HEALTH CONSULTATION

Technical Support Document for Assessment of Perfluorinated Chemicals and Selection of a Perfluorooctane Sulfonate (PFOS) Reference Dose as the basis for Michigan Fish Consumption Screening Values (FCSVs)

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Acronyms and Abbreviations

µM	micromolar
ADHD	attention deficit/hyperactivity disorder
AEC	absolute eosinophil count
AFFF	aqueous film-forming foam
ALT	alanine transaminase
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
BCF	bioconcentration factor
CAR	constitutive androstane receptor
CI	confidence interval
CL	clearance
COPD	chronic obstructive pulmonary disease
DHA	docosahexaenoic acid
ECP	eosinophillic cationic protein
EPA	eicosapentaenoic acid
FAWCAC	Fish and Wildlife Contaminant Advisory Committee
FCSVs	fish consumption screening values
FOSA	heptadecafluorooctane sulfonamide
fT4	free thyroxine
FXR	farnesoid X receptor
g	grams
GGT	γ-glutamyltransferase
GOT	glutamic-oxaloacetic transaminase
GPT	glutamic-pyruvic transaminase
GTP	γ-glutamyl transpeptidase
h	hour
HDL-C	high-density lipoprotein cholesterol
IgE	immunoglobulin E
ip-PFNA	perfluoro-7-methyloctanoic acid
kg	kilogram
L	liter
LDL	low-density lipoproteins
L-PFDS	sodium perfluoro-1-decanesulfonate
LXRα	liver X receptor α
MDCH	Michigan Department of Community Health
MDEQ	Michigan Department of Environmental Quality
MFCMP	Michigan Fish Contaminant Monitoring Program
mg	milligrams
mL	milliliter
MNDOH	Minnesota Department of Health
MOE	Ministry of the Environment
n-3 PUFAs	omega-3 polyunsaturated fatty acids
ng	nanograms

NHANES	National Health and Nutrition Examination Survey
NOAEL	no observed adverse effects level
OH-PCBs	hydroxylated metabolites of polychlorinated biphenyls
OR	odds ratio
PBDE	polybrominated diphenyl ether
PCB	polychlorinated biphenyl
PFAAs	perfluoroalkyl acids
PFBA	perfluorobutanoic acid
PFBS	perfluorobutane sulfonate
PFCs	perfluorinated chemicals
PFDA	perfluorodecanoic acid
PFDcS	perfluorodecane sulfonate
PFDoA	perfluorododecanoic acid
PFHpA	perfluoroheptanoic acid
PFHxA	perfluorohexanoic acid
PFHxS	perfluorohexane sulfonate
PFNA	perfluorononanoic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonate
PFOSA	perfluorooctane sulfonamide
PFOSE	perfluorooctane sulfonamidoethanol
PFPeA	perfluoropentanoic acid
PFPeDA	perfluoropentadecanoate
PFTeA	perfluorotetradecanoic acid
PFTriA	perfluorotridecanoic acid
PFUnA	perfluoroundecanoic acid
POPs	persistent organic pollutants
PPAR	peroxisome proliferator-activated receptor
ppb	parts per billion
ppm	parts per million
PXR	pregnane X receptor
RfD	reference dose
RNA	ribonucleic acid
SHE	Syrian hamster embryo
$t_{1/2}$	half-life
T3	triiodothyronine
T4	total thyroxine
T4-TTR	T4 bound to TTR
TBG	thyroxine-binding globulin
TSH	thyroid-stimulating hormone
TTR	transthyretin
U.S. EPA	United States Environmental Protection Agency
V_d	volume of distribution

Summary

The Michigan Department of Community Health (MDCH) issues fish consumption advisories for sport-caught fish and issued Michigan's first fish advisory due to a perfluorinated chemical (PFC), perfluorooctane sulfonate (PFOS) in 2012. This fish advisory was based on provisional PFOS fish consumption screening values (FCSVs). MDCH reviewed the available published literature regarding PFCs, to make recommendations regarding PFCs of concern and reference doses.

While several PFCs will accumulate in fish tissue, PFOS is currently identified as the most bioaccumulative. PFOS is not metabolized in fish or humans, and other PFCs, such as perfluorooctane sulfonamide (PFOSA), may be converted to PFOS in the body. In the case of other PFCs, such as perfluorooctanoic acid (PFOA), people may accumulate higher levels of these chemicals due to contaminated drinking water rather than consumption of contaminated fish. In certain cases, both exposure pathways may need to be assessed.

MDCH's conclusions regarding PFCs in fish:

1. *MDCH concludes that unlimited consumption of sport-caught fish with elevated PFOS levels could harm people's health.* Based on published information and other states' investigations, perfluorooctane sulfonate (PFOS) accumulates in fish to much higher levels than the other PFCs. Other studies indicated that consumption of fish with elevated PFOS levels is people's primary route of exposure. Exposure to PFOS has been linked to alterations in thyroid hormones, cholesterol levels, neurodevelopment, and immune function.

2. *MDCH concludes that additional site-specific evaluation of PFOA levels in fish may be needed when people are also exposed to PFOA by other routes.* PFOA does not bioaccumulate to a great extent in fish. However, at sites where people may have exposure to PFOA through contaminated groundwater or other routes of exposure, consumption of PFOA-containing fish may need to be included as part of a total exposure dose evaluation.

3. *MDCH concludes that, under certain circumstances, PFOSA levels in fish may need additional evaluation.* PFOSA has the potential to be metabolized into PFOS. At certain sites, PFOSA levels in fish might need additional evaluation.

4. *MDCH concludes that existing data is inadequate to evaluate other PFCs or the cumulative effects of exposure to multiple PFCs.* As additional information becomes available, MDCH will evaluate other relevant PFCs.

Next steps: The Michigan Fish Contaminant Monitoring program will monitor PFCs in fish in various locations throughout Michigan.

MDCH will update the provisional PFOS FCSVs and issue consumption advisories when applicable.

MDCH will address the presence of other PFCs in fish as needed and as toxicity information on other PFCs becomes available.

Purpose and Health Issues

MDCH issued the first fish advisory for PFOS in 2012 based on provisional fish consumption screening levels (FCSVs). The purpose of this document is to review the available published literature on PFCs and previously derived reference doses to recommend reference doses for use in the Michigan Fish Consumption Advisory Program to ensure that the consumption advice is protective of public health.

Background

Perfluorinated chemicals (PFCs) are human-made chemicals with fluorines attached to the carbon chain (Lindstrom, et al. 2011). PFCs are hydrophobic (water-repelling) and oleophobic (oil-repelling) and have many industrial and commercial uses (ATSDR 2009). PFCs have been used in surfactants, fire-fighting foams, stain-resistant coatings, paints, adhesives, and in electroplating applications (MDEQ 2011).

PFCs have also been used as pesticides. One, sulfluramid, is an insecticide used in bait stations. Sulfluramid is rapidly metabolized to PFOSA, then to the terminal metabolite PFOS, in rats (EPA 2001)

PFOA, along with PFOS, are final degradation products of numerous precursor chemicals (Lau, et al. 2007). Natural mechanisms of PFOS degradation have yet to be identified (Lindstrom, et al. 2011). Table 1 presents names and formulas of select PFCs.

Table 1: List of select perfluorinated chemicals.

Carbons	Abbreviation	Formula	Name	CAS Number
C4	PFBA	$C_4HF_7O_2$	perfluorobutanoic acid	375-22-4
C4	PFBS	$C_4HF_9O_3S$	perfluorobutane sulfonate	375-73-5
C5	PFPeA	$C_5HF_9O_2$	perfluoropentanoic acid	2706-90-3
C6	PFHxA	$C_6HF_{11}O_2$	perfluorohexanoic acid	307-24-4
C6	PFHxS	$C_6HF_{13}O_3S$	perfluorohexane sulfonate	355-46-4
C7	PFHpA	$C_7HF_{13}O_2$	perfluoroheptanoic acid	375-85-9
C8	PFOA	$C_8HF_{15}O_2$	perfluorooctanoic acid	335-67-1
C8	PFOS	$C_8HF_{17}O_3S$	perfluorooctane sulfonate	1763-23-1
C8	PFOSA	$C_8H_2F_{17}NO_2S$	perfluorooctane sulfonamide	754-91-6
C9	PFNA	$C_9HF_{17}O_2$	perfluorononanoic acid	375-95-1
C10	PFDA	$C_{10}HF_{19}O_2$	perfluorodecanoic acid	335-76-2
C11	PFUnA	$C_{11}HF_{21}O_2$	perfluoroundecanoic acid	2058-94-8

C12	PFDoA	$C_{12}HF_{23}O_2$	perfluorododecanoic acid	307-55-1

PFOS fish consumption advisories

Although several PFCs may be found in fish, the Minnesota Department of Health has only found PFOS at levels of concern in fish (MDH 2014). Minnesota has PFOS-driven fish consumption advisories on several lakes and rivers, including the Mississippi River. Wisconsin has PFOS-driven fish advisories listed for some Mississippi River locations. Table 2 presents Minnesota Department of Health's PFOS fish screening levels. There is only one area in Michigan, in Iosco County, with fish consumption advisories for PFOS (as of May 2014).

Table 2: Minnesota Department of Health's PFOS screening levels (in parts per billion [ppb]) for fish (MDH 2008).

Levels of PFOS in fish (ppb)	Meal Category
≤ 40	Unrestricted
> 40 – 200	1 meal/ week
> 200 – 800	1 meal / month
> 800	DO NOT EAT

Ontario, Canada, has five inland waterbodies with PFOS-driven fish consumption advisories. Ontario's PFOS consumption restrictions start at fish tissue levels of 80 parts per billion (ppb). At levels over 160 ppb, for sensitive populations, and 640 ppb, for the general population, do not eat advisories are issued. Ontario has also analyzed Lake Ontario, St. Lawrence River, Lake Huron, St. Marys River, and Lake Superior fish for PFCs (MOE 2013).

Discussion

Various studies measuring PFCs may have used different protocols for analysis. In a worldwide interlaboratory comparison study, the laboratories were most consistent for measuring PFOS and PFOA in human serum and blood (65% concordance among laboratories). Human serum and blood levels of the other eleven PFCs had less agreement than levels measured for PFOS and PFOA. Also, PFC levels were underestimated in fish tissue samples analyzed by most laboratories (Lau, et al. 2007). This may mean that various measurements may not be directly comparable between studies. However, general trends would still be relevant even if exact numbers may not be directly comparable. For example, PFOS, PFOA, and PFHxS are the most often detected PFCs and tend to be at the highest concentrations in human samples (Lau, et al. 2007), with PFOS being the predominant PFC identified in other biological samples worldwide (Houde, et al. 2011).

Environmental Contamination

PFC levels in Great Lakes fish and food chains

Levels of PFCs have been studied in Great Lakes fish. Fifteen PFCs were measured, including PFOS, PFOA, PFOSA, and PFHxS. PFOS was identified as the major PFC in 4-year-old lake trout, collected in 2001, from Lakes Superior, Huron, Erie, Ontario, and Michigan. Levels of

PFOS (mean ± standard error) were highest in whole body samples of lake trout from Lake Erie (102 ± 3 ppb), followed by Lakes Ontario (25 ± 8 ppb), Huron (14 ± 1 ppb), Michigan (13 ± 2 ppb), and Superior (5.1 ± 0.5 ppb) (Furdui, et al. 2007).

In a later study, using fish collected between 2006 and 2008, 11 PFCs, including PFOA, PFOS, PFDA, and PFHxS, were measured in whole bodies of lake trout from Lakes Superior, Huron, Erie, and Ontario and walleye from Lake Erie. PFOS levels (mean ± standard error) were the highest of the 11 measured PFCs. Lake Erie lake trout (96 ± 0.3 ppb) and walleye (54 ± 0.29 ppb) had the highest levels of PFOS, followed by lake trout from Lakes Ontario (52 ± 0.16 ppb), Huron (17 ± 3.9 ppb), and Superior (2.3 ± 0.46 ppb) (De Silva, et al. 2011).

Kannan et al. (2005) identified PFOS as the most commonly detected PFC in samples of various biota in Great Lakes food chains. This pattern was identified even though PFOA water concentrations were slightly higher than PFOS water concentrations. Fish at different trophic levels (round goby and smallmouth bass) had similar levels of PFOS; however, this was a comparison of PFC levels from round goby whole body samples versus muscle tissue from smallmouth bass. PFOS was identified in benthic invertebrates, round goby, Chinook salmon, mink, and bald eagles. Bioconcentration between benthic invertebrates or round goby and water was estimated to be greater than biomagnification between the trophic levels. PFOA biomagnification appeared to be less than PFOS biomagnification (Kannan, et al. 2005).

PFC levels in fish from inland lakes and rivers

Ten PFCs (PFBS, PFHxS, PFOS, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, and PFDoA) were analyzed in bluegill filets collected from Minnesota and North Carolina. The authors selected bluegill as they have been shown to accumulate levels of PFCs similar to higher trophic level fish and they are a common recreationally caught fish. The Minnesota bluegill (n=30) were collected in 2006 from the St. Croix River (background location), Lake Calhoun (no known PFC contamination), and four locations in the Mississippi River with historical PFC contamination. Of the 10 PFCs, only PFOS, PFDA, PFUnA, and PFDoA were detected in the Minnesota bluegill. PFOS levels ranged from 1.22 to 7.17 ppb in St. Croix River bluegill, 205 to 339 ppb in Lake Calhoun bluegill, and 23.7 to 428 ppb in the Mississippi River bluegill. PFOS was the most prevalent PFC in all bluegill filets and was about 82% of the total PFC concentration in the Minnesota filets. The North Carolina bluegill were collected in 2007 from the Haw River (n=31) and the Deep River (n=30), neither with known PFC contamination. PFOS, PFDA, PFUnA, and PFDoA were the only PFCs detected in the North Carolina bluegill. PFOS levels in the North Carolina bluegill ranged from 15.9 to 136 ppb. PFOS levels were only about 43% of the total PFCs in Haw River bluegill filets and 80% of the total PFCs in Deep River bluegill filets. North Carolina bluegill filets had higher levels of PFDA, PFUnA, and PFDoA compared to Minnesota bluegill filets (Delinsky, et al. 2009).

Delinsky et al. (2010) again investigated PFC levels in Minnesota fish. Fish collected in this study were bluegill, black crappie, and pumpkinseed. Fish were collected from the Mississippi River and 59 lakes throughout Minnesota in 2007. Several locations included in this study have known PFC contamination from local sources. Filets were composited and analyzed for ten PFCs (PFBS, PFHxS, PFOS, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, and PFDoA). Each composite sample consisted of one to 17 filets from the same fish species. PFOS was detected in

73% of the composites from the Mississippi River and 22% of the composites from the lakes. A majority (88%) of the composite samples from the Minnesota lakes had less than 3 ppb PFOS. PFOS levels, ranging from <1 to 2,000 ppb, were the highest of the ten PFCs measured in the samples. Levels of PFDA, PFUnA, and PFDoA were above the level of quantification in less than 10% of the samples. PFHxS was only detected above the level of quantification in one sample. PFBS, PFHxA, PFHpA, PFOA, and PFNA were below the level of quantification in all samples (Delinsky, et al. 2010).

Mississippi River fish (bluegill, carp, freshwater drum, smallmouth bass, and white bass) were collected in 2009 and filets were analyzed for 13 PFCs (PFOA, PFOS, PFBS, PFOSA, PFPeA, PFDA, PFUnA, PFDoA, PFHxA, PFHpA, PFNA, PFHxS, and PFBA). The same 13 PFCs were measured in water samples from fish collection locations. Locations selected had known historical PFC contamination. PFBA was detected in all of the water samples and PFOA, PFHxA, and PFOS were detected in more than 40% of the water samples. PFPeA, PFBS, PFHxS, and PFHpA were detected in less than 20% of the water samples. In contrast, PFOS was detected in almost all of the fish samples while PFDA, PFOSA, and PFUnA were detected in around 15 to 30% of the fish samples. PFDoA, PFOA, PFBA, PFHxS, PFHxA, PFHpA, and PFNA were detected in less than 10% of the fish samples. Table 3 presents the levels of PFOS in these fish. No relationship was identified between PFOS concentrations in the fish samples and fish length, age, gender, or percent lipids. The most migratory fish sampled, white bass, had the least variation among the collection locations (MPCA 2010).

