carbon chain length. Toxicology 184, 135-40.

Olsen, G.W., Burris,J.M. ,Ehresman, D.J., Froehlich, J.W., Seacat, A.M., Butenhoff, J.L., Zobel, L.R. (2007). Half-life of serum elimination of perfluorooctane sulfonate perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environ. Health Perspect. 115, 1298–1305.

Olsen, G.W., Ellefson, M.E., Mair, D.C., Church, T.R., Goldberg, C.L., Herron, R.M., Medhdizadehkashi, Z., Nobiletti, J.B., Rios, J.A., Reagen, W.K., Zobel, L.R. (2011). Analysis of a homologous series of perfluorocarboxylates from American Red Cross adult blood donors, 2000-2001 and 2006. Environ. Sci. Technol. 45, 8022-9.

Olson, C.T., Andersen, M.E. (1983). The acute toxicity of perfluorooctanoic and perfluorodecanoic acids in male rats and effects on tissue fatty acids. Toxicol. Appl. Pharmacol. 70, 362-372.

Onischenko, N., Fischer, C., Wan Ibrahim, W.N., Negri, S., Spulber, S., Cottica, D., Ceccatelli, S. (2010). Prenatal exposure to PFOS or PFOA alters motor function in mice in a sex-related manner. Neurotox. Res. 19, 452–461.

Post, G.B., Louis, J.B., Cooper, K.R., Boros-Russo, B.J., Lippincott, R.L. (2009a). Occurrence and potential significance of perfluorooctanoic acid (PFOA) Detected in New Jersey public drinking water systems. Environ. Sci. Technol. 43, 4547–4554.

Post, G.B., Louis, J.B., Cooper, K.R., Lippincott, R.L. (2009b). Response to comment on ‘‘Occurrence and Potential Significance of Perfluorooctanoic Acid (PFOA) Detected in New Jersey Public Drinking Water Systems.’’ Environ. Sci. Technol. 43, 8699–8700.

Post, G.B., Cohn, P.D., and Cooper, K.R. (2012). Perfluorooctanoic acid (PFOA), an emerging drinking water contaminant: a critical review of recent literature. Env. Res. 116, 93-117.

Post, G.B., Louis, J.B., Lippincott, R.L., and Procopio, N.A. (2013). Occurrence of perfluorinated chemicals in raw water from New Jersey public drinking water systems. Environ. Sci. Technol. 47, 13266-75.

Power, M. C., T. F. Webster, A. A. Baccarelli and M. G. Weisskopf (2013). Cross-sectional association between polyfluoroalkyl chemicals and cognitive limitation in the National Health and Nutrition Examination Survey. Neuroepidemiology 40(2): 125-132.

Prevedouros, K., Cousins, I.T., Buck, R.C., Korzeniowski, S.H. (2006). Sources, fate and transport of perfluorocarboxylates. Environ. Sci. Technol. 40, 32−44.

Quist, E.M., Filgo, A.J,, Cummings, C.A., Kissling, G,E., Hoenerhoff, M.J., Fenton, S.E. (2015). Hepatic mitochondrial alteration in CD-1 mice associated with prenatal exposures to low doses of perfluorooctanoic acid (PFOA). Toxicol. Pathol. 43:546-57.

Rahman, M.F., Peldszus, S., Anderson, W.B. (2014). Behaviour, and fate of perfluoroalkyl and polyfluoroalkyl substances (PFASs) in drinking water treatment: a review. Water Res. 50, 318-40.

Rayne, S., Forest, K. (2010). Theoretical studies on the pKa values of perfluoroalkyl carboxylic acids. J. Mol. Struct. (Theochem) 949, 60–69.

Rockwell, C.E., Turley, A.E., Cheng, X., Fields, P.E., Klaassen, C.D. (2013). Acute Immunotoxic Effects of Perfluorononanoic Acid (PFNA) in C57BL/6 Mice. Clin. Exp. Pharmacol. S4: 002. doi:10.4172/2161-1459.S4-002

Rosen, M.B., Schmid, J.R., Zehr, R.D., Das, K.P., Ren, H., Abbott, B.D., Lau, C. (2010). Toxicogenomic profiling of perfluorononanoic acid in wild-type and PPARα-null mice. The Toxicologist 114: 47. Abstract #219.

Rosen, M.B., Das, K.P., Wood, C.R., Wolf, C.J., Abbott, B.D., Lau, C. (2013). Evaluation of perfluoroalkyl acid activity using primary mouse and human hepatocytes. Toxicology 308, 129-37.

Roux Associates Inc. (2013). West Deptford Plant PFC Usage and Emissions. November 15, 2013. Posted at http://www.delawareriverkeeper.org/resources/Reports/Solvay%20Plan%20PFC_Usage_Solvay_WestDeptfordPlant_11_15_2013.pdf

Russell, M.H., Berti, W.R., Szostek, B., Buck, R.C. (2008). Investigation of the biodegradation potential of a fluoroacrylate polymer product in aerobic soils. Environ. Sci. Technol. 42, 800–807.

Shin, H.M., Vieira, V.M,, Ryan, P.B., Detwiler, R., Sanders, B., Steenland, K., Bartell, S.M. (2011). Environmental fate and transport modeling for perfluorooctanoic acid emitted from the Washington Works Facility in West Virginia. Environ. Sci. Technol. 45: 1435-42.

Sibinski, L.J. (1987). Final report of a two year oral (diet) toxicity and carcinogenicity study of fluorochemical FC-143 (perfluorooctanoate ammonium carboxylate) in rats. Vols. 1–4, 3M Company/RIKER. No.0281CR0012; 8EHQ-1087-0394, October 16, 1987.

Starling, A. P., S. M. Engel, D. B. Richardson, D. D. Baird, L. S. Haug, A. M. Stuebe, K. Klungsoyr, Q. Harmon, G. Becher, C. Thomsen, A. Sabaredzovic, M. Eggesbo, J. A. Hoppin, G. S. Travlos, R. E. Wilson, L. I. Trogstad, P. Magnus and M. P. Longnecker (2014). Perfluoroalkyl substances during pregnancy and validated preeclampsia among nulliparous women in the Norwegian Mother and Child Cohort Study. Am J Epidemiol 179(7): 824-833.

Stein, C.R., Wolff, M.S., Calafat, A.M., Kato, K., Engel, S.M. (2012). Comparison of polyfluoroalkyl compound concentrations in maternal serum and amniotic fluid: a pilot study.

Reprod Toxicol. 34:312-6.

Stump, D.G., Holson, J.F., Murphy, S.R., Farr, C.H., Schmit, B., Shinohara, M. (2008). An oral two-generation reproductive toxicity study of S-111-S-WB in rats. Reprod. Toxicol. 25, 7−20.

Swedish Environmental Agency (2013). Proposal for Harmonized Classification and Labeling Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2. Substance Name: 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-Heptadecafluorononanoic acid and its sodium and ammonium salts. Prepared by Swedish Environmental Agency. December 2013. http://echa.europa.eu/documents/10162/13626/clh_proposal_2_9_heptadecafluorononanoic_en.pdf

Takahashi, M., Ishida, S., Hirata-Koizumi, M., Ono, A., Hirose, A. (2014). Repeated dose and reproductive/developmental toxicity of perfluoroundecanoic acid in rats. J. Toxicol. Sci. 39, 97-108.

Tao, L., Ma, J., Kunisue, T., Libelo, E.L., Tanabe, S., Kannan, K. (2008a). Perfluorinated compounds in human breast milk from several Asian countries, and in infant formula and dairy milk from the United States. Environ. Sci. Technol. 42, 8597–8602.

Tao, L., Kannan, K., Wong, C.M., Arcaro, K.F., Butenhoff, J.L. (2008b). Perfluorinated compounds in human milk from Massachusetts, USA. Environ. Sci. Technol. 42, 3096–3101.

Tatum-Gibbs, K., Wambaugh, J.F., Das, K.P., Zehr, R.D., Strynar, M.J., Lindstrom, A.B., Delinsky, A., Lau, C. (2011). Comparative pharmacokinetics of perfluorononanoic acid in rat and mouse. Toxicology 281, 48−55.

Taylor, K.W., Hoffman, K., Thayer, K.A., Daniels, J.L. (2014). Polyfluoroalkyl chemicals and menopause among women 20-65 years of age (NHANES). Environ. Health Perspect. 122, 145-50.

Thomford, P.J. (2002). 104-Week dietary chronic toxicity and carcinogenicity study with perfluorooctane sulfonic acid potassium salt (PFOS; T-6295) in rats. St. Paul, MN: 3M (cited in ATSDR, 2009). .

TOEFCO. Understanding uses for Kynar. <http://toefco.com/understanding-uses-for-kynar/> Accessed March 1, 2014.

Tucker, D.K., Macon, M.B., Strynar, M..J, Dagnino, S., Andersen, E., Fenton, S.E. (2014). The mammary gland is a sensitive pubertal target in CD-1 and C57Bl/6 mice following perinatal perfluorooctanoic acid (PFOA) exposure. Reprod Toxicol. Online Dec 12. doi: 10.1016/j.reprotox.2014.12.002 [Epub ahead of print]

University of Wisconsin. (2014). Animal Specific Training: Mice (Mus Musculus). <http://www4.uwm.edu/usa/acp/training/manual/manual_mice.cfm> Accessed 2/13/14.

USEPA (2000). Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health. Office of Science and Technology. Office of Water. Washington, DC. EPA 822-B-00-004. October

2000. http://water.epa.gov/scitech/swguidance/standards/upload/2005_05_06_criteria_humanhealth_method_complete.pdf

USEPA (2003). United States Environmental Protection Agency. Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds. Part III: Integrated Summary and Risk Characterization for 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds. NAS Review Draft. December 2003. <http://www.epa.gov/ncea/pdfs/dioxin/nas-review/pdfs/part3/dioxin_pt3_full_oct2004.pdf>.

USEPA (2005). United States Environmental Protection Agency. Draft Risk Assessment of the Potential Human Health Effects Associated with Exposure to Perfluorooctanoic Acid and Its Salts. Office of Pollution Prevention and Toxics. January 4, 2005. <http://www.epa.gov/oppt/pfoa/pubs/pfoarisk.pdfs> (accessed August 16, 2011).

USEPA (2006). United States Environmental Protection Agency. Science Advisory Board Review of EPA's Draft Risk Assessment of Potential Human Health Effects Associated with PFOA and Its Salts, May 30, 2006. http://www.epa.gov/sab/pdf/sab_06_006.pdf. Accessed June 20, 2014.

USEPA (2008). United States Environmental Protection Agency. Child-Specific Exposure Factors Handbook. Chapter 3 -Water Ingestion. Table 3-1. Accessible at "Downloads" at <http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=199243>.

USEPA (2009). United States Environmental Protection Agency. Existing Chemical Action Plans. Office of Pollution Prevention and Toxics. Long-Chain Perfluorinated Chemicals (PFCs) Action Plan. December 30, 2009. <http://www.epa.gov/opptintr/existingchemicals/pubs/pfcs_action_plan1230_09.pdf>.

USEPA (2010). United States Environmental Protection Agency. Office of Pollution Prevention and Toxics. 2010/2015 PFOA Stewardship Program. <http://www.epa.gov/opptintr/pfoa/pubs/stewardship/index.html>. Last updated April 29, 2010.

USEPA. (2011). Exposure Factors Handbook 2011 Edition (Final). U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-09/052F. http://www.epa.gov/ncea/efh/pdfs/efh-complete.pdf

USEPA (2012a). United States Environmental Protection Agency. Perfluorooctanoic Acid (PFOA) and Fluorinated Telomers 2011 Annual Progress Reports. Posted February 6, 2012. <http://www.epa.gov/oppt/pfoa/pubs/stewardship/preports5.html>.

USEPA (2012b). United States Environmental Protection Agency. The Third Unregulated Contaminant Monitoring Rule (UCMR 3). Fact Sheet for Assessment Monitoring of List 1 Contaminants. May 2012. <http://water.epa.gov/lawsregs/rulesregs/sdwa/ucmr/ucmr3/upload/UCMR3_FactSheet_List1.pd f>.

USEPA (2015a). United States Environmental Protection Agency. UCMR 3 (2013-2015) Occurrence Data. Last updated January 2015. http://water.epa.gov/lawsregs/rulesregs/sdwa/ucmr/data.cfm

USEPA (2012c) Preamble to IRIS toxicologic reviews. In: Draft Toxicological Review of Trimethylbenzenes (CAS No. 25551-13-7, 95-63-6, 526-73-8, and 108-67-8)In Support of Summary

Information on the Integrated Risk Information System (IRIS), June 2012.

USEPA (2015b). United States Environmental Protection Agency. Benchmark Dose Software (BMDS). <http://www.epa.gov/ncea/bmds/>.