Table 3: Levels of PFOS (in parts per billion [ppb]) in Mississippi River fish collected by Minnesota agencies in 2009 (MPCA 2010).

Species	Number of fish	Range in ppb	Mean ± standard deviation in ppb
Bluegill	57	8-1,350	110 ± 219
Carp	60	5-1,340	77 ± 203
Freshwater Drum	60	5-3,580	229 ± 602
Smallmouth Bass	60	13-612	94 ± 141
White Bass	60	38-764	97 ± 95

Fish were collected from locations, ten per river, in the Ohio, Missouri, and Upper Mississippi Rivers. Whole fish were composited by species. Small fish composite samples included 20 to 200 individuals. Small fish species were emerald shiner, red shiner, river shiner, spotfin shiner, gizzard shad, and threadfin shad. Large fish composite samples included three to five fish. Large fish species were channel catfish, flathead catfish, freshwater drum, largemouth bass, smallmouth bass, sauger, white bass, common carp, river carpsucker, golden redhorse, and shorthead redhorse. Each composite sample, one small and one large fish species per location, was analyzed for 10 PFCs (PFBS, PFHxS, PFOS, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, and PFDoA). Of the 60 samples analyzed, PFBS, PFUnA, PFOA, and PFHpA were detected in less than half of the samples. PFOS was detected in 82% of the samples and represented approximately 86% of the amount of total PFCs in fish from all three rivers. Table 4 presents the levels of PFCs in the composite samples from the three rivers. Different PFC profiles were observed for each composite sample (Ye, et al. 2008).

Table 4: Number of detections of PFCs and range (in parts per billion [ppb]) in whole fish composite samples from the Missouri, Ohio, and Upper Mississippi Rivers (twenty samples per river) (Ye, et al. 2008).

PFC	Missouri River		Ohio River		Upper Mississippi River		Percent of detections (60 samples)
	Number detected	Range in ppb	Number detected	Range in ppb	Number detected	Range in ppb	
PFOS	13	<10.0-532	16	<10.0-1250	20	10.0-250	82%
PFDA	4	<0.20-2.0	17	<1.00-9.01	18	<0.40-5.16	65%
PFHxA	15	<4.00-12.6	8	<10.0-18.4	14	<2.00-11.1	62%
PFHxS	13	<0.20-8.14	12	<0.20-1.96	10	<0.20-3.33	58%
PFNA	10	<0.20-1.48	14	<0.20-5.89	11	<0.20-5.38	58%
PFDoA	10	<0.20-2.88	15	<1.00-4.13	5	<0.40-1.25	50%
PFUnA	2	<1.00-8.60	17	<0.40-45.3	5	<0.40-48.0	40%
PFHpA	18	<1.00-4.03	0	<4.00	3	<0.20-1.83	35%
PFOA	0	<1.00	1	<1.00-2.10	5	<0.20-0.60	10%
PFBS	2	<0.40-0.64	0	<0.40	1	<0.20-0.29	5%

PFOS and PFOA were measured in chub, river goby, and water from the Roter Main River, in Bayreuth, Germany in 2007. Sources of PFCs to this river were from the effluent released from a municipal waste water treatment plant. River water had higher levels of PFOS than PFOA. River goby muscle tissue had a mean PFOS level of 80 ppb, with a range of 65 to 106 ppb. The mean level of PFOS in river goby organs was more than double the mean muscle level. Chub muscle had a mean PFOS level of 13 ppb, with a range of 7.5 to 15.6 ppb. Mean PFOS levels in chub organs (liver, kidneys, gonads, and heart) were more than 4 times higher than in chub muscle. PFOS levels in the river goby muscle were about 6-fold higher than the chub muscle. PFOA levels in chub muscle were lower than the quantification limit of 1.5 ppb, while the mean PFOA level in river goby muscle was 5.9 ppb, with a range of 2.0 to 9.8 ppb. PFOA levels in chub organs tended to be higher than in chub muscles, while river goby organ PFOA levels were lower than muscle levels. River goby feed on benthic invertebrates living in the sediment, and the authors suggest that bioaccumulation from the sediment to the benthic invertebrates could explain this difference in PFC levels between the fish (Becker, et al. 2010).

Environmental Fate

PFCs, including PFOA and PFOS, have been identified in a variety of oceans and U.S. waters, including the Great Lakes and in waters throughout Michigan. PFCs persist in the environment, are resistant to degradation in soil and sediment, and are resistant to hydrolysis in water. Although it is anticipated that PFCs will be in the air only at low concentrations, these chemicals are also resistant to atmospheric photooxidation as well (ATSDR 2009).

PFCs also seem to move between environmental media. PFCs in contaminated soil amendments, spread over about 1,000 agricultural sites in Germany, were later identified in surface water used

as drinking water. A drinking water advisory was issued until PFOA levels were lower than a guidance value of 0.3 ppb (Wilhelm, et al. 2008).
Aqueous film-forming foam (AFFF) firefighting agents were often used during fire-training activities at the Wurtsmith Air Force Base, in Oscoda, Michigan. The base was decommissioned in 1993. Groundwater samples taken from ten locations between the fire-training area and a nearby marsh were analyzed for PFCs (PFOS, PFHxS, PFOA, and PFHxA) in November 1998 or June 1999. Levels of PFOS, PFHxS, PFOA, and PFHxA ranged from 4.0 to 110 ppb, 9.0 to 120 ppb, <3.0 to 105 ppb, and <3.0 to 20 ppb, respectively. These PFCs persisted in the groundwater at least five years after release (Moody, et al. 2003).

Release of AFFF has also resulted in contamination of biota along with environmental media. In June of 2000, 22,000 liters of AFFF were released into Spring Creek, Spring Creek Pond (a constructed pond), and eventually Etobicoke Creek in Toronto, Ontario, Canada. PFOS was the main PFC in the AFFF and found to be the predominant PFC in water, sediment, fish (whole without the liver), and fish liver. Samples of all four media were collected in 2003, 2006, and 2009. The highest levels of PFOS in water were from the 2003 samples collected closest to the release site, however, sediment PFOS levels were similarly elevated in samples closest to the release site collected in all three years. PFOS levels in fish livers were higher than levels in the rest of the fish. PFOS levels declined by about 80% over the duration of the study, based on a comparison of fish and fish liver samples collected in 2003 and 2009. Short-chain PFCs, PFHxS, PFHpA, and PFOA, which were detected in the water samples, were not found in the fish. Higher PFOS levels were identified in samples taken close to the spill location about a decade after the AFFF release. Water and fish levels, collected in 2009 from areas impacted by the AFFF spill, were around 2 to 10 times higher than upstream locations. The authors attributed this to long-term impacts of the spill along with urbanization (Awad, et al. 2011).

During an investigation in 2010, PFCs were measured in snapping turtle plasma collected in 2007-2008 from what was then considered a reference site, Lake Niapenco, in Hamilton, Ontario, Canada. As PFC levels were higher in those turtles than at the industrial locations, a study was carried out to determine if a nearby airport contributed to PFC levels in snapping turtles, amphipods (small crustaceans), fish, and shrimp along with water from Lake Niapenco and the Welland River. The airport has a fire-fighting training area that drains into the Welland River, which drains into the lake (de Solla, et al. 2012). The fire-fighting training area was used between 1985 and 1994, with a reported 15,000 liters of AFFF (PFOS-based) sprayed per year (Gewurtz, et al. 2014). Levels of PFOS found in snapping turtle plasma samples from the Welland River (n=1) and Lake Niapenco (n=18) were more than 40 times higher than those from other sites. PFOS levels represented about 99% of the total PFCs in snapping turtle plasma downstream of the airport, but only between 72 to 94% of the total PFC levels from the other sites. Although the authors could not confirm the PFCs were from airport runoff, a PFC identified in aircraft hydraulic fluid was also found at low levels in nearby biota (de Solla, et al. 2012).

PFC levels were measured in sport fish, collected between 2009 and 2012, from Lake Niapenco and the Welland River. The species collected were black crappie, bluegill, brown bullhead, channel catfish, common carp, freshwater drum, largemouth bass, northern pike, pumpkinseed, rock bass, smallmouth bass, walleye, white crappie, white sucker, and yellow perch. Elevated

PFC levels were identified in the fish, even though AFFF releases ended more than 15 years previously. Fish downstream of the airport, as far as 40 kilometers away, had elevated levels of PFCs, possibly transported in the water combined with persistence in the sediment. Levels in fish did not appear to vary temporally and PFOS was the only PFC with a spatial trend (Gewurtz, et al. 2014).

Trophic Transfer of PFCs

Juvenile rainbow trout were given food spiked with PFPA, PFHxA, PFHpA, PFOA, PFDA, PFUnA, PFDoA, PFTA, PFBS, PFHxS, and PFOS for 34 days. Levels of PFCs in the food ranged from 0.32 to 1.2 parts per million (ppm). Fish were sampled on days 4, 7, 14, 21, 28, and 34. After the 34 days, the remaining fish were fed untreated food for 41 days (days 35 to 75). Fish given the untreated food were sampled on days 41, 48, 55, 62, 68, and 75. PFCs were measured in the liver, blood, and carcass (with the gut removed). Carboxylates with less than six perfluoralkyl carbons (PFPA, PFHxA, and PFHpA) were not detected in the fish tissue and PFBS was only detected in fish sampled on day 21, 28, 34, and 41. The bioaccumulation of these PFCs was expected to be very low. Bioaccumulation factors for PFCs in the carcass were calculated and were approximately one or lower for PFOA, PFDA, PFUnA, PFDoA, PFTA, PFOS, and PFHxS. The bioaccumulation factor for PFOS was about ten times higher than the bioaccumulation factor for PFOA in the carcass. The half-life of PFOS was approximately five times higher than the half-life for PFOA in both the carcass and liver. The authors concluded that dietary PFC exposure would not result in biomagnification in juvenile rainbow trout (Martin, et al. 2003a).

Juvenile rainbow trout were placed in water containing PFPA, PFHxA, PFHpA, PFOA, PFDA, PFUnA, PFDoA, PFTA, PFBS, PFHxS, and PFOS for 288 hours (12 days). Average levels of the PFCs ranged from 0.014 to 1.7 micrograms per liter in the water. The longer chain PFCs had lower water solubility. Fish were sampled at 4.5, 9 18, 36, 72, 144, and 288 hours. After the 288 hours of exposure, the trout were transferred to water without PFCs. Fish were sampled 4.5, 8, 18, 36, 72, 144, 288, 456, and 792 hours (33 days) after being transferred to the PFC-free water. Perfluorinated carboxylates with more than six carbons (PFOA, PFDA, PFUnA, PFDoA, and PFTA) and perfluorinated sulfonates with more than five carbons (PFHxS and PFOS) were detected in the blood, liver, and carcass at all timepoints. PFCs were found in the kidney, liver, gall bladder, blood plasma, blood cells, gonads, adipose and muscle tissue, as well as the gills. Levels of PFCs tended to be highest in the blood, kidney, liver, and gall bladder. Levels were the lowest in the muscle tissue, followed by adipose tissue and gonads. The authors estimated that 22% of the total PFOS, 61% of the total PFHxS, and 81% of the total PFOA body burden could be in the muscle, if muscle represented about 67% of rainbow trout weight. The shortest half-life was for PFOA in the carcass, blood, and liver. Half-lives for PFDA, PFUnA, PFDoA, PFTA, PFOS, and PFHxS were about three times longer. All PFCs had half-lives that were similar in the carcass, blood, and liver. The bioconcentration factor (BCF) for PFOA was more than 100 times smaller than the BCF for PFOS. Higher BCFs tended to be in the blood for the carboxylates, while for PFOS and PFHxS, the blood and liver BCFs were similar. The authors determined that sulfonates bioconcentrated to a greater extent than carboxylates with the same number of carbons due to higher uptake rates and lower elimination rates (Martin, et al. 2003b).

PFOS, PFOA, PFHxS, and PFOSA were measured in surface water and livers of fish and waterfowl in New York.[1] Surface water samples were collected from nine freshwater lakes and rivers, while livers were taken from 38 smallmouth bass, 28 largemouth bass, and 10 waterfowl (varied species). Water, fish, and waterfowl samples were collected in different years within a decade. In seven of the nine waterbodies, PFOS levels were lower than PFOA levels. In all of the waterbodies, PFHxS levels were lower than PFOS levels. PFOSA was not detected in any water sample. PFOS was detected in all 66 of the fish liver samples and the levels were higher (9 to 315 ppb wet weight) than the levels of PFOA (<1.5 to 7.7 ppb wet weight). Ninety percent of the fish liver samples had detectable PFOA. PFOSA was detected in fewer fish liver samples (only 62%) at levels similar to PFOA (<1.5 to 11.4 ppb wet weight). PFHxS was not detected in any of the fish samples, with a detection limit of 1.5 ppb. All waterfowl liver samples had detectable PFOS, but none had detectable PFOA, PFOSA, or PFHxS. Based on the water and fish liver samples, the authors calculated a BCF of 8,850 for PFOS and 184 for PFOA. No BCF was calculated for PFOSA (Sinclair, et al. 2006).

Based on the above and other similar studies, there are a few general points regarding the bioaccumulation of PFCs. Most importantly, bioaccumulation and biomagnification is dependent on the number of perfluorinated carbons in a specific PFC. Sulfonates appear to be more bioaccumulative than carboxylates with the same number of fluorinated carbons. PFOA, and other carboxylates with seven or fewer perfluorinated carbons, do not appear to biomagnify or to be bioaccumulative (Conder, et al. 2008). It should be noted that PFOA seems to bioaccumulate in human serum, but not other biological matrices (Lindstrom, et al. 2011).

Exposure Pathways

An exposure pathway contains five elements: (1) the contaminant source, (2) contamination of environmental media, (3) an exposure point, (4) a human exposure route, and (5) potentially exposed populations. An exposure pathway is complete if there is a high probability or evidence that all five elements are present. Table 5 describes human exposure to PFOS from ingestion of fish.

Table 5: Exposure pathway for human exposure to PFOS from fish.

Source	Environmental Medium	Exposure Point	Exposure Route	Exposed Population	Time Frame	Exposure
Point sources, fire-fighting or fire suppression activities, food chain contamination	Fish (contamination from the water and sediments magnifying in the food web)	Inland lake, river, or Great Lake fish filets	Ingestion	Recreational anglers and individuals who eat sport-caught fish	Past Present Future	Potential Potential Potential

Fish can be a major source of exposure to PFCs for humans (Lindstrom, et al. 2011). Domingo (2012) provided a summary of foods that were relevant for people's exposure to PFOS and other

[1] PFCs in livers were measured because at the time of the publication, measuring PFCs in the liver was a better established method and would represent the highest level in the fish or waterfowl (Sinclair, et al. 2006).

PFCs. Fish intake was a contributor of dietary PFOS based on studies in Poland, Spain, and Norway (Domingo 2012).

Del Gobbo et al. (2008) examined the effect of cooking on PFOS levels in seafood. PFOS, along with PFOA, PFNA, PFDA, PFUA, PFDoA, PFTeA, and PFOSAs (n-ethyl- and n-methyl-PFOSAs) was measured in raw, fried, and boiled seafood. Skin was left on the finfish tested. PFOS was detected in 24% of the samples with a range of 0.21 to 1.68 ppb wet weight. PFOSAs were only detected in scallops. PFOA was detected in 10% of the samples with levels ranging from 0.06 to 1.78 ppb wet weight. Two longer chain carboxylates, PFNA and PFUnA, were detected in 11% of samples with levels ranging from 0.39 to 2.11 ppb wet weight. Levels of PFCs detected in seafood tended to decrease after cooking, although in some samples, the levels increased. The authors speculated on several possible explanations for this, but recommended further investigation in the effect of cooking on PFC levels (Del Gobbo, et al. 2008). Follow-up information indicated that cooking fish did not change the overall PFOS amounts present in the samples (Bhavsar 2013).