Viberg, H., Lee, I., Eriksson, P. (2013). Adult dose-dependent behavioral and cognitive disturbances after a single neonatal PFHxS dose. Toxicology 304, 185−191.

Vieira, V.M., Hoffman, K., Shin, H.M., Weinberg, J.M., Webster, T.F., Fletcher, T. (2013). Perfluorooctanoic acid exposure and cancer outcomes in a contaminated community: A geographic analysis. Environ. Health. Perspect. 121, 318−233.

Wang, N., Szostek, B., Buck, R.C., Folsom, P.W., Sulecki, L.M., Capka, V., Berti, W.R., Gannon, J.T. (2005). Fluorotelomer alcohol biodegradation-direct evidence that perfluorinated carbon chains breakdown. Environ Sci Technol. 39:7516-28.

Wang, N., Szostek, B., Buck, R.C., Folsom, P.W., Sulecki, L.M., Gannon, J.T. 8-2 fluorotelomer alcohol aerobic soil biodegradation: pathways, metabolites, and metabolite yields. Chemosphere. 75:1089-96.

Wang, I.J., Hsieh, W.S., Chen, C.Y., Fletcher, T., Lien, G.W., Chiang, H.L., Chiang, C.F., Wu, T.N., Chen, P.C. (2011). The effect of prenatal perfluorinated chemicals exposures on pediatric atopy. Environ. Res. 111, 785–791.

Wang, Y., Starling, A.P., Haug, L.S., Eggesbo, M., Becher, G., Thomsen, C., Travlos, G., King, D., Hoppin, J.A., Rogan, W.J., Longnecker, M.P. (2013). Association between perfluoroalkyl substances and thyroid stimulating hormone among pregnant women: a cross-sectional study. Environ. Health. 12, 76.

Wang, J., Yan, S., Zhang, W., Zhang, H., Dai, J. (2015). Integrated proteomic and miRNA transcriptional analysis reveals the hepatotoxicity mechanism of PFNA exposure in mice. J Proteome Res. 14:330-41.

Washington, J.W., Ellington, J., Jenkins, T.M., Evans, J.J., Yoo, H., Hafner, S.C. (2009). Degradability of an acrylate-linked, fluorotelomer polymer in soil. Environ. Sci. Technol. 43, 6617–6623.

Watkins, D.J., Josson, J., Elston, B., Bartell, S.M., Shin, H.M., Vieira, V.M., Savitz, D.A., Fletcher, T., Wellenius, G.A. (2013). Exposure to perfluoroalkyl acids and markers of kidney function among children and adolescents living near a chemical plant. Environ Health Perspect. 121, 625-30.

Weaver, Y.M., Ehresman, D.J., Butenhoff, J.L., Hagenbuch, B. (2010). Roles of rat renal organic anion transporters in transporting perfluorinated carboxylates with different chain lengths. Toxicol. Sci. 113, 305–314.

Wen, L.L, Lin, L.Y, Su, T.C, Chen, P.C, and Lin, C.Y. (2013). Association Between Serum Perfluorinated Chemicals and Thyroid Function in U.S. Adults: The National Health and Nutrition Examination Survey 2007–2010. J. Clin. Endocrinol. Metab. 98, E1456-1464.

WIL Research Laboratories, LLC. (2006). Study number WIL-497002. Cited in Mundt et al. (2007).

Wolf, C.J., Takacs, M.L., Schmid, J.E., Lau, C., Abbott, B.D. (2008). Activation of mouse and human peroxisome proliferator-activated receptor alpha by perfluoroalkyl acids of different functional groups and chain lengths. Toxicol. Sci. 106, 162−71.

Wolf, C.J., Zehr, R.D., Schmid, J.E., Lau, C., Abbott, B.D. (2010). Developmental effects of perfluorononanoic acid in the mouse are dependent on peroxisome proliferator-activated receptor-alpha. PPAR Res. 2010, pii: 282896.

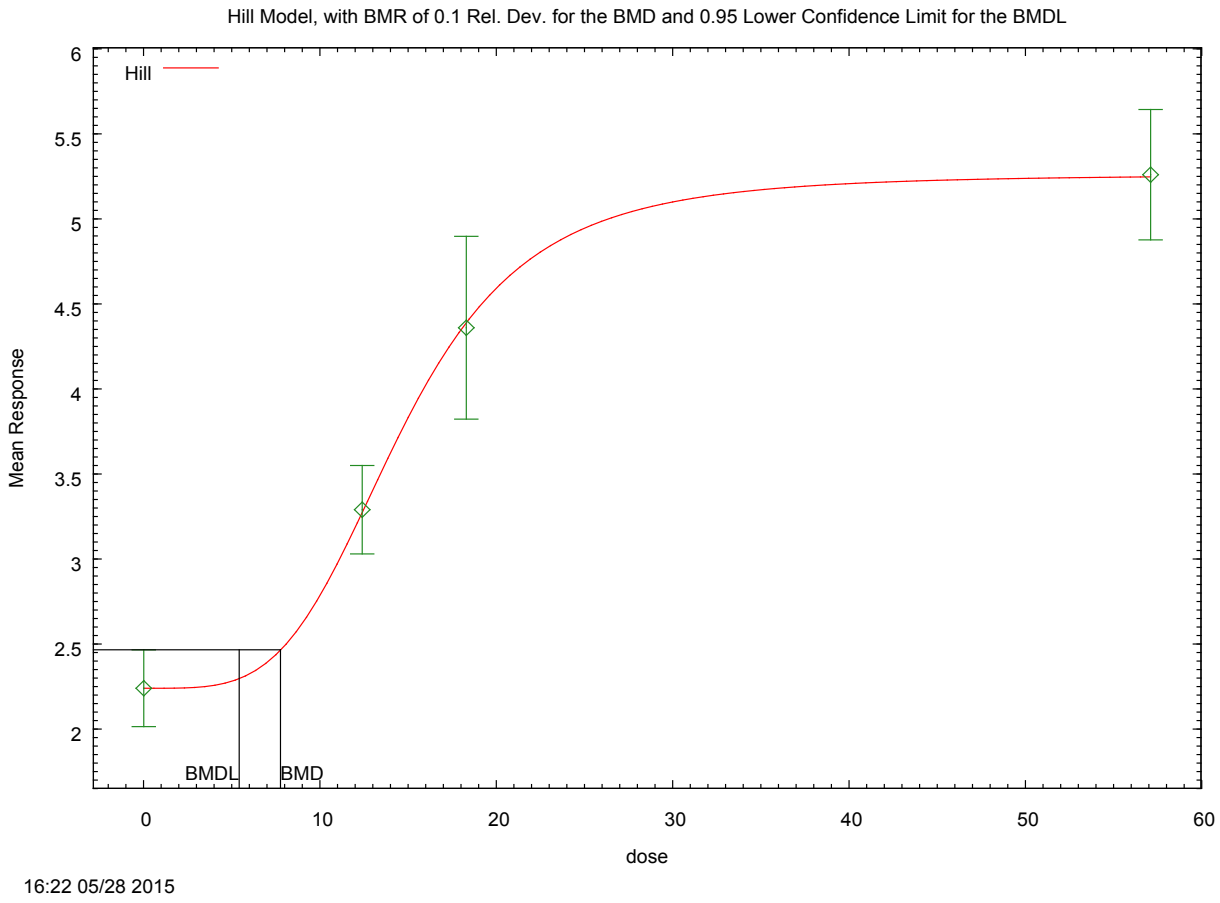
Wolf, C.J., Schmid, J.E., Lau, C., Abbott, B.D. (2012). Activation of mouse and human peroxisome proliferator-activated receptor-alpha (PPARa) by perfluoroalkyl acids (PFAAs): Further investigation of C4–C12 compounds. Reprod. Toxicol. Epub 2011 November.