Xiao et al. (2012) measured PFCs in raw or unprocessed foods. Food was purchased from a farmer's market in downtown Minneapolis or two grocery stores in Minneapolis in the summer of 2010. Vegetables (bell peppers, cabbage, carrots, cauliflower, celery, cucumbers [peeled], crimini mushrooms, iceberg lettuce, tomatoes, sweet onions [peeled], eggplant, and potatoes [peeled]), meats (ground pork, beef, and chicken), cereals and cereal products (rice, oats, and yellow beans), and unbleached flour were analyzed for PFHpA, PFOA, PFNA, PFOS, PFDA, and PFUnDA. PFOS was detected in the unbleached flour samples (mean ± SD = 0.095 ± 0.042 ng/g dry weight) and in cauliflower from a farmer's market (at levels below the limit of quantification). All other samples had no detectable PFCs. However, PFOS and other PFCs in tap water used for washing and cooking increased the levels in the foods (Xiao, et al. 2012).

Estimating human PFOS intake

Llorca et al. (2010) measured PFOA, PFOS, perfluoro-7-methyloctanoic acid (ip-PFNA), PFNA, PFDA, and sodium perfluoro-1-decanesulfonate (L-PFDS) in human milk (20 samples), baby cereals (2 brands), and infant formulas (3 brands). Human milk had detectable levels of PFOA, PFOS, i,p-PFNA, PFDA, and L-PFDS, with PFOS and i,p-PFNA being detected in 95% of the samples. All of the measured PFCs were detected in the cereal and formula samples, with the highest levels found for PFDA and PFOS. Table 6 presents the levels of the PFCs. The authors calculated infant's possible exposure to PFOA and PFOS from human milk, baby cereals, and infant formula and found that all potential exposures were at or below the tolerable daily intakes (Llorca, et al. 2010).

Table 6: Range of PFCs (PFOA, PFOS, perfluoro-7-methyloctanoic acid (ip-PFNA), PFNA, PFDA, and sodium perfluoro-1-decanesulfonate (L-PFDS) in parts per billion (ppb) measured in human milk, baby cereals, and infant formulas (Llorca, et al. 2010).

PFC	Human milk (ppb)	Baby cereals (ppb)	Infant formula (ppb)
PFOA	ND – 0.907	0.166 – 0.438	0.374 – 0.723
PFOS	0.028 – 0.865	0.162 – 0.458	0.229 – 1.098
ip-PFNA	0.021 – 0.260	0.166 – 0.438	0.374 – 0.723
PFNA	ND	0.044 – 0.138	0.118 – 0.219
PFDA	ND – 1.095	0.236 – 0.266	0.693 – 1.289
L-PFDS	ND – 0.070	0.0561 – 0.0906	0.0551 – 0.719

Berger et al. (2009) measured PFCs (PFBS, PFHxS, PFOS, PFOSA, PFDcS, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTriA, PFTeA, and PFPeDA) in fish from Lake Vättern, in Sweden, and the Baltic Sea. For perch, burbot, and whitefish, PFCs were measured in the skinless filet and for salmon and brown trout, muscle samples were taken around the dorsal fin. For almost all of the samples, PFPeDA, PFBS, PFDcS, PFHxA, and PFHpA were not detected. Levels of PFOS were higher than all other PFCs measured. For the Lake Vättern fish, median PFOS levels were highest in burbot and perch and lowest in whitefish (burbot, perch>salmon>brown trout>whitefish). For the Baltic Sea fish, whitefish had the highest median PFOS level and the lowest level was in salmon (whitefish>perch>burbot>brown trout>salmon). Lake Vättern fish had higher median PFOS concentrations than the same species of Baltic Sea fish. The authors concluded that differences in waterbody characteristics and food web differences may be responsible for this difference. The authors attributed the difference in PFOS levels in the different fish species to species-specific feeding habits and toxicokinetics (Berger, et al. 2009).

The authors went on to calculate PFOS intake using the median Lake Vättern PFOS levels (7.5 ppb) for 37 to 87-year-old women (65 kilogram [kg]) with a high consumption of fish, and 34 to 64-year-old men (75 kg) using the median Baltic Sea PFOS levels (1.4 ppb) with a moderate or high consumption of fish. The number of meals, each assumed to be 125 grams (g) of fish per meal, was previously assessed using surveys. High consuming women ate two to 20 meals per month, while moderate and high consuming men ate 0 to five and two to 58 meals per month, respectively. For the women, the calculated PFOS intake ranged from 0.92 to 9.6 ng/kg/day, with a median of 2.7 ng/kg/day. For the men, the moderate consumers were calculated to have an intake of 0 to 0.39 ng/kg/day, with a median of 0.15 ng/kg/day. The high consumers of Baltic Sea fish were calculated to have a PFOS intake ranging from 0.15 to 4.5 ng/kg/day, with a median of 0.62 ng/kg/day (Berger, et al. 2009).

Although these calculated intakes were below the tolerable daily intake of 150 ng/kg/day, the authors caution that the contribution of other dietary PFOS was not included. Based on other studies, the levels of PFOS calculated for high consumers of Lake Vättern and the Baltic Sea fish were similar or slightly higher than PFOS intake from total diet or duplicate portion studies. The authors concluded that consuming fish from PFOS contaminated freshwater could be a significant source of human PFOS exposure (Berger, et al. 2009).

Egeghy and Lorber (2011) estimated human PFOS intake both back-calculated from the National Health and Nutrition Examination Survey (NHANES) serum PFOS levels and forward-modeled from measurements of PFOS levels in drinking water, dust, air, and food. Back-calculation from the serum PFOS levels used the geometric mean from NHANES 2003-2004 (20.7 nanograms per milliliter [ng/mL]) and a one-compartment model. The authors assumed that PFOS exposure had occurred for a sufficient amount of time for levels to reach steady state. Two different volumes of distribution were used: 3000 and 200 mL/kg. (Volume of distribution is the apparent volume for an amount of chemical that would result in a given plasma concentration. The authors used two different volumes of distribution as there are no human values.) The modeled PFOS intake based on PFOS serum levels (back-calculated level) ranged from 1.6 to 24.2 ng/kg/day, depending on the volume of distribution used (Egeghy and Lorber 2011).

Three exposure scenarios (typical PFOS exposure conditions, exposure in environments highly contaminated with PFOS, and exposure to PFOS plus precursors) were used for the forward-modeled PFOS intake for a 2-year-old child and for an adult. Under the typical exposure scenario, the largest percentage of PFOS intake comes from food ingestion for both the 2-year-old child (42%) and an adult (72%). Dust ingestion was also a potentially large source of PFOS for a 2-year-old (36%). For the contaminated environment scenario, contaminated drinking water was included, but ingestion of contaminated fish or other wildlife was not. Under the contaminated environment scenario, the largest percentage of PFOS intake is from water ingestion for both the child and adult. When PFOS precursors, perfluorooctane sulfonamidoethanol (PFOSEs) and PFOSAs, are included in the scenario, the largest percentage of PFOS intake comes from dust ingestion for a child (51%) and food ingestion for an adult (59%). The median PFOS intake for an adult, including the precursors, was 4.2 ng/kg/day. The typical exposure scenario resulted in a median PFOS intake of 2.3 ng/kg/day, and the contaminated environment scenario resulted in a median PFOS intake of 31.4 ng/kg/day (Egeghy and Lorber 2011).

The calculated intake from the exposure scenarios is within or similar to the range obtained for the intake modeled from serum data (1.6 to 24.2 ng/kg/day). Egeghy and Lorber noted the uncertainties in the modeling and concluded that, due to those, the range calculated could not be narrowed. They also noted that the inclusion of the precursors in the intake may overestimate the actual intake (Egeghy and Lorber 2011).

Loccisano et al. (2011) developed a model for PFOS and PFOA toxicokinetics for monkeys and humans. This model included multiple compartments to incorporate information on PFOS and PFOA partitioning in the body and reabsorption in the kidney. Renal reabsorption in the proximal tubules is one explanation for the longer half-lives of PFOS and PFOA in monkeys and humans compared to rodents. Once there was agreement between experimental data for intravenous and oral exposure to PFOA and PFOS in monkeys and modeling results, the model was refined for humans. Renal reabsorption parameters were also used for humans, however the transporter capacity was adjusted to account for the longer half-lives of PFOA and PFOS in humans (three to five years in humans compared to about 20 days to six months in monkeys) (Loccisano, et al. 2011).

The model was run with data from populations exposed to PFOA in drinking water in Ohio and Germany and with serum levels from the general population (95% confidence intervals for the geometric means from 1999-2000 and 2003-2004 NHANES data). Two half-lives were used for the PFOA intake, as one was obtained from a worker study (3.8 years) and the other was from residents drinking PFOA-contaminated water (2.3 years). With a half-life of 2.3 years, the estimated PFOA exposure for the general population ranges from 0.33 to 0.52 ng/kg/day. With a half-life of 3.8 years, the estimated PFOA intake for the general population ranges from 0.2 to 0.32 ng/kg/day. Using a half-life of 5.4 years, the estimated PFOS intake was 1.6 to 3.0 ng/kg/day. The authors noted that, although the model incorporated renal reabsorption and was able to describe experimental data, renal reabsorption may not be solely responsible for the differences observed with PFOA and PFOS serum half-lives in laboratory animals and humans (Loccisano, et al. 2011).

Jain (2014) examined the influence of diet and other factors on the serum levels of PFHxS, PFNA, PFOA, and PFOS in NHANES participants (individuals ages 12 or older enrolled in 2003-2004, 2005-2006, and 2007-2008 cycles). The four PFCs were selected as they were detected in more than 60% of the samples. Data from 5,586 individuals were used for the PFHxS analysis. The PFHxS geometric mean was 2.32 ppb in males and 1.53 ppb in females. Levels of PFHxS were significantly positively associated with age and serum protein. PFHxS levels were significantly negatively associated with serum albumin, and calories consumed. Levels of PFHxS were not associated with any specific food consumption (Jain 2014).

Data from 5,591 individuals were used for the PFNA, PFOA, and PFOS analyses. The PFNA geometric means were 1.30 ppb in males and 1.06 ppb in females. PFNA levels were significantly positively associated with age, serum cholesterol, calories consumed, alcohol consumed, amounts of milk without cheese consumed, fish consumed, and non-alcoholic beverages consumed. PFNA levels were significantly negatively associated with age squared, fat consumed, and body mass index (Jain 2014).

The PFOA geometric means were 4.69 ppb in males and 3.51 ppb in females. Levels of PFOA were significantly positively associated with age squared, serum cholesterol, serum albumin, body mass index, and non-alcoholic beverages consumed. Serum PFOA levels were significantly negatively associated with amount of caffeine consumed, amounts of milk without cheese consumed, and amounts of dry beans consumed (Jain 2014).

The PFOS geometric means were 19.83 ppb in males and 14.32 ppb in females. Serum levels of PFOS were significantly positively associated with age squared, serum cholesterol, serum albumin, amount of meat consumed, amount of fish consumed, alcoholic beverages consumed, and fat consumed. PFOS levels were significantly negatively associated with amount of caffeine consumed, calories consumed, amount of milk without cheese consumed, amount of cheese consumed, amount of fats and oils consumed, and amount of non-carbonated water consumed. PFOS levels also decreased over time, based on the NHANES cycles used. Jain cautions that further investigation is needed to determine if these associations are relevant to people's health (Jain 2014).

Table 7 summarizes the estimated PFOS intakes from the studies described above.

Table 7: Summary of estimated PFOS intakes.

Population	Estimated PFOS intake	Basis of calculation	Reference
High fish consuming Swedish women (37-87 years old)	0.92 to 9.6 ng/kg/day (median = 2.7 ng/kg/day)	Between 2-20 meals (125 g/meal) per month of Lake Vattern fish with a median PFOS level of 7.5 ppb	Berger et al. 2009
Moderate fish consuming Swedish men (34-64 years old)	0 to 0.39 ng/kg/day (median = 0.15 ng/kg/day)	Between 0-5 meals (125 g/meal) per month of Baltic Sea fish with a median PFOS level of 1.4 ppb	Berger et al. 2010
High fish consuming Swedish men (34-64 years old)	0.15 to 4.5 ng/kg/day (median = 0.62 ng/kg/day)	Between 2-58 meals (125 g/meal) per month of Baltic Sea fish with a median PFOS level of 1.4 ppb	Berger et al. 2011
U.S. general population	1.6-24.2 ng/kg/day	geometric mean PFOS levels of 20.7 ng/mL from NHANES (2003-2004), one compartment model using two different volumes of distribution	Egeghy and Lorber 2011
Typical PFOS exposure for an adult in the U.S.	2.3 ng/kg/day (median)	PFOS levels in drinking water, dust, air, and food	Egeghy and Lorber 2011
High PFOS exposure for an adult in the U.S.	31.4 ng/kg/day (median)	PFOS levels in dust, air, food, and PFOS contaminated drinking water	Egeghy and Lorber 2011
Typical PFOS + PFOS precursor exposure for an adult in the U.S.	4.2 ng/kg/day (median)	PFOS and PFOS precursor levels in drinking water, dust, air, and food	Egeghy and Lorber 2011
U.S. general population	1.6-3.0 ng/kg/day	95% confidence intervals for the PFOS geometric means from the 1999-2000 and 2003-2004 NHANES, multi-compartment model, PFOS half-life of 5.4 years	Loccisano et al. 2011

Biomonitoring of the general population and fish-eating humans

Levels of PFCs were measured in serum samples from NHANES participants. Four PFCs were detected in almost all of the 2003-2004 participants: PFOS (99.9%), PFOA (99.7%), PFHxS (98.3%), and PFNA (98.9%). PFOS levels significantly correlated with PFOA, PFHxS, and PFNA levels. Participants in 2003-2004 NHANES had significantly lower levels of PFOS, PFOA, and PFNA than those measured in 1999-2000 NHANES (based on comparisons of the geometric means for all age groups, sex, and race) (Calafat, et al. 2007). Tables 8, 9, 10, and 11 present levels of PFOS, PFOA, PFHxS, and PFNA in NHANES 2003-2004 participants, respectively.

Table 8: Geometric mean and percentiles (95% confidence intervals) for serum levels of perfluorooctanesulfonate (PFOS) in parts per billion (ppb) for participants, ages 12 and older, in National Health and Nutrition Examination Survey (NHANES) 2003–2004 participants (Calafat, et al. 2007).

Variable	Geometric mean	10th percentile	25th percentile	50th percentile	75th percentile	95th percentile	Number
All	20.7 (19.2–22.3)	9.8 (9.0–10.8)	14.6 (13.8–15.2)	21.1 (19.8–22.4)	29.9 (27.5–32.8)	54.6 (44.0–65.9)	2,094
12–19 years	19.3 (17.5–21.4)	9.9 (9.5–10.9)	14.4 (12.5–15.7)	19.9 (17.6–21.9)	27.1 (23.6–30.2)	42.2 (35.1–52.1)	640
20–39 years	18.7 (17.3–20.1)	8.9 (8.2–10.2)	12.6 (11.2–14.2)	18.7 (17.7–20.4)	27.4 (24.9–29.7)	44.3 (38.6–60.8)	490
40–59 years	22.0 (19.7–24.5)	10.6 (9.2–12.3)	15.3 (14.1–18.0)	22.2 (20.2–24.2)	32.2 (27.4–35.4)	61.5 (43.8–81.8)	387
≥ 60 years	23.2 (20.8–25.9)	9.9 (7.7–13.0)	16.6 (15.0–17.9)	23.9 (20.9–27.2)	34.7 (30.0–39.3)	69.4 (49.6–90.0)	577
Female	18.4 (17.0–20.0)	9.0 (7.8–9.9)	12.4 (11.5–13.8)	18.2 (16.8–19.7)	27.3 (23.6–30.0)	45.7 (42.3–61.5)	1,041
Male	23.3 (21.1–25.6)	12.3 (10.4–13.5)	17.7 (15.9–18.9)	23.9 (22.3–25.3)	32.1 (28.7–35.7)	62.7 (43.8–81.8)	1,053

Table 9: Geometric mean and percentiles (95% confidence intervals) for serum levels of perfluorooctanoic acid (PFOA) in parts per billion (ppb) for participants, ages 12 and older, in National Health and Nutrition Examination Survey (NHANES) 2003–2004 participants (Calafat, et al. 2007).