Wolterbeek, APM. (2004). Combined oral repeated dose toxicity study with the reproductive/developmental toxicity screening test with S-111-S-WB in rats. TNO Report, no 4833 (cited in Mertens et al., 2010, and Stump et al., 2008).

Zhang, Y., Beesoon, S., Zhu, L., Martin, J.W. (2013a). Biomonitoring of perfluoroalkyl acids in human urine and estimates of biological half-life. Environ. Sci. Technol. 47, 10619-27.

Zhang, T., Sun, H., Lin, Y., Qin, X., Zhang, Y., Geng, X., Kannan, K. (2013b). Distribution of poly- and perfluoroalkyl substances in matched samples from pregnant women and carbon chain length related maternal transfer. Environ. Sci. Technol. 47, 7974-81.

APPENDIX 1. Detailed Benchmark Dose Modeling Results for 10% Increase in Maternal Liver Weight on GD 17 (Das et al., 2015)



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

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


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

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


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


          Asymptotic Correlation Matrix of Parameter Estimates

               lalpha          rho    intercept            v            n            k

    lalpha          1        -0.97        -0.14         0.15       -0.079        0.051

       rho      -0.97            1         0.12        -0.16        0.081       -0.053

 intercept      -0.14         0.12            1        -0.42         0.13         0.27