Variable	Geometric mean	10th percentile	25th percentile	50th percentile	75th percentile	95th percentile	Number
All	3.9 (3.6–4.3)	1.9 (1.8–2.1)	2.7 (2.6–3.0)	4.0 (3.8–4.4)	5.8 (5.2–6.3)	9.8 (7.4–14.1)	2,094
12–19 years	3.9 (3.5–4.4)	2.2 (1.9–2.3)	2.9 (2.6–3.2)	3.9 (3.3–4.4)	5.4 (4.6–6.1)	8.6 (5.9–12.6)	640
20–39 years	3.9 (3.6–4.2)	1.8 (1.5–2.1)	2.7 (2.5–3.0)	4.1 (3.7–4.5)	5.8 (5.4–6.1)	9.6 (8.4–11.1)	490
40–59 years	4.2 (3.8–4.8)	2.0 (1.8–2.4)	2.9 (2.6–3.2)	4.2 (3.9–4.8)	6.3 (5.3–7.2)	10.6 (7.4–16.9)	387
≥ 60 years	3.7 (3.3–4.1)	1.8 (1.5–2.1)	2.7 (2.4–2.9)	3.9 (3.5–4.3)	5.4 (4.9–5.9)	9.5 (6.9–14.1)	577
Female	3.5 (3.2–3.8)	1.6 (1.5–1.9)	2.5 (2.2–2.7)	3.6 (3.2–3.9)	5.2 (4.6–5.7)	8.4 (7.4–10.6)	1,041
Male	4.5 (4.1–4.9)	2.3 (2.0–2.4)	3.2 (3.1–3.5)	4.6 (4.2–5.0)	6.3 (5.6–7.1)	10.4 (7.4–17.5)	1,053

Table 10: Geometric mean and percentiles (95% confidence intervals) for serum levels of perfluorohexane sulfonate (PFHxS) in parts per billion (ppb) for participants, ages 12 and older, in National Health and Nutrition Examination Survey (NHANES) 2003–2004 participants (Calafat, et al. 2007).

Variable	Geometric mean	10th percentile	25th percentile	50th percentile	75th percentile	95th percentile	Number
All	1.9 (1.7–2.2)	0.7 (0.6–0.7)	1.0 (0.9–1.2)	1.9 (1.6–2.1)	3.3 (2.8–3.9)	8.3 (7.1–9.7)	2,094
12–19 years	2.4 (2.1–2.9)	0.6 (0.5–0.8)	1.2 (1.0–1.4)	2.3 (1.7–3.0)	4.8 (3.9–6.0)	13.1 (9.9–19.6)	640
20–39 years	1.8 (1.6–2.0)	0.5 (0.5–0.6)	1.0 (0.9–1.2)	1.7 (1.5–2.0)	2.8 (2.5–3.3)	6.7 (4.9–9.4)	490
40–59 years	1.9 (1.6–2.2)	0.7 (0.5–0.8)	1.0 (0.9–1.2)	1.6 (1.4–2.0)	3.1 (2.3–4.5)	6.7 (5.5–8.2)	387
≥ 60 years	2.0 (1.7–2.4)	0.8 (0.5–0.9)	1.1 (1.0–1.3)	1.9 (1.6–2.1)	3.2 (2.6–3.7)	10.2 (7.0–12.6)	577
Female	1.7 (1.6–1.9)	0.6 (0.5–0.6)	0.9 (0.8–1.0)	1.5 (1.4–1.8)	2.9 (2.5–3.5)	8.2 (6.7–10.0)	1,041
Male	2.2 (1.9–2.5)	0.8 (0.7–1.0)	1.3 (1.1–1.4)	2.0 (1.8–2.4)	3.3 (2.8–4.4)	8.5 (6.4–10.5)	1,053

Table 11: Geometric mean and percentiles (95% confidence intervals) for serum levels of perfluorononanoic acid (PFNA) in parts per billion (ppb) for participants, ages 12 and older, in National Health and Nutrition Examination Survey (NHANES) 2003–2004 participants (Calafat, et al. 2007).

Variable	Geometric mean	10th percentile	25th percentile	50th percentile	75th percentile	95th percentile	Number
All	1.0 (0.8–1.1)	0.4 (0.3–0.4)	0.6 (0.5–0.6)	1.0 (0.9–1.1)	1.5 (1.2–1.7)	3.2 (1.8–7.7)	2,094
12–19 years	0.9 (0.7–1.0)	0.3 (0.3–0.4)	0.5 (0.5–0.6)	0.7 (0.6–0.9)	1.2 (0.9–1.5)	2.7 (1.3–6.3)	640
20–39 years	1.0 (0.8–1.1)	0.3 (0.2–0.5)	0.6 (0.6–0.7)	0.9 (0.8–1.1)	1.4 (1.2–1.7)	2.8 (1.9–6.1)	490
40–59 years	1.1 (0.9–1.4)	0.5 (0.4–0.5)	0.7 (0.6–0.7)	1.0 (0.9–1.2)	1.7 (1.2–2.4)	4.3 (1.7–9.3)	387
≥ 60 years	0.8 (0.7–1.0)	0.3 (0.2–0.3)	0.5 (0.5–0.6)	0.9 (0.8–1.0)	1.3 (1.1–1.5)	3.0 (1.6–6.5)	577
Female	0.9 (0.7–1.0)	0.4 (0.3–0.4)	0.6 (0.5–0.6)	0.9 (0.7–0.9)	1.2 (1.0–1.6)	3.0 (1.7–6.1)	1,041
Male	1.1 (0.9–1.3)	0.5 (0.4–0.5)	0.6 (0.6–0.7)	1.0 (0.9–1.2)	1.6 (1.3–1.8)	4.0 (1.8–8.7)	1,053

PFCs were measured in the blood of anglers (PFOA, PFOS, PFHxS, PFHxA, PFPA, and PFBS) and in fish (perch, eels, pikes, ciscos, and roaches) filets (PFDoA, PFUnA, PFDA, PFNA, PFOA, PFOS, PFHpA, PFHxA, PFHxS, and PFBS) from Lake Möhne, a freshwater lake in Germany. PFCs were also measured in drinking water samples (PFBA, PFBS, PFPA, PFHxA, PFHxS, PFOA, PFOS, PFHpA, PFNA, and PFDA). In the fish filets, PFOS was the predominant PFC measured. PFOS levels ranged from 4.5 to 150 ppb. The authors noted that the different fish

species had different PFOS levels depending on feeding behavior. Piscivorous fish tended to have higher PFOS levels than plankton-, spawn-, or fry-eating fish, which tended to have greater levels of PFOS than non-predatory, ground-feeding fish. PFOA was only detectable in 20% of the fish filets, with a maximum level of 2.3 ppb. None of the other PFCs (PFDoA, PFUnA, PFDA, PFNA, PFHpA, PFHxA, PFHxS, and PFBS) were detected in fish filets (Holzer, et al. 2011).

All of the 105 anglers (99 men and 6 women) had detectable PFOS, PFOA, and PFHxS in their blood plasma. The levels ranged from 1.1 to 650 ppb for PFOS, 2.1 to 170 ppb for PFOA, and 0.4 to 17 ppb for PFHxS. Only 10% had detectable PFBS, and two PFCs (PFHxA and PFPA) were not detected in any human sample. People who ate fish two to three times a month had seven times the level of PFOS than those who never ate fish from Lake Möhne. The geometric means for PFOS ranged from 7.1 ppb (never ate fish from Lake Mohne) to 80 ppb (more than three servings/month). The geometric mean PFOS intake from fish consumption was calculated to range from 0.4 (two servings per year) to 5.4 ng/kg/day (more than three servings/month). Levels of PFOA and PFHxS were higher in people who ate fish more than three times a month compared to those who infrequently or never ate fish from Lake Möhne. Geometric means for PFOA and PFHxS were 7.9 (never ate fish from Lake Mohne) to 16 (more than three servings/month) ppb and 2.3 (never ate fish from Lake Mohne) to 3.8 (more than three servings/month) ppb, respectively (Holzer, et al. 2011).

PFCs were only detected in 57% of the tapwater samples, with PFBA the most frequently detected. PFOA levels in tapwater ranged from 20 to 47 ng/L while PFOS levels ranged from 11 to 59 ng/L. PFHxS was not detected in any tapwater sample. While the PFOS and PFHxS levels were associated with fish consumption, PFOA blood levels were associated with levels in tapwater. All three PFCs were associated with age (Holzer, et al. 2011).

PFCs were measured in 31 participants, four women and 27 men, in the New York State Angler Cohort Study (NYSACS). Between 1995 and 1997, participants completed a survey on their medical history and sportfish and game consumption, and also provided a blood sample. Eight PFCs (PFDA, PFHpA, PFNA, PFOA, PFUnA, PFHxS, PFOS, and PFOSA) were measured in the participant's serum samples. Thyroid hormones were also measured. Participants ate a median of one to 6 New York state sportfish meals, with a minimum of zero and a maximum of 24 to 36 meals, from June 1994 to June 1995 (Bloom, et al. 2010).

PFOS, with a range of 7.25 to 76.88 ppb, and PFHxS, with a range of 0.16 to 4.60 ppb, were detected in all serum samples. PFOA was detected in almost all samples (~94%), with a range of 0.57 to 2.58 ppb. PFDA (~65%) and PFUnDA (~52%) were detected in about half of the samples, with ranges of 0.14 to 1.14 ppb and 0.14 to 0.92 ppb, respectively. Based on the summed amount of PFCs, PFOS levels were about 83% of the total. PFOA levels were about 6% of the total, while PFDA and PFUnDA levels were each about 1% of the total (Bloom, et al. 2010).

PFOS was not associated with fish consumption, but both PFDA and PFUnDA were significantly associated with New York state sportfish fish meals. Serum PFOS levels were significantly correlated with levels of PFDA, PFNA, and PFUnDA. All of the participants had

free thyroxine (FT4) levels within the normal laboratory range (0.80-1.80 nanograms per deciliter [ng/dL]) and only one participant had TSH levels higher than the normal laboratory range (0.40-5.00 microInternational Unit/milliliter [µIU/mL]). No significant associations were identified between serum PFC levels and thyroid hormones; however, the authors noted that substantially increased participant numbers may be needed to identify significant associations (Bloom, et al. 2010).

Levels of PFOS and plasma lipids were measured in Nunavik Inuit adults (n=720) in 2004. The plasma PFOS arithmetic mean was 25.7 ppb, with men (28.2 ppb) having a significantly higher mean PFOS level than women (23.1 ppb). PFOS levels tended to increase with age in both genders, with PFOS levels significantly higher in people older than 45 years old (Chateau-Degat, et al. 2010).

Higher high-density lipoprotein cholesterol (HDL-C) levels were significantly associated with higher plasma PFOS levels in both men and women after adjustment for confounders, including levels of omega-3 polyunsaturated fatty acids (n-3 PUFAs) (eicosapentaenoic acid [EPA]+ docosahexaenoic acid [DHA]). Lower total cholesterol/HDL-C ratios were significantly associated with higher plasma PFOS levels in both men and women. Lower triacylglycerol levels were significantly associated with higher PFOS levels only in women. Associations between total cholesterol/HDL-C ratios or triacylglycerol levels and PFOS levels were significant after adjustment for confounders (Chateau-Degat, et al. 2010).

The authors note that these results are contrary to those identified in studies with other human populations and laboratory animals, which identified decreased HDL-C with increasing PFOS levels. A possible explanation is that the Inuit population in this study consumes very high n-3 PUFA levels through a traditional diet, which can result in an increase in HDL-C. The authors speculate that PFOS exposure may result in similar effects in addition to those already attributable to n-3 PUFA exposure and that further investigation into these effects is necessary (Chateau-Degat, et al. 2010).

Toxicological Evaluation

People tend to be exposed to multiple PFCs. However, although these chemicals can be grouped, there are metabolic and toxicologic differences that prevent assessing these chemicals together. Scialli et al. (2007) examined studies exposing rats to PFOS, PFOA, and PFBS. The authors could not identify a scaling system that would allow assessing combinations of these chemicals together. Based on the studies that were compared, the authors suggested that the relative toxicity of the different PFCs may change with the endpoint assessed (Scialli, et al. 2007). Therefore, while human epidemiological studies are briefly described below, the interrelatedness of different PFC levels may make it too difficult to select a point-of-departure, or even choose one speculatively, at this time.

Human epidemiology studies

Health effects related to PFOA, PFOS, and other PFCs have been examined in two large populations. One is the C8 Health Project and the other is NHANES. These are epidemiology studies that focus on identifying health effects associated with the presence of PFCs in the body.

Limited information is available regarding any participant's specific PFOS intake and, in some cases, the levels of different PFCs are associated with one another. This further complicates the identification of health effects associated with individual PFC exposures. Therefore, while human data are usually preferred over animal data, the health effects found to be associated with the C8 Health Project and NHANES participants will be used to guide the evaluation of rodent and non-human primate studies rather than to select a point of departure for an RfD.

Health effects identified in participants in the C8 Health Project

The C8 Health Project participants are individuals from Ohio and West Virginia primarily exposed to PFOA in drinking water, but would also have typical U.S. background exposures to PFCs. While PFOS was measured in participants' serum samples, about half of the studies (at the time of this document) primarily focus on PFOA. This limits the usefulness of the C8 Health Project results for assessing human health effects of PFOS.

Participants in the C8 Health Project live in areas of Ohio and West Virginia where there is water pollution from the DuPont Washington Works plant. The plant contaminated the Ohio River and groundwater in the area with PFOA. More than 65,000 people who had been exposed to PFOA-contaminated water for at least a year completed health surveys and submitted blood samples for PFC analysis. The 10 PFCs measured were PFPeA, PFHxA, PFHxS, PFHpA, PFOA, PFOS, PFNA, PFDA, PFUnA, and PFDoA (Frisbee, et al. 2009).

Almost all participants had measurable levels of PFHxS (97.9%), PFOA (99.9%), PFOS (99.6%), and PFNA (97.7%). About half of the participants had measurable levels of PFHxA (53.2%), while less than half had measurable PFHpA (37.5%) and PFDA (46.3%). Less than 10% of the participants had measurable levels of PFPeA (4.9%), PFUnA (8.7%), and PFDoA (0.7%). Participants had PFOA levels that were 6- to 8-fold higher (geometric mean ± SD: 32.9 ± 240.8 ppb) than levels measured in NHANES participants. Levels of PFOS were slightly lower in the C8 participants (19.2 ± 15.6 ppb) than those measured in NHANES participants (Frisbee, et al. 2009).

Lipids (total cholesterol, high- and low-density lipoproteins [HDL and LDL], and triglycerides) were measured in adult participants (over 18 years old). Samples from adults taking cholesterol-lowering medication were excluded from the analysis, with 46,294 participants being included. PFOA levels ranged from 0.25 to 17,556.6 ppb, with a mean of 80.3 ppb. PFOS levels ranged from 0.25 to 759.2 ppb, with a mean of 22.4 ppb (Steenland, et al. 2009).

Higher serum PFOS or PFOA was associated with higher levels of total cholesterol, LDL, and triglycerides. Serum levels of PFNA and PFHxS were also positively associated with higher levels of cholesterol. This may be because PFNA and PFHxS levels were correlated with PFOA and PFOS levels. The authors noted that this correlation made it difficult to pick apart the possible roles of each of the measured PFCs. However, the more important predictors of lipid level variance were age, gender, and body mass index. In addition, due to the study design, the authors were unable to determine if PFOS or PFOA caused the increased cholesterol or occurred concurrently with the increased cholesterol (Steenland, et al. 2009).

Lipids (total cholesterol, HDL and LDL, and triglycerides) were also measured in blood samples from the child participants, less than 19 years old, to determine if there are associations between serum PFOA and PFOS and lipids. More than 10,000 of the child participants, out of 12,476, had serum PFC and lipids levels available. Levels of PFOA were much higher in 12- to 19-year-old participants (29.3 ppb) than in 12- to 19-year-olds in the 2003-2004 NHANES (3.9 ppb). Twelve- to 19-year-olds had serum PFOS levels (19.1 ppb) similar to levels obtained from the 2003-2004 NHANES (19.3 ppb) (Frisbee, et al. 2010).