         v       0.15        -0.16        -0.42            1        -0.43         0.49

         n     -0.079        0.081         0.13        -0.43            1        -0.38

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

Parameter Estimates

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

Table of Data and Estimated Values of Interest

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

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

Model Descriptions for likelihoods calculated

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

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

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

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

Likelihoods of Interest

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

Explanation of Tests

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

Tests of Interest

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

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

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

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

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

Benchmark Dose Computation

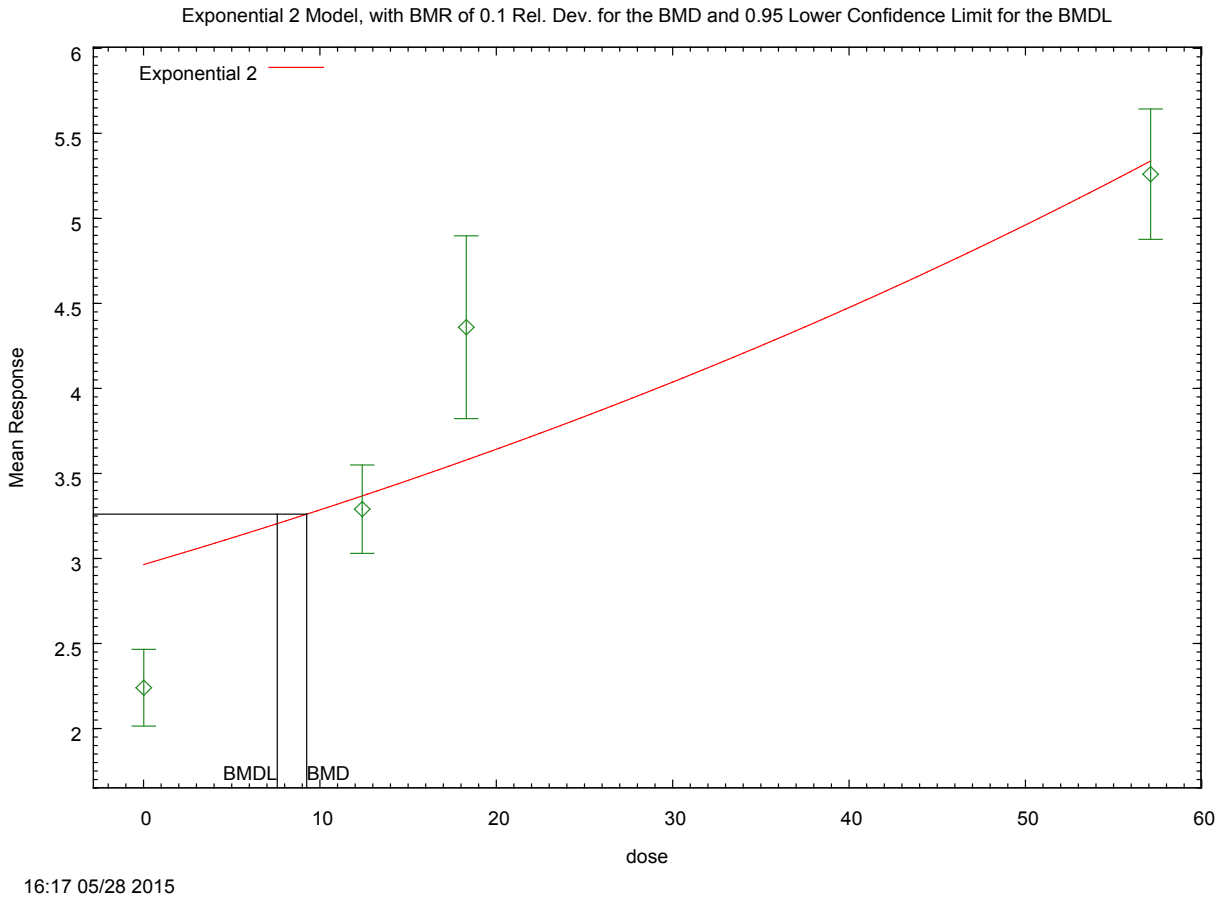
Specified effect = 0.1

Risk Type = Relative deviation

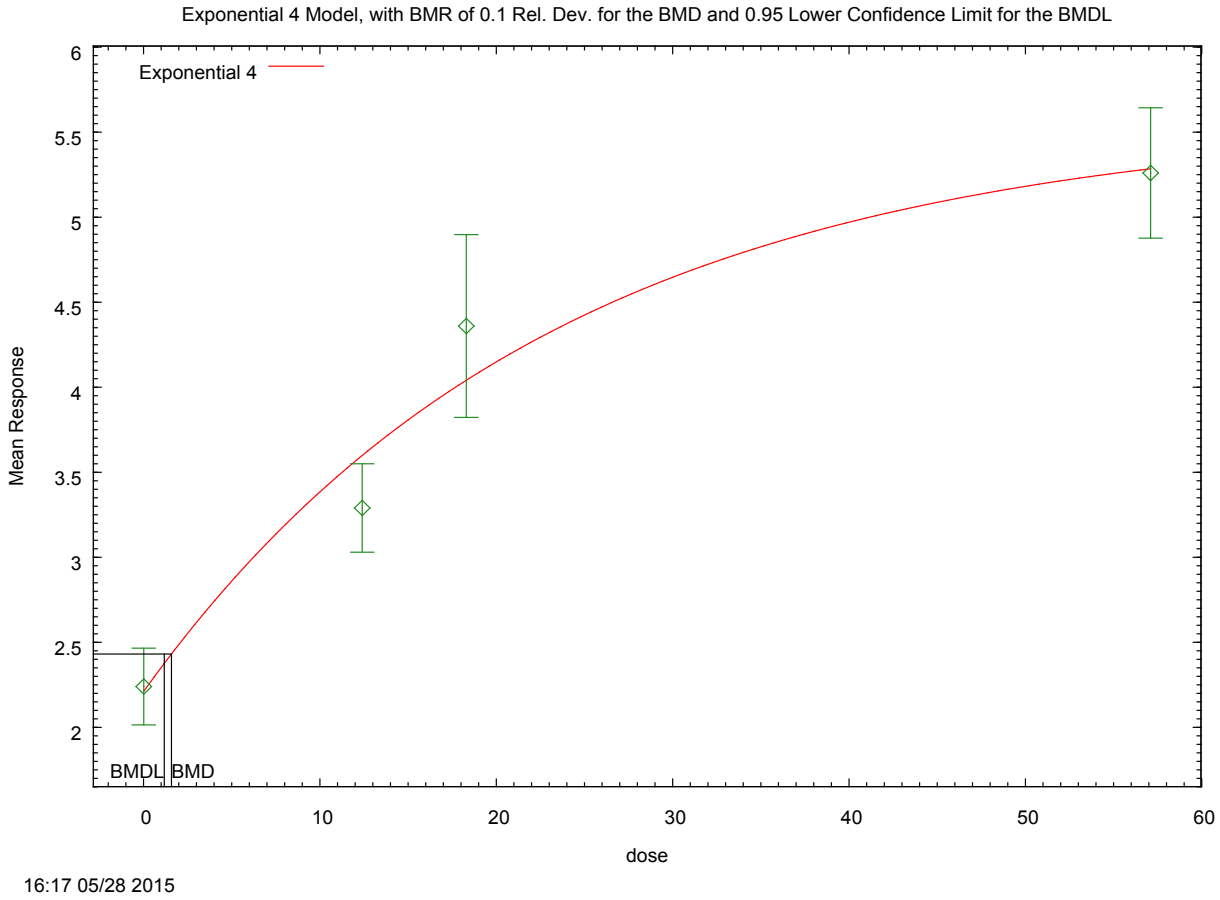
Confidence level = 0.95

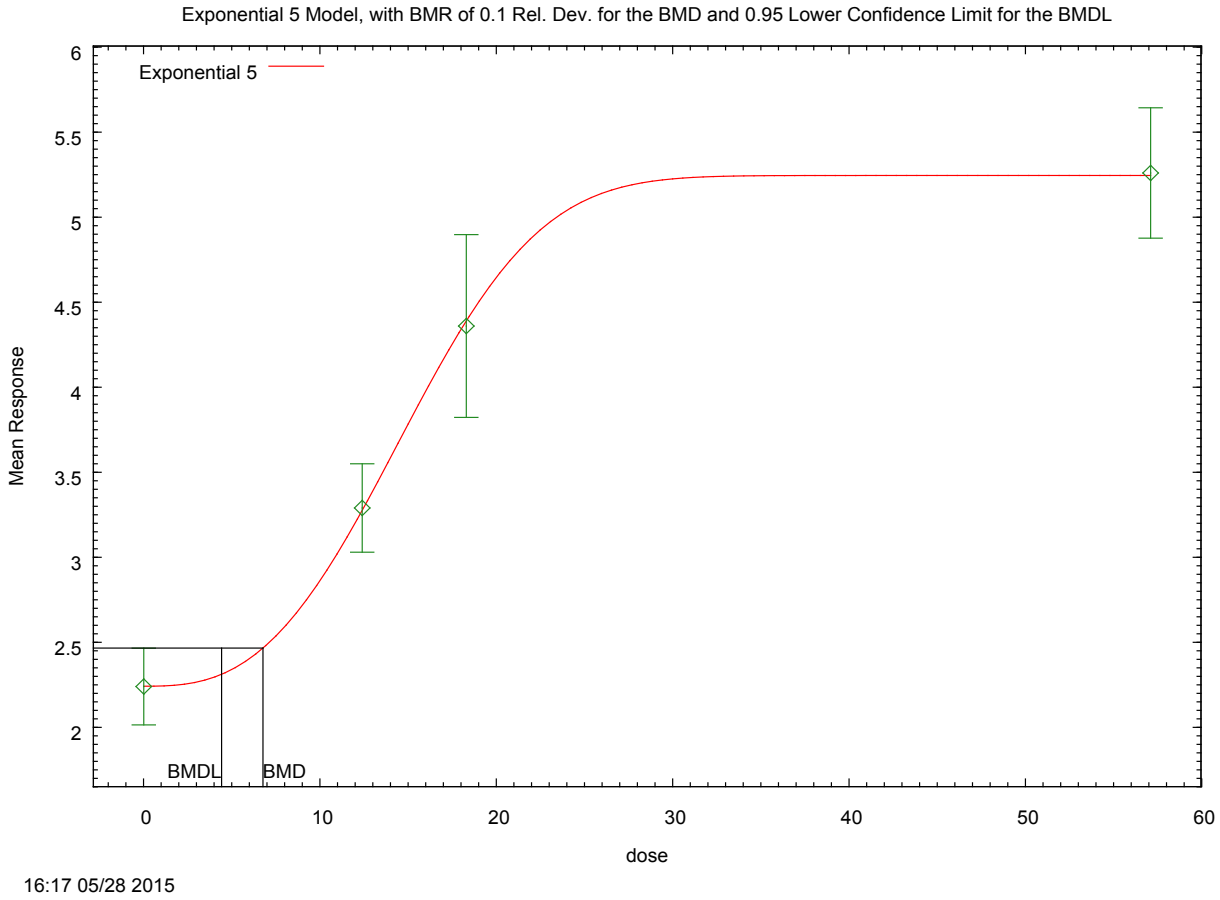
BMD = 7.7643

BMDL = 5.42534



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


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


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

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

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

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


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

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

   MLE solution provided: Exact


                              Initial Parameter Values

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

    * Indicates that this parameter has been specified
```

Parameter Estimates by Model

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

-- Indicates that this parameter does not appear in model

Std. Err. Estimates by Model

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

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

Table of Stats From Input Data

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

Estimated Values of Interest

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

Other models for which likelihoods are calculated:

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

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

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

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

Likelihoods of Interest

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

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

Explanation of Tests

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

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

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

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

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	77.78	6	< 0.0001
Test 2	8.042	3	0.04516
Test 3	1.887	2	0.3892
Test 4	34.52	2	< 0.0001
Test 5a	34.52	2	< 0.0001
Test 5b	-5.739e-011	0	N/A
Test 6a	9.314	1	0.002274

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

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

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

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

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

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

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

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

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

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

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

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

Benchmark Dose Computations:

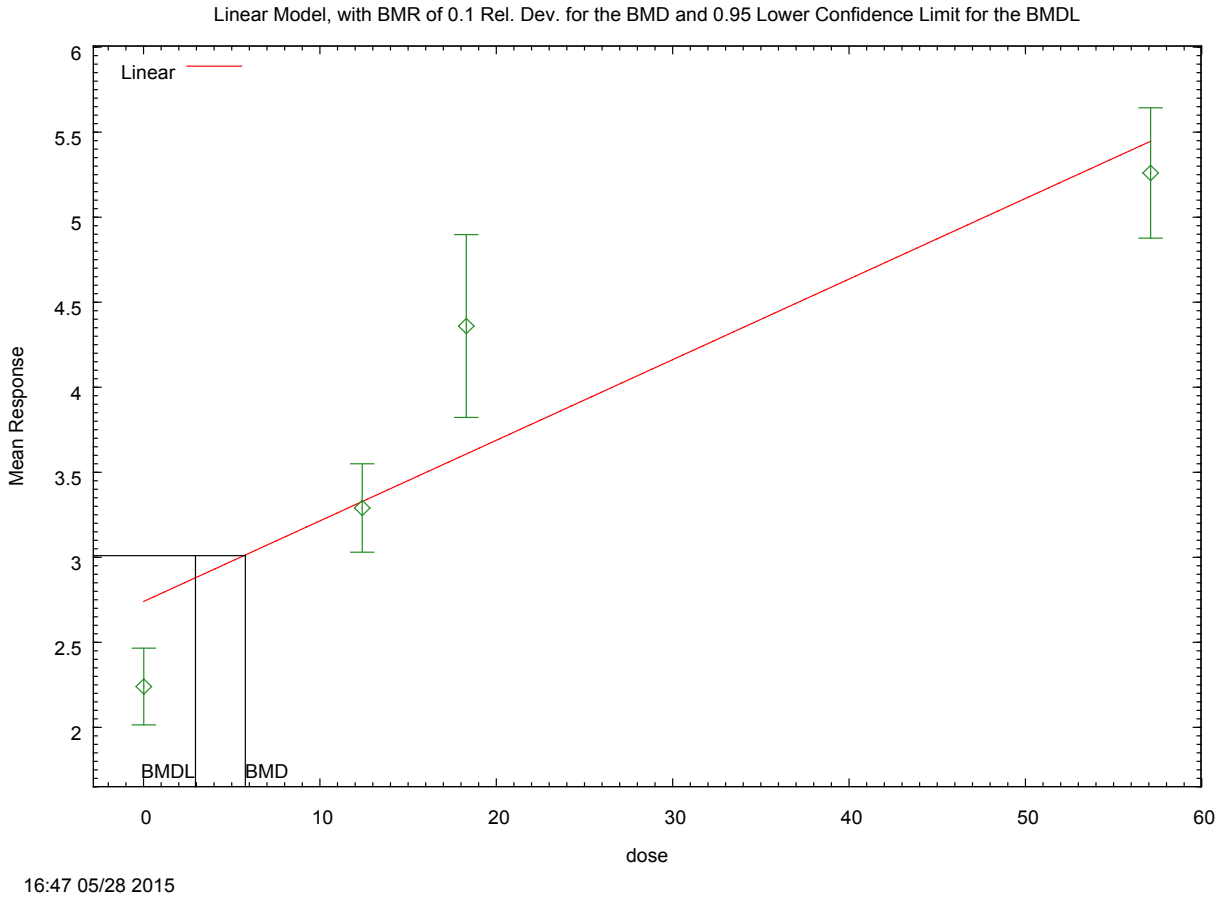
Specified Effect = 0.100000

Risk Type = Relative deviation

Confidence Level = 0.950000

BMD and BMDL by Model

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


Linear Model, with BMR of 0.1 Rel. Dev. for the BMD and 0.95 Lower Confidence Limit for the BMDL
Linear
Mean Response
dose
BMDL
BMD
6
5.5
5
4.5
4
3.5
3
2.5
2
0
10
20
30
40
50
60
16:47 05/28 2015

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

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


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

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



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


          Asymptotic Correlation Matrix of Parameter Estimates

               lalpha          rho       beta_0       beta_1

    lalpha          1        -0.99         0.63         -0.7

       rho      -0.99            1        -0.63          0.7

    beta_0       0.63        -0.63            1        -0.85

    beta_1       -0.7          0.7        -0.85            1


                              Parameter Estimates

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


     Table of Data and Estimated Values of Interest
```