Increases in total cholesterol and LDL were significantly associated with increases in serum PFOA and PFOS levels for boys and girls combined. Increasing PFOA levels and decreasing PFOS levels were significantly associated with increasing triglycerides levels in 12- to 18-year-old girls only. Increasing PFOS levels were also significantly associated with increasing HDL levels. The authors noted that the larger increases in lipid levels were seen at the lower levels of PFOA, indicating that the relationship may be nonlinear. While there may be associations between PFOS, PFOA, and total cholesterol and LDL, additional investigation is necessary to determine if PFOS or PFOA can cause increases in serum lipids (Frisbee, et al. 2010)

Pregnancy outcomes, from up to five years before enrollment, were examined in the C8 participants. For the analyses using PFOA levels, women were included if they lived in the same water district from the start of the pregnancy to enrollment in the project. All women were included in the PFOS analyses, as PFOS exposure was not dependent on the water district. Data from 5,624 women were included in this study. The mean PFOA level was 48.8 ppb and the mean PFOS level was 15.0 ppb. PFOA and PFOS levels were only minimally, but significantly, correlated (Stein, et al. 2009).

The authors found no association between PFOA or PFOS levels and miscarriage and only a very weak association between PFOS levels and preterm birth. PFOS and PFOA levels were weakly associated with preeclampsia and may be associated with low birth weight, but the authors noted that this link should be further studied. Birth defects were not associated with PFOS levels, and only weakly associated with PFOA levels. Similar results were obtained for analyses of participants with pregnancies within three years of enrollment (1,197 pregnancies) (Stein, et al. 2009).

Adults (20 years or older) were included in an analysis of PFOA and PFOS levels with serum uric acid levels. Elevated levels of uric acid have been linked to development of hypertension and may be a risk factor for cardiovascular, kidney, or metabolic disease. Serum PFC and uric acid levels were obtained from 53,458 adult participants. Uric acid levels were within typical levels for an adult population (mean ± SD = 5.58 ± 1.55 mg/dL). The means for serum PFOA and PFOS were 86.4 and 23.4 ppb, respectively (Steenland, et al. 2010).

Serum uric acid increased with increasing levels of serum PFOA or PFOS. Serum PFOS levels were not as strongly associated with uric acid as PFOA levels were. The authors did note that PFOA and PFOS levels were correlated, but that PFOA appeared to be more strongly associated with uric acid levels. Two possible mechanisms suggested by the authors were that induction of oxidative stress or that shared usage, by PFOA or PFOS and uric acid, of organic anion transporters may result in less uric acid being secreted. The authors noted that, because of the

study design, there was no way to know if the PFOA or PFOS levels resulted in higher uric acid levels or if the higher uric acid levels cause retention of PFOA or PFOS (Steenland, et al. 2010).

Children (8 to 18 years old) from the C8 Health Project were assessed to determine if PFOA or PFOS was associated with sexual maturation. Measures of sexual maturation were blood levels of sex steroid hormones and self-reported menarche status. A total of 6,007 children (3,076 boys and 2,931 girls) participated in the study. Total and free testosterone was measured in the boys' blood and estradiol was measured in the girls' blood (Lopez-Espinosa, et al. 2011).

Reduced odds of boys reaching puberty at the time of the study were found with increasing levels of PFOS. No association was found with PFOA. For girls, both PFOA and PFOS were associated with reduced odds of postmenarche, with a longer delay in the highest quartile. Only PFOS was associated with a delay based on estradiol levels. Based on the results, puberty was delayed by about three to six months across the range of serum PFOA or PFOS levels. The authors noted that PFC levels were measured in some of the children after puberty, and changes associated with puberty could have altered PFC levels (Lopez-Espinosa, et al. 2011).

Stein and Savitz (2011) examined associations between PFOA, PFOS, PFHxS, and PFNA with attention deficit/hyperactivity disorder (ADHD) or a learning problem in child participants, ages 5 to 18, in the C8 Health Project. For the 10,546 child participants, the mean serum PFOA, PFOS, PFHxS, and PFNA levels were 66.3, 22.9, 9.3, and 1.7 ppb, respectively. While PFOA, PFHxS, and PFNA levels were higher in the C8 Health Project children, the PFOS levels were similar to NHANES participants. About 5% of the child participants were counted as ADHD cases and 12% of the participants had learning problems. Some positive associations were identified between PFOA, PFOS, or PFHxS and ADHD, however, only PFHxS levels were associated with learning problems. Overall, although some associations between PFC levels and ADHD were identified, there were no consistent associations and further research into potential developmental effects is needed (Stein and Savitz 2011).

The association between markers of liver function and serum PFOA and PFOS was examined in 46,452 adult participants (18 years or older) of the C8 Health Project. Median levels of PFOA and PFOS were 28 and 20.3 ppb, respectively. Women had significantly lower levels of PFOA and PFOS compared to men. Women also had significantly lower levels of liver function markers (alanine transaminase [ALT], γ-glutamyltransferase [GGT], and direct bilirubin) compared to men (Gallo, et al. 2012).

Higher PFOA and PFOS levels were significantly associated with abnormally high serum ALT levels. Individuals with higher serum PFOA concentrations also tended to have higher serum GGT levels, but this trend was not observed with PFOS levels. Higher serum PFOS, but not PFOA, levels tended to be associated with higher bilirubin levels. Although this study could not determine causation, the results indicated that higher serum PFOA and PFOS levels were associated with increased levels of a marker of liver injury. The authors noted that this is consistent with other human epidemiology and laboratory animal studies (Gallo, et al. 2012).

Associations between thyroid function and modeled *in utero* PFOA concentrations or measured serum PFOA, PFOS, and PFNA concentrations were assessed in child participants (ages 1 to

17) of the C8 Health Project. Serum PFC measurements and thyroid hormone measurements (thyroid-stimulating hormone [TSH] and total thyroxine [T4]) or presence of thyroid disease was available for 10,725 children. Median serum PFOA, PFOS, and PFNA levels for all the children were 29.3, 20.0, and 1.50 ppb, respectively. *In utero* PFOA levels were modeled for 4,713 child participants. The median *in utero* modeled PFOA level was 11.5 ppb. Based on thyroid hormone levels, children without thyroid disease and/or medication use were grouped into categories of subclinical hypothyroidism and hyperthyroidism (Lopez-Espinosa, et al. 2012).

Increases in PFOS and PFNA tended to be associated with an increase in total T4 levels in girls (ages 1 to 17) and boys (ages 10-17). There were no associations between PFOS or PFNA and TSH. Higher serum PFOA levels tended to be associated with increased number of children with thyroid disease. For children 5 years or younger, modeled *in utero* PFOA levels tended to be associated with an increase in total T4, and serum PFOA tended to be associated with a drop in TSH levels in girls. While serum PFOA may be associated with thyroid disease, there were no associations found between PFOA, PFOS or PFNA and subclinical hyper- or hypothyroidism. Levels of PFOA, PFOS, and PFNA were positively correlated and modeled in utero PFOA levels positively correlated with serum PFOA levels. While results are suggestive of an association between PFCs and thyroid hormone levels, further investigation is needed (Lopez-Espinosa, et al. 2012).

C8 study participants' (144 men and 146 women) peripheral blood cell ribonucleic acid (RNA) was isolated to determine if PFOA or PFOS levels were associated with changes in expression of genes involved in cholesterol metabolism or transport. Expression of thirteen genes, including several peroxisome proliferator-activated receptor (PPAR) genes, was measured using quantitative polymerase chain reaction. The geometric means for PFOS and HDL levels were significantly different between men and women (Fletcher, et al. 2013).

Increasing serum levels of PFOA were significantly associated with reduced gene expression for three genes dealing with cholesterol transport. Increasing levels of serum PFOS were significantly associated with increased expression of one gene for cholesterol mobilization and reduced expression of another gene involved in cholesterol transport. The genes significantly associated with PFOA and PFOS were not the same. The authors note that the RNA was from whole blood and may not reflect changes in the liver (Fletcher, et al. 2013). Vanden Heuvel (2013) additionally commented that changes in amounts of macrophages in individual blood samples may have also been responsible for these associations. It was further noted that the geometric means of both cholesterol and LDL were normal, and not elevated, in the population from the Fletcher et al. (2013) study and that the C8 participants were not at greater risk of coronary artery disease or stroke (Vanden Heuvel 2013). This does not preclude elevated cholesterol or LDL levels in some of the participants, but further investigation is needed.

The C8 Health Project Panel focused on PFOA, as that was the chemical at elevated levels in people's drinking water. The panel issued findings linking PFOA to several health effects, but also listed health effects that did not appear to be linked to the participants PFOA exposure (C8SciencePanel 2012). As PFOS was not a focus of the panel, there are no probable link

reports for health effects that may result from PFOS. Table 12 presents the findings of the probable effects reports.

Table 12: C8 Health Project Panel findings (C8SciencePanel 2012).

Health effects probably linked to PFOA exposure:	Health effects that do not appear to be linked to PFOA exposure:
• pregnancy-induced hypertension • testicular and kidney cancer • thyroid disease • ulcerative colitis • hypercholesterolemia	• preterm birth or low birthweight • miscarriage or still birth • birth defects • Type II diabetes • stroke • asthma or chronic obstructive pulmonary disease (COPD) • neurodevelopmental disorders in children (including attention deficit disorders and learning disabilities) • common infections (including influenza in children or adults) • Parkinson's disease • osteoarthritis • liver disease • chronic kidney disease • hypertension • coronary artery disease • certain autoimmune diseases (rheumatoid arthritis, lupus, Type I diabetes, Crohn's disease, and multiple sclerosis)

Health effects associated with PFOS in National Health and Nutrition Examination Survey (NHANES) participants

Child participant data from the 1999-2000 and 2003-2004 NHANES were used in a study to assess associations between serum PFC levels (PFOS, PFOA, PFHxS, and PFNA) and attention deficit/hyperactivity disorder (ADHD). Children (n=571), ages 12-15, were included, with 48 reported to have ADHD. Almost all (more than 96%) of the children had detectable serum levels of PFOS, PFOA, PFHxS, and PFNA. Median levels (range) of PFOS, PFOA, PFHxS, and PFNA were 22.6 (2.1-87.2), 4.4 (0.4-21.7), 2.2 (ND-64.1), and 0.6 (ND-5.9) ppb, respectively (Hoffman, et al. 2010).

Increased serum PFOA, PFOS, and PFHxS levels were significantly associated with an increased risk of having ADHD. Increased serum PFNA levels were also associated with increased risk of having ADHD, but not significantly. Increases in serum PFOS were significantly associated with an increased risk of having ADHD, even after accounting for confounding variables, such as age, sex, race, maternal smoking during pregnancy and

prescription medication use. Although results suggest an association between serum PFC levels and ADHD, further investigation into the relationship between serum PFC levels and ADHD is needed. Because of the study design, the authors could not determine a causal relationship between PFC levels and ADHD (Hoffman, et al. 2010).

Melzer et al. (2010) examined associations between PFOA or PFOS serum levels and thyroid disease in NHANES participants. NHANES data were from the 1999-2000, 2003-2004, and 2005-2006 cohorts. Information on thyroid disease from participants less than 20 years old was not collected, so only individuals 20 years old or older were included in the analysis. Serum PFOA and PFOS levels and thyroid disease information were available for 3,966 participants (1,900 men and 2,066 women). The geometric mean serum PFOA levels for men and women were 4.91 and 3.77 ppb, respectively. The geometric mean serum PFOS levels for men and women were 25.08 and 19.14 ppb, respectively. Mean serum PFOA and PFOS levels were significantly higher in men than in women (Melzer, et al. 2010).

Thyroid disease was significantly associated with higher survey-weighted serum PFOA quartiles in women, but not men. Women also had a significant association between currently medicated thyroid disease and higher survey-weighted serum PFOA quartiles. Men only had a significant association between currently medicated thyroid disease and higher survey-weighted serum PFOS quartiles. No other diseases (arthritis, asthma, diabetes, COPD, heart disease, or liver disease) were associated with higher levels of PFOA or PFOS in women or men. The highest serum PFOS quartile was associated with lower COPD, but the authors noted that additional investigation should be done to rule out a false positive. Although associations have not been found in populations with higher serum PFOA levels than the NHANES population, the authors caution that further work is needed to investigate this association (Melzer, et al. 2010).

The associations between serum PFC levels in 1999-2000 and 2003-2008 NHANES participants and effects on cognition in older adults were assessed. The authors hypothesized that the ability of PFCs to suppress immune function may reduce inflammation observed in neurodegenerative diseases. NHANES participants from 60 to 85 years (n = 1,766), with a mean age of 70.3, were included in this study. Geometric means for serum PFOS, PFOA, PFNA, and PFHxS were 22.63, 4.08, 1.01, and 2.05 ppb, respectively (Power, et al. 2012).

The authors found that higher serum PFC levels were associated with lower self-reported limitation due to difficulty remembering or periods of confusion. Levels of the PFCs (PFOS, PFOA, PFNA, and PFHxS) were correlated, and the effects of individual PFCs could not be assessed. The model was adjusted for changes in serum volume or kidney function (possibly from complications of diabetes) and fish consumption. The association was the strongest in non-medicated diabetics. The authors posit that this protective effect may occur via PPAR signaling, but note that additional research is necessary (Power, et al. 2012).

Geiger et al. (2013) used NHANES data from 1999-2000 and 2003-2008 to investigate whether serum PFOA or PFOS levels were associated with hyperuricemia (elevated serum uric acid) in participants 12 to 18 years old (n = 1,772). Hyperuricemia cases were defined as individuals with serum uric acid levels of 6 mg/dL or more. Hyperuricemia has been associated with risk of

hypertension, childhood metabolic syndrome, and other cardiometabolic risk factors. Increases in serum PFOA levels were significantly associated with increases in serum uric acid levels, even after adjusting the model for age, sex, race/ethnicity, body mass index, total cholesterol, and other factors. While increasing serum levels of PFOS were significantly associated with increases in serum uric acid in the unadjusted model, there was no significant association in the adjusted model. There were significant associations between increasing serum PFOA or PFOS levels and increase in percentage of individuals with hyperuricemia. The authors speculate that competition between PFCs and uric acid for binding with organic anion transporters may limit the amount of uric acid that can be excreted, but note that further investigation is needed (Geiger, et al. 2013).

Other populations

Workers with PFOS exposure were assessed for increased incidence of bladder cancer. Workers enrolled in the study worked at least 365 days before 1998. Participants had been previously categorized as having no, low, high, or combined low or high PFOS exposure. Information on incidence of bladder cancer was collected in 2002 from 1,400 participants and 185 death certificates. Eleven cases of bladder cancer were identified, with five of the 11 identified from the death certificates. The authors found that incidence of bladder cancer was similar to the U.S. population. Because of the limited size of the population included in the study, the authors could not rule out the possibility that a small increased risk (1.5 to 2-fold) of developing bladder cancer occurred in the higher PFOS-exposed workers (Alexander and Olsen 2007).

Participants from a birth cohort from the Faroe Islands were involved in assessing the effects of PFOA and PFOS on the serum antibody concentrations against tetanus and diphtheria in 5- or 7-year-old children. Prenatal (maternal serum at week 32 of pregnancy) and postnatal (child's serum at age 5) PFC (PFOS, PFOA, PFHxS, PFNA, and PFDA) levels were measured. Geometric means for prenatal (maternal) serum PFOS, PFOA, PFHxS, PFNA, and PFDA were 27.3, 3.20, 4.41, 0.60, and 0.28 ppb, respectively. Geometric means for postnatal (child) serum PFOS, PFOA, PFHxS, PFNA, and PFDA were 16.7, 4.06, 0.63, 1.00, and 0.28 ppb, respectively. PFC levels within the prenatal and postnatal datasets were interrelated, but were only weakly correlated with polychlorinated biphenyl (PCB) levels (sum of PCB-138, -153, and -180, times two). Pre- and postnatal PFC levels were weakly associated (Grandjean, et al. 2012).