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

Model Descriptions for likelihoods calculated

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

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

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

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

Likelihoods of Interest

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

Explanation of Tests

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

Tests of Interest

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

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

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

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

The p-value for Test 4 is less than .1. You may want to try a different model

Benchmark Dose Computation

Specified effect = 0.1

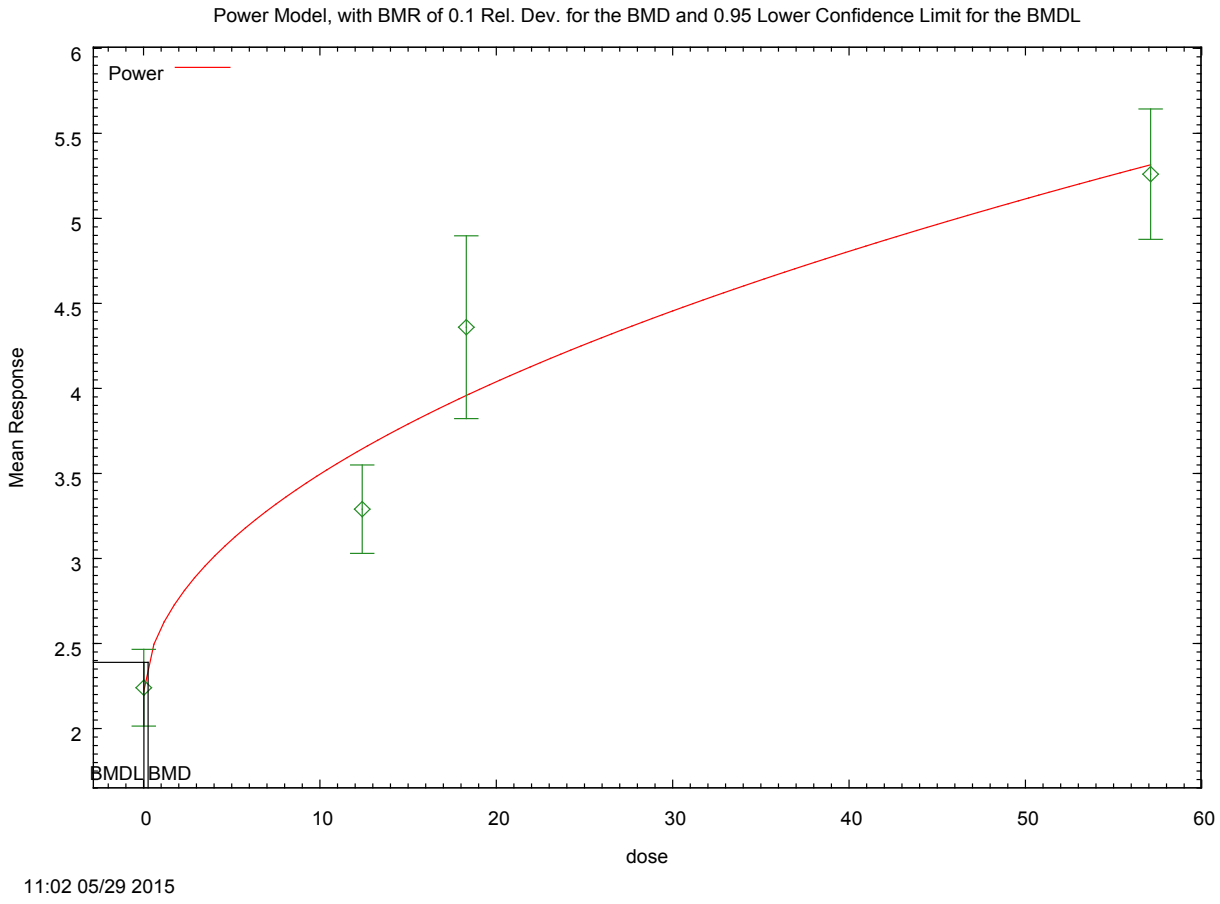
Risk Type = Relative deviation

Confidence level = 0.95

BMD = 5.77161

BMDL = 2.94582

BMDL computation failed for one or more point on the BMDL curve. The BMDL curve will not be plotted


Power Model, with BMR of 0.1 Rel. Dev. for the BMD and 0.95 Lower Confidence Limit for the BMDL
Power
Mean Response
6
5.5
5
4.5
4
3.5
3
2.5
2
BMDL BMD
0
10
20
30
40
50
60
dose
11:02 05/29 2015

```
================================================================
        Power Model. (Version: 2.18;  Date: 05/19/2014)
        Input Data File: C:/BMDS260/Data/pow_Lau maternal liver wt without resorption
5-28-15_Opt.(d)
        Gnuplot Plotting File:  C:/BMDS260/Data/pow_Lau maternal liver wt without
resorption 5-28-15_Opt.plt
                                              Fri May 29 11:02:48 2015
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 BMDS Model Run
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   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = Mean
   Independent variable = Dose
   The power is not restricted
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

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



                  Default Initial Parameter Values
                         lalpha =       -1.504
                            rho =            0
                        control =         2.24
                          slope =     0.260376
                          power =        -9999


           Asymptotic Correlation Matrix of Parameter Estimates

                 lalpha          rho      control        slope        power

    lalpha            1        -0.98        -0.16        0.054       -0.023

       rho        -0.98            1         0.15       -0.058        0.024

   control        -0.16         0.15            1        -0.63         0.53

     slope        0.054       -0.058        -0.63            1        -0.98

     power       -0.023        0.024         0.53        -0.98            1
```

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	-3.96586	1.265	-6.44521	-1.48651
rho	1.93299	0.949011	0.0729611	3.79301
control	2.17191	0.120445	1.93584	2.40798
slope	0.422924	0.144482	0.139745	0.706103
power	0.495715	0.0886516	0.321961	0.669469

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0.013	8	2.24	2.22	0.27	0.298	0.18
12.4	8	3.29	3.65	0.311	0.481	-2.09
18.3	8	4.36	3.96	0.643	0.52	2.18
57.1	10	5.26	5.31	0.536	0.692	-0.241

Model Descriptions for likelihoods calculated

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

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

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

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

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	10.695847	5	-11.391693
A2	14.716640	8	-13.433281
A3	13.772953	6	-15.545906
fitted	7.468885	5	-4.937769
R	-24.175471	2	52.350943

Explanation of Tests

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

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	77.7842	6	<.0001
Test 2	8.04159	3	0.04516
Test 3	1.88738	2	0.3892
Test 4	12.6081	1	0.0003841