Children were vaccinated for tetanus and diphtheria at three, five and 12 months of age and received a booster vaccination when they were 5 years old. Antibody concentrations were measured at ages five (prior to the booster) and seven. Higher maternal serum PFOS and PFOA were associated with lower antibody concentrations against diphtheria. Higher PFC levels in children's serum were also associated with lower antibodies against diphtheria and tetanus. Adjustments for PCB levels in milk and serum did not alter these associations. The authors also examined whether increased PFC levels were related to antibody levels falling below a clinically protective level. The odds ratio for antibodies against diphtheria at 5 years of age were 2.48 (95% CI, 1.55 to 3.97) for maternal serum and 1.60 (95% CI, 1.10 to 2.34) for child serum. Similar odds ratios were found with the antibody concentrations for both diphtheria (for PFOS, odds ratio [OR], 2.38 [95% confidence interval (CI), 0.89 to 6.35] and for PFOA, OR 3.27

[95% CI, 1.43 to 7.51]) and tetanus (for PFOS, OR, 2.61 [95% CI, 0.77 to 8.92] and for PFOA, OR 4.21 [95% CI, 1.54 to 11.44]) measured at age seven (Grandjean, et al. 2012).[2]

In a study with the Danish National Birth Cohort, Andersen et al. (2010) investigated whether maternal PFOA or PFOS levels were associated with children's weight, length, and body mass index. Maternal plasma levels of PFOA and PFOS and child's weight and length at 5 months and 12 months were available for approximately 1,100 mother-child pairs. Women were enrolled in the study during 1996 to 2002. Maternal PFOS levels ranged from 6.4 to 106.7 ppb and PFOA levels ranged from the below the level of quantification (1 ppb) to 21.9 ppb (Andersen, et al. 2010).

For boys, there were statistically significant associations between weight and body mass index at 5 months and 12 months and both maternal PFOA and PFOS levels. There were no associations between the length of boys and PFC levels. For girls, there were no statistically significant associations between maternal PFOA or PFOS levels and weight or length. The authors speculated that endocrine-disrupting activities of PFCs may explain the gender differences (Andersen, et al. 2010).

Women in the Norwegian Mother and Child Cohort Study, enrolled in 2003-2004, participated in a study to assess the relationship between thyroid stimulating hormone (TSH) and PFCs. Data from 903 women were included in the analysis. Levels of PFOS, PFOA, PFHpA, PFNA, PFDA, PFUnDA, PFDoA, PFTrDA, PFTeA, PFBA, PFHxS, PFHpS, PFOSA, and TSH were measured in plasma collected during the 18th week of gestation. Seven of the PFCs (PFDA, PFHpS, PFHxS, PFNA, PFOA, PFOS, and PFUnDA) were detected in about 70% or more of the plasma samples. The geometric mean for PFOS, 12.77 ppb, was the highest followed by the geometric mean for PFOA, 2.13 ppb (Wang, et al. 2013).

Significant positive correlations were identified between PFOS and PFOA levels and also between PFOS and PFHpS levels. Before adjusting for confounders, PFHpS, PFNA, and PFOS were positively associated with TSH levels. After adjusting for confounding variables, only PFOS levels were positively associated with TSH levels. There were no associations between PFC levels and self-reported thyroid abnormalities. The authors noted that the sample volume was insufficient to measure other thyroid hormones, but that the association between PFOS and TSH was small and may not be clinically significant (Wang, et al. 2013).

Inuit from Nunavik who were at least 18 years of age were enrolled in a study to assess associations between levels of plasma PFOS, and other halogenated chemicals, and thyroid hormones. Most of the participants had thyroid hormones within the normal range. PFOS levels were available for 621 participants, and a food frequency questionnaire was completed by 536 participants. PFOS was detected in all participants with a geometric mean of 18.28 ppb and a

[2] This same population also had reduced antibody production associated with increased PCB levels. Although Grandjean and Budtz-Jorgensen (2013) calculated a benchmark dose for PFOS based on the reduction in antibody production, that value will not be discussed as it may be higher or lower than the calculated value (exposure to PCB may increase or decrease the amount of PFOS needed to alter antibody production). (Grandjean and Budtz-Jorgensen 2013)

range of 0.48 to 470 ppb (wet weight). PFOS levels correlated with PCB congeners, other organochlorine chemicals, and polybrominated diphenyl ether (PBDE)-153 (Dallaire, et al. 2009).

Higher PFOS levels were significantly associated with lower thyroid stimulating hormone (TSH), triiodothyronine (T3), and thyroxine-binding globulin (TBG) levels. Higher PFOS levels were significantly associated with higher free thyroxine (fT4) levels. Several statistically significant associations were identified between TSH, T3, TGB and PCBs, other organochlorine chemicals, and PBDE-47. Further work is needed to explore the link between PFOS and thyroid hormone levels and any potential association with disease in adults (Dallaire, et al. 2009).

Inuit women from Nunavik (n = 120) participated in a study to investigate whether plasma levels of PFOS, hydroxylated metabolites of polychlorinated biphenyls (OH-PCBs), and chlorophenols were associated with decreased amounts of T4 bound to transthyretin (TTR). Several OH-PCBs and chlorophenols, along with PFOS, were measured in this population. PFOS was detected in all samples, with a geometric mean of 10.924 ppb and a range of 9.842 to 12.125 ppb. TSH, free T4, total T4, TTR, T4 bound to TTR (T4-TTR), free T3, total T3, and thyroxin-binding globulin(TBG) were measured, and all except TBG and total T4 were within the reference ranges. There were no significant associations between the chemicals and T4-TTR levels. The authors caution that, although significant associations were not identified, binding of these chemicals to TTR could increase fetal exposure (Audet-Delage, et al. 2013).

Pregnant Taiwanese women, in their third trimester, were enrolled in the Taiwan Birth Panel cohort study. This cohort participated in a study to examine whether prenatal PFC exposure affected immune function and allergic disease in children, as measured by immunoglobulin E (IgE) levels and development of atopic dermatitis. Cord blood was collected from 483 children; however, 239 were excluded due to insufficient blood volume, missing data, or loss to follow-up. PFOA (66%), PFOS (99.6%), and PFNA (59.4%) were detected in a majority of the samples, but PFHxS was only above the detection limit in 10.7% of the samples. The median (range) PFOA, PFOS, PFNA, and PFHxS levels were 1.71 (0.75-17.40), 5.50 (0.11-48.36), 2.30 (0.38-63.87), and 0.035 ppb (0.035-0.420 ppb), respectively. PFHxS was not included in the analysis as close to 90% of the samples were below the detection limit. Male infants tended to have higher average cord blood PFC levels than female, and higher PFC levels tended to occur in infants with low birth weight and decreased gestational ages, but these were not statistically significant. IgE levels were measured in cord blood serum and in blood serum collected at two years of age (Wang, et al. 2011a).

No significant correlation was identified when comparing cord blood PFC levels and total IgE levels at two years of age, even when data from boys and girls were separately analyzed. There was a significant positive correlation between cord blood PFOA and PFOS levels with cord blood IgE even after adjusting for confounding variables. When the data were analyzed by gender, only boys had a significant positive correlation between cord blood PFOA and PFOS levels and cord blood IgE levels. Atopic dermatitis was identified by a three-question survey and dermatologist's examination of a sub-group of the children. Forty-three of the children developed atopic dermatitis. After adjusting for confounding variables, there were no significant correlations between PFC levels and atopic dermatitis. For atopic dermatitis analysis, the

authors noted that blood PFC levels at two years of age could have been a confounding factor that was not accounted for in the present study. The authors recommended follow-up studies as prenatal PFOA and PFOS did positively correlate with cord blood IgE levels (Wang, et al. 2011a).

Childhood neurodevelopment and associations with PFOA and PFOS levels were investigated in a subset of participants from the Taiwan Birth Panel Study. The Comprehensive Developmental Inventory for Infants and Toddlers was administered to 239 children at two years of age to evaluate their neurodevelopment. PFOA and PFOS levels were measured in cord blood, with mean concentrations of 2.5 ppb for PFOA and 7.0 ppb for PFOS. Lower testing results for the whole test, and cognitive, language, gross-motor, fine-motor, social, and self-help sub-domains were significantly associated with higher serum PFOS levels. The largest differences were identified with the whole test and the gross-motor sub-domain. No consistent associations were identified between serum PFOA levels and testing results. Although neurodevelopment was measured and recall bias was not an issue, the authors noted that the subset of participants had different characteristics, such as educational level and family income, than the whole cohort, indicating that selection bias may be a concern (Chen, et al. 2013).

Children in the Genetic and Biomarkers study for Childhood Asthma in Taiwan participated in a case-control study to examine the potential link between PFCs and asthma. Two hundred and thirty-one children (cases) between the ages of 10 and 15 were enrolled between 2009 and 2010, after having been diagnosed with asthma in the previous year. The control population (n = 225) was selected from a previous cohort (a population from seven public schools in Northern Taiwan). Serum IgE absolute eosinophil counts (AEC), and serum eosinophillic cationic protein (ECP) were measured in children. Age, height, weight, sex, environmental tobacco smoke exposure, month of survey completion, IgE levels, AEC, and ECP were significantly different between children with and without asthma. Ten PFCs (PFOS, PFOA, PFBS, PFDA, PFDoA, PFHpA, PFHxA, PFHxS, PFNA, and PFTA) were measured in serum. All PFCs were detected in more than 70% of the children with asthma and more than 50% of the children without asthma. PFHpA was not detected in enough of the samples to include it in the analysis (Dong, et al. 2013).

There were significant differences in levels of nine of the PFCs between the children with and without asthma. Increasing odds of asthma were associated with increased PFC levels for all PFCs except PFHxA and PFTA. PFC levels were not associated with serum IgE levels or AEC in children without asthma. However, levels of serum ECP were significantly positively associated with serum PFDA or PFDoA levels in children without asthma. In children with asthma, levels of PFOS, PFOA, PFDA, PFDoA, and PFNA were significantly positively associated with serum IgE, AEC, and serum ECP. Serum PFBS was significantly positively associated with AEC; serum PFHxS was significantly positively associated with AEC and serum ECP; and serum PFTA was significantly positively associated with serum IgE and AEC in children with asthma. The authors noted some limitations of the study: a single serum PFC sample was taken and, due to the cross-sectional study design, the causal relationship between PFCs and asthma cannot be investigated (Dong, et al. 2013).

The associations between serum levels of PFOS, PFOA, and PFHxS and metabolic function and lipid levels were investigated in participants of the Canadian Health Measures Survey (Cycle 1 2007-2009). Non-pregnant participants (up to 1,297 men and 1,403 women) over the age of 18 were included in this study. The geometric means for PFOS, PFOA, and PFHxS for all participants were 8.40, 2.46, and 2.18 ppb, respectively. Serum levels of PFOS, PFOA, and PFHxS were positively correlated with each other (Fisher, et al. 2013).

Serum levels of PFHxS were significantly associated with total cholesterol, LDL, non-HDL, and the HDL/total cholesterol ratio. Significant associations were only identified for PFOS and PFOA in unweighted models. The weighted model was adjusted for the probability based sampling strategy. No significant associations were identified between any of the PFCs and insulin and glucose measurements. Serum PFOA, PFOS, and PFHxS levels were not significantly associated with metabolic syndrome, nor were serum PFOA or PFOS levels and high cholesterol. Increasing serum PFHxS levels were significantly associated with increasing odds ratios for high cholesterol in both the unweighted and weighted models. Increasing levels of serum PFOA and PFOS were only significantly associated with increasing odds ratios for high cholesterol in the unweighted models. These results provided limited evidence that serum PFOA or PFOS levels were associated with cholesterol. The authors note that there were associations between cholesterol and serum PFHxS levels, but due to the cross-sectional study design, no causal relationships could be investigated (Fisher, et al. 2013).

Japanese men and women, ages 15 to 76, participated in a study to investigate factors associated with PFOS and PFOA levels. Participants (n = 608, 307 men and 301 women) were enrolled between 2008 and 2010. Along with PFOS and PFOA measurements, levels of omega-3 fatty acids (DHA and EPA) and liver enzymes (γ-glutamyl transpeptidase [GTP], glutamic-oxaloacetic transaminase [GOT], and glutamic-pyruvic transaminase [GPT]) were measured. Participants were also given a survey, which included occupational history, medical history, and dietary habits. The median PFOS and PFOA levels for all participants were 5.8 and 2.1 ppb, respectively. Men had significantly higher PFOS levels compared to women and levels were significantly different among five age categories. Only women had significant differences in PFOA levels based on age. Women who had a pregnancy had significantly lower PFOS and PFOA levels compared to women who had not had a pregnancy. PFOS levels were significantly positively correlated with frequency of boiled fish in broth, sliced raw fish, and coastal fish intake. Serum levels of DHA and EPA, biomarkers of fish intake, were also significantly positively correlated with PFOS and PFOA levels. Serum levels of GOT and GPT, indicators of liver cell damage, were significantly positively correlated with both PFOS and PFOA levels. However, since this study was cross-sectional, causal relationships cannot be determined (Yamaguchi, et al. 2013).

Toxicokinetics

PFOS and PFOA are efficiently absorbed after oral exposures, with more than 90% of an oral dose absorbed in rats. After absorption, PFOS and other PFCs bind to serum albumin and other proteins in plasma, such as lipoproteins. Once absorbed, PFCs are distributed throughout the body with the highest amounts typically in the serum and liver. Humans have liver to serum ratios around 1:1, as do cynomolgus monkeys, while rats appear to retain higher levels of PFCs in the liver. No metabolism has been identified with either PFOA or PFOS (ATSDR 2009).

Excretion occurs through the kidney and in the bile in humans, monkeys, and rodents. PFOS reabsorption by organic anion transporters reduces the amount excreted in urine in both humans and monkeys compared to rats. These chemicals are also reabsorbed in the gastrointestinal tract when excreted in bile (ATSDR 2009). Andersen et al. (2006) modeled the toxicokinetics of PFOS and PFOA in cynomolgus monkeys and adjusted the model based on human kinetic data. In the model, urinary excretion was slower for PFOS compared to PFOA, no matter the species.

PFOS half-lives vary widely among humans and laboratory animals. This can be seen in half-lives for PFOS, PFHxS, and PFOA calculated from repeat serum measurements from retired fluorochemical workers. Blood samples were taken from twenty-six retired workers (24 men and 2 women) for an average of five years (range of 3.1 to 5.3 years). The average PFC half-lives were calculated as 5.4 years for PFOS (range 2.4 to 21.7 years), 8.5 years for PFHxS (range 2.2 to 27 years), and 3.8 years for PFOA (range 1.5 to 9.1 years). The maximum half-lives for PFOS, PFHxS, and PFOA were calculated from different individuals (Olsen, et al. 2007).

PFOS half-lives in nonhuman primates range from 110 to 170 days (ATSDR 2009). PFOS half-lives in male rats were calculated to be between 7.5 days to approximately 41 days, while female rats have had half-lives between 2 and 71 days after an oral exposure to PFOS. Shorter term follow-up tended to result in short calculated half-lives. Male and female Sprague-Dawley rats had half-lives around 40 and 60-70 days, respectively, after at least a 10 week follow-up time. Male and female CD-1 mice were found to have a PFOS half-life of about 40 days (Chang, et al. 2012). A difference in reabsorption and recirculation between humans and laboratory animals may be one possible explanation for the observed half-life differences (ATSDR 2009).

Tan et al. (2008) developed a toxicokinetic model to examine the role of renal resorption in the half-lives of PFOS and PFOA in rats and monkeys. Observational data was used from male and female monkeys (a single intravenous dose and a 26 week oral dosing study) and male rats (a single IV dose and an 89 day oral dosing study). In the final model, male rats had a higher liver: blood partition coefficient and had additional binding in the liver compared to male monkeys, indicating that male rats retained more PFOS in the liver. Monkeys also had increased renal reabsorption compared to rats (Tan, et al. 2008).

Mode of Action and Carcinogenicity

PFOS and PFOA activate a class of nuclear receptors called peroxisome proliferator-activated receptors (PPARs), with PFOA a stronger activator of PPARα than PFOS (Ren, et al. 2009, Rosen, et al. 2010). PPARα activation regulates genes involved in lipid metabolism, peroxisome biogenesis, proteasome activation, and inflammation. Wild-type and PPARα knock-out mice[3] were gavaged with PFOS (0, 3, 10 mg/kg) for seven days. In both wild-type and PPARα knock-out mice, PFOS exposure led to altered expression of genes linked to lipid metabolism, inflammation, and xenobiotic metabolism through a PPARα-independent mechanism. PFOS treatment also caused altered expression of genes linked to ribosome biogenesis, oxidative phosphorylation, and cholesterol biosynthesis in PPARα knock-out but not wild-type mice. It

[3] PPARα knock-out mice are mice with little or no expression of PPARα. Wild-type mice are those with the same genetic background as the knock-out mice, but with normal expression of PPARα.

should be noted that both wild-type and PPARα knock-out mice exposed in the 10 mg/kg groups had significantly increased liver weights compared to control mice (Rosen, et al. 2010).