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

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

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

The p-value for Test 4 is less than .1. You may want to try a different model

Benchmark Dose Computation

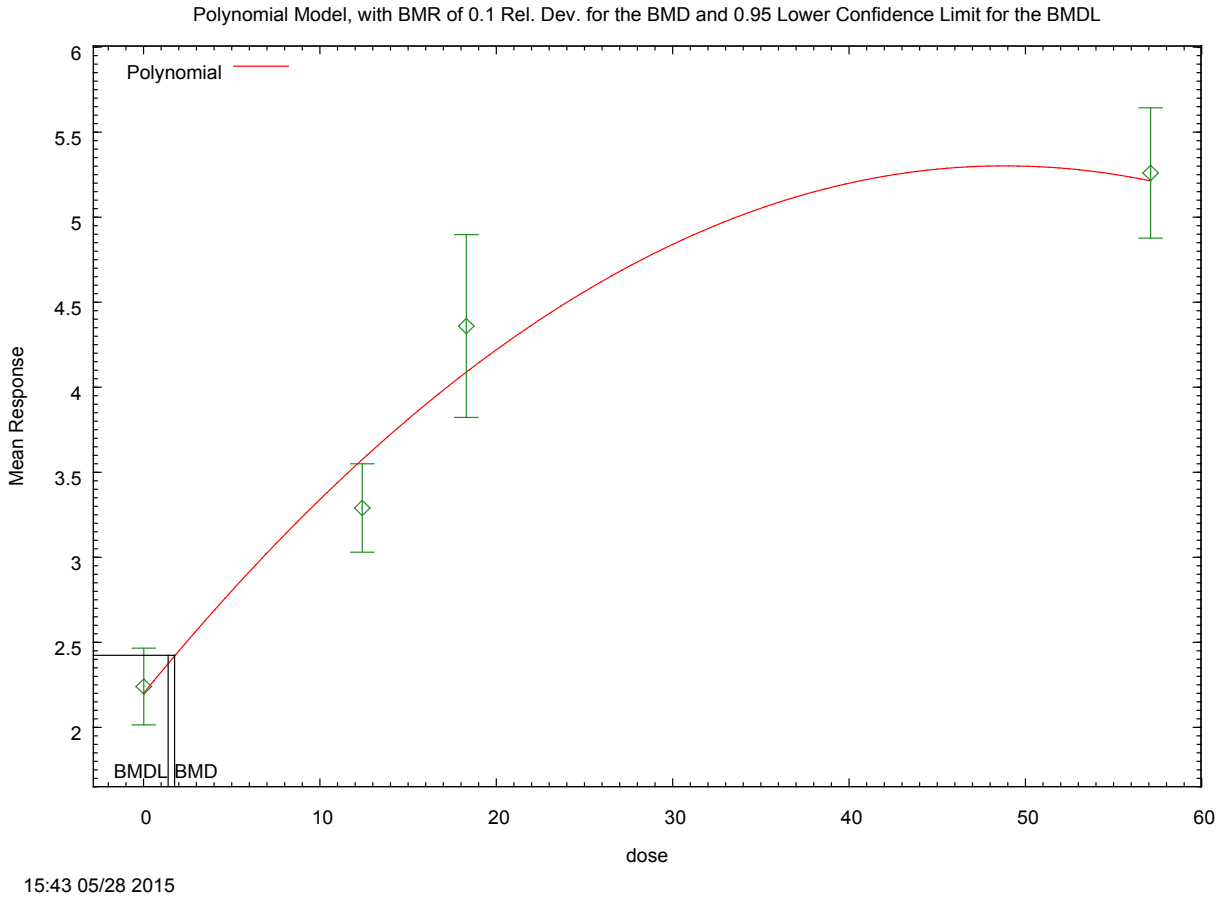
Specified effect = 0.1

Risk Type = Relative deviation

Confidence level = 0.95

BMD = 0.260709

BMDL = 0.0210435


Polynomial Model, with BMR of 0.1 Rel. Dev. for the BMD and 0.95 Lower Confidence Limit for the BMDL
Polynomial
Mean Response
6
5.5
5
4.5
4
3.5
3
2.5
2
BMDL
BMD
0
10
20
30
40
50
60
dose
15:43 05/28 2015

```
================================================================
        Polynomial Model. (Version: 2.20;  Date: 10/22/2014)
        Input Data File: C:/BMDS260/Data/ply_Lau maternal liver wt without resorption
5-28-15_Opt.(d)
        Gnuplot Plotting File:  C:/BMDS260/Data/ply_Lau maternal liver wt without
resorption 5-28-15_Opt.plt
                                              Thu May 28 15:43:34 2015
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

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


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

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



                  Default Initial Parameter Values
                         lalpha =       -1.504
                            rho =            0
                         beta_0 =      2.16211
                         beta_1 =     0.132156
                         beta_2 =  -0.00136126


           Asymptotic Correlation Matrix of Parameter Estimates

                lalpha          rho       beta_0       beta_1       beta_2

    lalpha           1        -0.98        -0.15         0.13       -0.089

       rho       -0.98            1         0.15        -0.12        0.071

    beta_0       -0.15         0.15            1        -0.61         0.49

    beta_1        0.13        -0.12        -0.61            1        -0.96

    beta_2      -0.089        0.071         0.49        -0.96            1


                                Parameter Estimates

                                                      95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
         lalpha         -4.07867          1.15936            -6.35097            -
1.80638
```

```
            rho          1.90932         0.867406            0.209232
3.6094
         beta_0           2.2034        0.0983742             2.01059
2.39621
         beta_1         0.127329        0.0136347            0.100606
0.154053
         beta_2      -0.00129539      0.000236025         -0.00175799        -
0.000832786
```

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0.013	8	2.24	2.21	0.27	0.277	0.357
12.4	8	3.29	3.58	0.311	0.44	-1.88
18.3	8	4.36	4.1	0.643	0.5	1.47
57.1	10	5.26	5.25	0.536	0.634	0.0479

Model Descriptions for likelihoods calculated

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

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

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

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

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	10.695847	5	-11.391693
A2	14.716640	8	-13.433281
A3	13.772953	6	-15.545906
fitted	9.944650	5	-9.889301
R	-24.175471	2	52.350943

Explanation of Tests

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

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value

Test 1	77.7842	6	<.0001
Test 2	8.04159	3	0.04516
Test 3	1.88738	2	0.3892
Test 4	7.6566	1	0.005656

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

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

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

The p-value for Test 4 is less than .1. You may want to try a different model

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Relative deviation

Confidence level = 0.95

BMD = 1.76206

BMDL = 1.39649

BMDL computation failed for one or more point on the BMDL curve.
The BMDL curve will not be plotted