PPARα activation also contributed to developmental toxicity in PFOA-, but not PFOS-exposed mice. Neonatal lethality and delayed eye opening, measures of developmental toxicity, were examined in wild-type and PPARα knock-out mice. Pregnant mice, both wild-type (0, 4.5, 6.5, 8.5, and 10.5 mg/kg/day) and PPARα knock-out (0, 8.5, and 10.5 mg/kg/day), were exposed to PFOS during the last four days of gestation (GD15-18). Neonatal deaths were increased in PPARα knock-out mice exposed to PFOS. Eye opening was delayed in both wild-type and knock-out mouse pups (Abbott, et al. 2009). While these measures of developmental toxicity were previously found to be PPARα-dependent for mice exposed to PFOA (Abbott, et al. 2007), these data indicate that PFOS exposure leads to similar effects through a PPARα-independent pathway (Abbott, et al. 2009).

Other nuclear receptors have also been implicated in biological responses to PFOA and PFOS. Human and rat primary hepatocytes were treated with 0 (vehicle control), 25 micromolar (μM) PFOA, or 25 μM PFOS for 24 hours. RNA was isolated and expression of genes under the control of PPARα, pregnane X receptor (PXR), constitutive androstane receptor (CAR), liver X receptor α (LXRα), or farnesoid X receptor (FXR) were measured. As expected, gene expression increased for genes controlled by PPARα in both rat and human hepatocytes. PXR and CAR regulate genes for drug metabolism and elimination. Genes regulated by PXR and CAR had increased expression in rat hepatocytes treated with PFOA and PFOS. However, in human hepatocytes, only PFOS treatment increased expression of genes under the control of PXR and CAR. LXRα regulates genes for fatty acid and cholesterol biosynthesis, carbohydrate metabolism, and bile acid synthesis and elimination. In rat hepatocytes, PFOS and PFOA treatment caused slight increases in two of the three genes controlled by LXRα. In human hepatocytes, PFOA treatment increased the expression of one gene and PFOS treatment increased the expression of another gene under the control of LXRα. Levels of a third gene under the control of LXRα were too low to accurately quantitate. FXR regulates genes involved in bile acid and carbohydrate metabolism. Expression of genes under the control of FXR were not altered in PFOA- or PFOS-treated rat hepatocytes or in PFOA-treated human hepatocytes. PFOS-treated human hepatocytes had increased expression of genes regulated by FXR (Bjork, et al. 2011).

The cytotoxicity and genotoxicity of PFOA and PFOS was investigated in a human hepatocellular carcinoma line (HepG2). Cells were treated with 5, 10, 50, 100, 200, 300, 400 μM PFOA or 5, 10, 50, 100, 200, 300 μM PFOS for one or 24 hours. None of the concentrations tested, including the initial concentrations ranging up to 600 μM PFOS and 800 μM PFOA, reduced cell viability after one hour of exposure. Concentrations over 300 μM PFOS and 200 μM for PFOA significantly reduced cell viability compared to control cells after 24 hours of exposure. DNA and chromosomal damage was measured with comet and micronucleus assays. After 24 hours of exposure to PFOA (10 and 200 μM), cells had significantly increased variation in measurements of DNA damage compared to the control cells. There were no significant changes in cells treated with PFOS. Neither 24 hours exposure to PFOS nor PFOA increased the number of HepG2 cells with micronuclei, a measure of chromosomal damage. PFOS or PFOA treatment for 24 hours did not increase the amount of reactive oxygen species in the cells. Taken

together, these results indicate that PFOS and PFOA, while cytotoxic, were not genotoxic (Florentin, et al. 2011).

The genotoxicity of PFOS was assessed in Syrian hamster embryo (SHE) cell culture. SHE cells were exposed to PFOS (0, 0.00002, 0.0002, 0.002, 0.02, 0.2, 2.0, 20, and 50 µg/mL) for seven days. These PFOS concentrations were not cytotoxic. Significantly increased cell transformation was observed at 0.2 and 2.0 µg/mL compared to the control. No damaged DNA was identified in cells treated with 0.0002 to 50 µg/mL PFOS. Transcription of PPAR genes (*ppar-α*, *ppar-β/δ*, and *ppar-γ* mRNA) was measured. Increased transcription of *ppar-β/δ* was identified in SHE cells treated with 0.2 µg/mL for 24 hours. Levels of *ppar-α* and *ppar-γ* in PFOS-treated cells were not significantly different from the control cells after five and 24 hours. After seven days of exposure, transcripts of *ppar-β/δ* were significantly increased, at PFOS concentrations of 0.2 and 2.0 µg/mL, compared to the controls. Levels of *ppar-γ* were significantly increased at all of the PFOS concentrations (0.02 to 20 µg/mL), while only cells treated with 0.2 µg/mL had a significantly increased level of *ppar-α* mRNA. Based on these results, PFOS may be carcinogenic through a non-genotoxic mechanism. The authors noted that the non-monotonic dose response observed (increased transcription at lower doses and no effect at higher doses) may be due to upregulation of nuclear receptors at low doses and downregulation at high doses seen with endocrine disruptors (Jacquet, et al. 2012).

Based on currently available information, PFOA does not seem to be genotoxic or react with DNA, but does appear to cause liver tumors through activation of PPARα. This pathway may be of limited relevance to humans as levels of PPARα are much lower in human livers compared to rat livers. A form of testicular cancer, Leydig cell tumors, also occurred in rats treated with PFOA, possibly through a hormonal mechanism. While this mechanism could occur in humans, there is insufficient data to conclude that PFOA affects humans the same way(Klaunig, et al. 2012). Pancreatic tumors were also observed in rats; however, increased mortality from pancreatic cancer was not identified in a studies of fluorochemical production workers (Klaunig, et al. 2012, Steenland and Woskie 2012).

Non-human primate study

Male and female cynomolgus monkeys were given PFOS potassium salt by intragastric intubation of a capsule dose for 26 weeks (182 days). The purpose of the study was to determine the earliest measurable response, and corresponding serum levels, in the treated monkeys. The treatment groups were: control (n=6/sex), 0.03 (n=4/sex), 0.15 (n=6/sex), and 0.75 (n=6/sex) mg/kg/day. After dosing ceased, two/sex in the control, 0.15, and 0.75 mg/kg/day groups were followed for one year. Two male monkeys in the 0.75 mg/kg/day group died during the treatment period, one during the 22nd week and the other during the 25th week. The death during the 22nd week was attributed to pulmonary necrosis, although the monkey did have hepatocellular hypertrophy similar to the other monkeys in the 0.75 mg/kg/day treatment group. For the death in the 25th week, it was possibly from hyperkalemia, but the monkey also had signs of liver damage. The authors speculated that these deaths may have been treatment-related as the observed effects were similar to the observed effects with cholesterol lowering (statin) drugs (Seacat, et al. 2002).

Blood samples for serum PFOS measurements were collected 27 days prior to treatment and during 11 of the treatment weeks (1, 2, 4, 6, 8, 12, 16, 20, 24, 26, and 27 [day 183] weeks) and 11 weeks out of the recovery year (27 [days 184, 185, and 187], 28, 29, 30, 31, 35, 39, 43, 47, 51, and 53 weeks). Serum PFOS levels increased linearly for the 0.03 and 0.15 mg/kg/day groups throughout the treatment period. Serum PFOS levels appeared to plateau for the 0.75 mg/kg/day groups around 20 weeks of treatment. Two monkeys each in the 0.15 and 0.75 mg/kg/day groups were followed a year after treatment ceased. Serum PFOS levels in these monkeys increased the week after treatment ceased and then declined to around control animal levels by the end of the recovery year. Elimination half-lives were about 200 days for monkeys in both the 0.15 and 0.75 mg/kg/day groups (Seacat, et al. 2002).

Body weights were significantly reduced and relative liver weights (liver weights expressed as a percentage of body weight) were significantly increased in the 0.75 mg/kg/day group at the end of the treatment period (183 days). Centrilobular vacuolation, hypertrophy, mild bile stasis, lipid-droplet accumulation, and increased glycogen content were visible in some of the 0.75 mg/kg/day dose group livers on day 184 (Seacat, et al. 2002).

Blood samples for hematology and serum chemistry measurements were collected 50, 40, and 27 days prior to treatment and on days 37, 62, 91, 153, and 182 of the treatment period. Cholesterol levels were significantly lower on days 91, 153, and 182 for both the males and females in the 0.75 mg/kg/day groups. HDL levels, only measured on days 153 and 182, were also significantly lower in the 0.03 and 0.75 mg/kg/day males and 0.15 and 0.75 mg/kg/day females compared to control monkeys. While differences in other measurements were observed in male monkeys, no consistent dose response was identified. Female monkeys in the PFOS treatment groups had no differences in other values compared to the control group (Seacat, et al. 2002).

Blood samples for hormone analyses were collected 50, 40, and 27 days prior to treatment and on days 37, 62, 91, and 182 of the treatment period. T3 levels were significantly lower in the 0.75 mg/kg/day male and female groups. T3 levels were also significantly lower in the 0.15 mg/kg/day male and female groups, though not in the confirmatory analysis of the samples taken at necropsy. Free T3 levels were also significantly lower in the 0.75 mg/kg/day male and female groups. TSH levels were increased in the 0.75 mg/kg/day treated monkeys. While testosterone levels were not affected by the treatment, levels of estradiol were lower in the 0.75 mg/kg/day male group. Two of the six female monkeys treated with 0.75 mg/kg/day had lower estradiol levels, however the mean value from the group was not significantly different from the control females. For the 0.75 mg/kg/day group, data indicated that hepatic peroxisome proliferation increased, though the authors noted the increase was less than two-fold, which could indicate a lack of biological significance. Based on the changes in the cholesterol and thyroid hormone levels, the NOAEL in the study was 0.03 mg/kg/day (Seacat, et al. 2002).

Summaries of rodent and *in vitro* studies can be found in Appendix A.

Minnesota Department of Health PFOS Reference Dose (RfD)

The Minnesota Department of Health calculated an RfD for PFOS in 2009. The point of departure used was 35 mg/L, which is a $BMDL_{10}$ from benchmark dose modeling of raw data from Seacat et al. (2002). The serum concentration was converted to a human equivalent dose of

0.0025 mg/kg/day. An uncertainty factor of 3 was used to account for animal to human variability (potential differences in toxicodynamics) and an uncertainty factor of 10 was used to account for variation among humans. The resulting RfD was $8x10^{-5}$ mg/kg/day (MDH 2009).

U.S. EPA PFOS Reference Doses

The U.S. EPA Office of Water developed a Provisional Health Advisory value for PFOS in 2009. The Office of Emergency Management and Office of Superfund Remediation and Technology Innovation (both in the Office of Solid Waste and Emergency Response) developed a subchronic PFOS RfD based on the Provisional Health Advisory value. The Provisional Health Advisory was based on the NOAEL of 0.03 mg/kg/day from a study in cynomolgus monkeys (Seacat et al. 2002). An uncertainty factor of 10 was used to account for variation among humans. A toxicodynamics uncertainty factor of 3 was used to account for variation among the monkeys and a toxicokinetic uncertainty factor of 13 was used to account for differences in the rate of PFOS clearance between humans and monkeys. The resulting subchronic RfD was $8x10^{-5}$ mg/kg/day (EPA 2009).

The U.S. EPA released a draft health effects document and draft RfD for PFOS in February 2014. This document is still within their review process. Because of the on-going review, MDCH will set an interim RfD. After the U.S. EPA document is finalized, MDCH will reassess the interim RfD.

Selection of an interim RfD for use in the Michigan Fish Consumption Advisory Program

The Seacat et al. (2002) study NOAEL of 0.03 mg/kg/day was selected as the basis for an interim RfD. This study was selected for multiple reasons. First, in the absence of applicable human study data, non-human primates are typically a better model for humans. Although cynomolgus monkeys and humans have differences in toxicokinetics, they are more similar to each other than rodent models. For example, the liver: blood partitioning is about 1:1 in both monkeys and humans, while it is about 6.5:1 in rats (ATSDR 2009). In addition, while humans have a PFOS half-life that is about 10 times longer than the half-life in monkeys, the human PFOS half-life is more than 200 times longer than the half-life in male rats and 30 times longer than female rats. Third, the initial cellular events are likely to have greater similarity between humans and monkeys rather than humans and rats. In rats, health effects are often through PFC activation of PPAR, along with other receptors (Ye, et al. 2012). However, humans tend to have smaller amounts of PPAR, which are activated differently, compared to rats (Rosen, et al. 2009). Health effects observed in Seacat et al. (2002) were liver changes and alterations of cholesterol, thyroid, and other hormone levels, as described above. These effects have been correlated to PFOS exposure in human epidemiological studies as well as being observed in other laboratory animal studies.

The time-integrated serum concentration (area under the curve [AUC]) associated with the NOAEL of 0.03 mg/kg/day, obtained through a physiologically-based pharmacokinetic model, was 22,100 mg/L*h.[4] The average serum concentration is considered to be the internal dose.

[4] The AUC was used by the Michigan Department of Environmental Quality Water Resources Division for derivation of a Rule 57 Human Noncancer Value for PFOS (MDEQ 2014).

$$average serum concentration(mg/L) = \frac{AUC(mg/L*h)}{(\exp osure duration(d) \times 24h/d)}$$

Where,

- AUC = 22,100 milligrams per liter hour (mg/L*h)
- Exposure duration = 182 days (d)
- 24 hours per day (h/d) = 24 h/d

The AUC, adjusted for duration of the study (182 days [d]), resulted in an average serum concentration of 5.06 mg/L at steady-state. The average serum concentration (5.06 mg/L) can be converted to a human equivalent dose at steady-state using information on PFOS clearance. At steady state, clearance from and dose to the body are equal and can be calculated using the equation below.

$$CL = V_d \times \left(\frac{\ln 2}{t_{1/2}} \right)$$

Where,

- CL = Clearance, in L/kg/d
- V_d = 0.23 liters/kilogram (L/kg) (volume of distribution [V_d] used in calculating MDEQ's RfD (MDEQ 2014) and used in a human pharmacokinetic model (Thompson, et al. 2010))
- ln 2 = natural logarithm of 2 = 0.693
- $t_{1/2}$ = 5.4 years x 365 days/year = 1,971 days (arithmetic mean PFOS half-life [$t_{1/2}$] in humans (Olsen, et al. 2007))

This results in a clearance of 8.1 x 10^{-5} L/kg/d. Using that value, the average monkey serum concentration (5.06 mg/L) can be converted to a human equivalent dose of 4.1x10^{-4} mg/kg/d.

$$5.06 mg/L \times 8.1 \times 10^{-5} L/kg/d = 4.1 \times 10^{-4} mg/kg/d$$

The human equivalent dose (4.1x10^{-4} mg/kg/d) associated with the NOAEL was divided by a total uncertainty factor of 30 (10 for human-to-human variability and 3 for animal-to-human toxicodynamic variability not accounted for in the human equivalent dose calculation[5]), resulting in an interim RfD of 1.4 x 10^{-5} mg/kg/d.

Children's Health Considerations

Children may have higher exposures to PFOS and other PFCs from sources other than food items based on their hand to mouth behaviors. In a modeled exposure scenario for a 2-year-old child, food ingestion was the primary source of PFOS (42%), followed by dust ingestion (36% of

[5] Although the Seacat et al. (2002) study is subchronic in duration, no additional subchronic-to-chronic uncertainty factor is needed as the physiologically-based pharmacokinetic model output is the average serum concentration at steady-state.

calculated PFOS intake). When the authors included possible PFOS precursors in the calculation, dust ingestion was the primary source of PFOS for the child scenario (Egeghy and Lorber 2011).

Children also have other unique exposures, in addition to dust. Beesoon et al. (2011) measured the amount of PFCs in house dust (n=18), but also measured the levels in maternal and umbilical cord serum. Maternal serum (n=20) was collected at 15 weeks of gestation, and cord serum (n=20) was collected at delivery. For the dust samples, PFOA, PFOS, and PFHxA all had similar median levels. PFOS, PFOA, PFHxS, and PFNA were the major PFCs in maternal and cord sera. PFOS, PFOA, PFNA, and PFHxS were significantly higher in maternal serum than in cord serum. PFUnA, PFDoA, and PFTA were detected in six or fewer maternal serum samples, but not in any cord serum samples. The transplacental transfer efficiencies were estimated by dividing the PFC concentrations in cord serum by the levels in maternal serum. The transplacental transfer efficiencies ranged from 0.10 to 0.58 for PFOS and 0.26 to 1.00 for PFOA. The authors noted that their values underestimated actual levels, as the maternal serum was collected at 15 weeks. Higher chain carboxylates (greater than 8 carbons) and sulfonates (greater than 7 carbons) had lower transplacental transfer efficiencies compared to lower chain chemical, leading to the conclusion that the placenta blocks longer chain better than shorter chain PFCs. (Beesoon, et al. 2011)

Along with prenatal exposure, children may be exposed to PFCs through breast milk. Levels of PFOA and PFOS were measured in samples of human milk. Twenty-one women were breastfeeding for the first time (primiparas) and 16 were breastfeeding for at least the second time (multiparas). PFOS was detected in 90% of the samples from the primiparous women and in 62% of the samples from the multiparous women. PFOA was detected in 81% of the samples from the primiparous women and in 46% of the samples from the multiparous women. PFOS levels ranged from 15 to 288 ppb (166 ppb in samples from multiparas). PFOA levels ranged from 24 to 241 ppb (100 ppb in samples from multiparas). (Barbarossa, et al. 2013)

As children have developing systems, prenatal and postnatal exposures may result in increased sensitivity to toxicity mediated by PFOS or other PFCs. Increased levels of PFOS and other PFCs have been associated with alterations in thyroid hormones levels (Lopez-Espinosa, et al. 2012), delayed puberty (Lopez-Espinosa, et al. 2011), and increased risk of having ADHD (Hoffman, et al. 2010). Additional investigation is needed because evidence is not currently available to show that PFCs cause these effects or at what level of exposure.

Roth and Wilks (2014) qualitatively assessed epidemiological studies investigating neurodevelopmental effects of PFCs, including PFOS, PFOA, and PFHxS. The authors noted that a meta-analysis could not be conducted due to lack of studies and the diversity of the endpoint evaluated in available studies. Eight studies were included, with five measuring head circumference and three assessing other neurodevelopmental or neurobehavioral endpoints. All examined associations between specific endpoints and levels of PFOS and PFOA in blood (maternal, child, or umbilical cord). Four other studies, using indirect evidence (questionnaire-based), were not qualitatively evaluated. Overall, the authors noted that associations with neurodevelopmental domains, such as fine motor skills or cognitive performance, were only identified with specific PFCs. No effects can be attributed to all PFCs. Specific limitations of the existing studies included a lack of power analyses, to determine if the number of participants

were sufficient to detect effects, and investigations into the causal relationship between neurodevelopmental toxicity and PFCs (Roth and Wilks 2014).

Conclusions

- MDCH concludes that unlimited consumption of sport-caught fish with elevated PFOS levels could harm people's health. Based on published information and other states' investigations, one of the PFCs, perfluorooctane sulfonate (PFOS) accumulates in fish to much higher levels than the other PFCs. Other studies indicated that consumption of fish with elevated PFOS levels is people's primary route of exposure. Because of this, the focus of this review is mostly on the available published studies investigating the health effects of PFOS. Exposure to PFOS has been linked to alterations in thyroid hormones, cholesterol levels, neurodevelopment, and immune function.
- MDCH concludes that additional site-specific evaluation of PFOA levels in fish may be needed when people are also exposed to PFOA by other routes. PFOA does not bioaccumulate to a great extent in fish. However, at sites where people may have exposure to PFOA through contaminated groundwater or other routes of exposure, consumption of PFOA-containing fish might need to be included as part of a total exposure dose evaluation.
- MDCH concludes that, under certain circumstances, PFOSA levels in fish may need additional evaluation. PFOSA has the potential to be metabolized into PFOS. At certain sites, PFOSA levels in fish might need additional evaluation.
- MDCH concludes that existing data is inadequate to evaluate other PFCs or the cumulative effects of exposure to multiple PFCs. As additional information becomes available, MDCH will evaluate other relevant PFCs.

Recommendations

- Use the interim RfD to update provisional PFOS fish consumption screening values (FCSV) and use these values to provide fish consumption advice in Michigan.
- Begin or continue monitoring of sport-caught fish in Michigan for PFOS and other relevant PFCs.
- Provide the Fish and Wildlife Contaminant Advisory Committee (FAWCAC) and other relevant groups (Great Lakes Sport Fish Advisory Task Force and Great Lakes Human Health Network) with a copy of this document.
- Develop an RfD for PFOA and PFOSA, if needed.

Public Health Action Plan

1. MDCH will issue fish consumption advisories using updated PFOS FCSVs.
2. The MDCH Analytical Chemistry Laboratory will analyze fish filets, collected for the Michigan Fish Contaminant Monitoring Program (MFCMP) (administered by the Department of Environmental Quality), for PFOS and other PFCs.
3. MDCH will provide access to this document to FAWCAC and other relevant groups.

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Appendix A: Summaries of rodent and in vitro studies

Rodent studies

Sprague-Dawley rats were exposed to PFOS for two years to investigate chronic toxicity and carcinogenicity of PFOS exposure. Rats (at least 11 per sex per group) were fed 0, 0.5, 2, 5, and 20 ppm PFOS in their diet for 104 weeks. Twenty male and female (10 each) rats were fed 20 ppm for 52 weeks and then placed on the control diet for an additional 52 weeks. Statistically significant increases in ALT, a liver enzyme, in the 20 ppm males on weeks 14 and 53; reductions in serum total cholesterol in male rats in the 20 ppm on weeks 14, 27, and 53; and reductions in serum cholesterol in 2, 5, and 20 ppm female rats on week 27 were identified. Serum glucose was significantly decreased in the 20 ppm males in weeks 4 and 53; 2 ppm females in week 53; 5 ppm females in week 14 and 53; and 20 ppm females in week 53. Serum urea nitrogen was statistically significantly different from controls in some weeks; however, the authors noted that there were no changes with microscopic renal findings. There were statistically significant increases in serum creatinine in the 2 ppm females at week 14. The authors concluded that the serum urea nitrogen changes were likely due to mild dehydration. Although a few statistically significant clinical chemistry effects were identified, they were not consistent over treatment time or dose. No urine chemistry or hematology changes were identified that the authors considered treatment related (Butenhoff, et al. 2012).

For the four unscheduled deaths (three males and one female in the 20 ppm group) before week 53, two of the males and the female had large, mottled, or diffusely dark livers. At the sacrifice in week 104, some rats in the 5 and 20 ppm groups had enlarged, mottled, diffusely darkened, or focally lightened livers. Increased amounts of non-neoplastic lesions were found in the livers of the PFOS-exposed rats. These include hepatocellular centrilobular hypertrophy, eosinophilic hepatocytic granules, and centrilobular hepatocytic pigment in the rats given 20 ppm and hepatocellular centrilobular hypertrophy in the 5 ppm rats. Male rats fed 20 ppm PFOS had a statistically significant increase in the incidence of hepatocellular adenoma and thyroid follicular cell adenoma. Female rats fed 20 ppm had a statistically significant increase in hepatocellular adenoma (Butenhoff, et al. 2012).

The authors estimated benchmark doses for liver tumor incidence in male and female rats. A lower limit on the benchmark dose resulting in a 10% increase in tumor incidence ($BMDL_{10}$) was 7.9 ppm for male rats and 8.0 ppm for female rats. The authors also estimated the $BMDL_{10}$ for serum PFOS levels after 14 weeks of dietary PFOS exposure, which was 62 µg/mL for males and 92 µg/mL for females. The authors note that use of rat serum levels may be protective of human exposure as rats have higher liver to serum ratios. Humans and other primates are expected to have lower levels in the liver than levels seen in rat livers based on serum levels (Butenhoff, et al. 2012).

Twelve-week-old Crl:CD (SD) female rats were given 0, 0.1, 0.3 and 1.0 mg/kg/day potassium PFOS salt (25 per group) from gestational day 0 to postnatal (PND) day 20. On PND 17, failure to habituate (increased activity during the testing; animals not used to the test environment) was identified in eight male rats (maternal dose of 1.0 mg/kg/day). Increased total and cumulative activity was also noted in males (0.3 mg/kg/day group) on PND 17 and females (1.0 mg/kg/day

group) on PND 21, but as the habituation was similar to the control groups and no other differences were noted, the authors concluded that this was not a K^+PFOS treatment related effect. There were no differences on the other locomotion, startle, or swim (testing swimming, learning, or memory) testing. No differences in brain weight, length, or width were identified in treated animals. The NOAEL was identified as 0.3 mg/kg/day for both development of the nervous system (increased motor activity and failure to habituate in males in the 1.0 mg/kg/day maternal dose group) and maternal effects (lower maternal food consumption, body weight-gains during gestation, and maternal body weights during lactation in the 1.0 mg/kg/day group). Based on additional work with another set of exposed rats, mean maternal serum PFOS levels were approximately 6.2 µg/mL in the 0.3 mg/kg/day group (Butenhoff, et al. 2009).

Adult male C57BL/6 mice were gavaged daily (12 mice per group) with a potassium salt of PFOS (0, 0.0167, 0.0833, 0.4167, or 0.8333 mg/kg/day) for 60 days. All endpoints were measured after the 60-day treatment. Mice gavaged with PFOS had significant increases in serum PFOS levels, with means ranging from 4,350 to 59,740 ppb. Body weight was significantly lower for the mice given 0.8333 mg/kg/day, and food intake for this group was also significantly reduced when compared to the control group (Dong, et al. 2012).

Spleen and thymus weight were significantly lower in the 0.8333 mg/kg/day group, while liver weight was significantly higher in the 0.0833 and 0.8333 mg/kg/day groups compared to the control group. The number of cells in the spleen and thymus were significantly reduced, compared to the control group, in the mice given 0.8333 mg/kg/day. Cell viability, in both the spleen and the thymus, was significantly reduced in the 0.0833 and 0.8333 mg/kg/day groups compared to the control group. Mitochondrial membrane potential, measured by the amount of fluorescence in a cell, significantly decreased in thymocytes from mice given 0.0833 mg/kg/day and in splenocytes and thymocytes from mice given 0.8333 mg/kg/day. Both 0.0833 and 0.8333 mg/kg/day treated mice had a significantly increased percentage of splenocytes expressing p53 and cytochrome c proteins compared to control mice. Only mice treated with 0.8333 mg/kg/day had a significantly increased, compared to control mice, percentage of thymocytes expressing p53 and cytochrome c proteins. Both 0.0833 and 0.8333 mg/kg/day treated mice had a significantly lower percentage of splenocytes expressing bcl-xl (anti-apoptotic) and pro-caspase-3 compared to control mice. Only mice treated with 0.8333 mg/kg/day had a significantly reduced percentage of thymocytes expressing bcl-xl and pro-caspase-3 compared to control mice. There were no significant differences in percentages of splenocytes or thymocytes expressing bcl-2 (anti-apoptotic) or bax (pro-apoptotic) proteins in any of the PFOS-treated groups. Based on these results, the authors concluded that mitochondria are involved in PFOS-induced apoptosis. The NOAEL for this study was 0.0167 mg/kg/day based on increased liver weight, reduced cell viability number of cells in the spleen and thymus, and changes in proteins involved in apoptosis (Dong, et al. 2012).

Adult male C57BL/6 mice were gavaged daily (6 per treatment group) with a potassium salt of PFOS (0, 0.0083, 0.0167, 0.0833, 0.4167, or 0.8333 mg/kg/day) for 60 days. Cytokines (interleukin [IL]-2, IL-10, IL-4, interferon [IFN]-γ, and immunoglobulin [Ig]M and IgG) were measured the day after treatment ceased (day 61). Serum PFOS levels, measured on day 61, were significantly increased in the 0.0833, 0.4167, or 0.8333 mg/kg/day groups compared to the control group. Serum PFOS means in those three groups ranged from 10.75 to 51.71 mg/L. The

final body weight was significantly lower in the 0.8333 mg/kg/day compared to the control group. Some organ weights were significantly different from the controls (Dong, et al. 2011).

In the 0.8333 mg/kg/day group, spleen and thymus masses were significantly decreased. Liver mass was significantly increased in the 0.4167 and 0.8333 mg/kg/day groups. Ex vivo cytokine production was measured from splenocytes cultured for 48 hours. The level of IFN- γ was significantly lower in the 0.8333 mg/kg/day group compared to the control group. IL-4 protein levels were significantly higher than the control in the 0.0833, 0.4167, and 0.8333 mg/kg/day groups. The number of splenocytes producing IL-2 protein was significantly lower in the 0.8333 mg/kg/day group compared to the control group. Significantly more splenocytes produced IL-10 in the 0.8333 mg/kg/day group compared to the control animals. Serum levels of sheep red blood cell (SRBC)-specific IgG, IgG1 and IgE were significantly higher in the 0.8333 mg/kg/day group compared to the control group. There was no difference in serum levels of SRBC-specific IgG2a or in delayed-type hypersensitivity response to SRBC, measured by footpad swelling, in the mice on day 61. Levels of SRBC-specific IgM were significantly lower in the 0.0833, 0.4167, or 0.8333 mg/kg/day groups compared to the control group (Dong, et al. 2011).

Serum corticosterone levels were not different in any of the groups, which suggest that the immune effects were not due to a stress response. The authors stated that PFOS exposure causes an enhanced humoral response (supported by increased TH2-type cytokines levels) and suppression of cellular response (supported by decreased IL-2, IFN-γ, and IgM production). Based on the above results, the NOAEL for this study was 0.0167 mg/kg/day (Dong, et al. 2011)

In an earlier study with adult male C57BL/6 mice gavaged daily with PFOS potassium salt for 60 days, the authors also examined immune system function. Mice, 10 per group, were treated with 0, 0.00833, 0.08333, 0.41667, 0.83333, and 2.08333 mg/kg/day. At the end of the treatment period, serum PFOS levels were significantly higher than the controls in all groups, and means ranged from 0.674 mg/L (0.00833 mg/kg/day treated group) to 120.67 mg/L (2.08333 mg/kg/day treated group). Body weight was significantly lower in the 0.41667, 0.83333, and 2.08333 mg/kg/day groups compared to the control animals on day 60. Food intake on day 60 was also significantly decreased in the 0.83333 and 2.08333 mg/kg/day groups from the baseline food intake (Dong, et al. 2009).

Spleen and thymus mass were significantly decreased compared to the control in the 0.41667, 0.83333, and 2.08333 mg/kg/day groups. Kidney mass was significantly lower in the 0.83333 and 2.08333 mg/kg/day groups compared to the control. Liver mass was significantly increased compared to the control in the 0.08333, 0.41667, 0.83333, and 2.08333 mg/kg/day groups. Serum corticosterone levels were significantly increased in the 0.83333 and 2.08333 mg/kg/day groups compared to the control group. Total number of cells in the spleen and thymus were reduced in the mice treated with 0.41667, 0.83333, and 2.08333 mg/kg/day compared to the control group. While all spleen CD4/CD8 subpopulations measured (CD4+, CD4+/CD8+, CD4-/CD8-, CD8+, and B220+) decreased in the 0.83333 and 2.08333 mg/kg/day groups, only CD4+, CD4+/CD8+, CD4-/CD8- subpopulations decreased in the 0.41667 mg/kg/day group compared to control animals. CD4 and CD8 are cell surface markers of T cells and B220 is a cell surface marker of B cells. All thymic CD4/CD8 subpopulations measured (CD4+, CD4+/CD8+, CD4-/CD8-, and CD8+) decreased in the 2.08333 mg/kg/day group compared to the control. The

thymic CD4+ subpopulation also decreased in the 0.83333 mg/kg/day group compared to the control group. Both the thymic CD4+/CD8+ and CD8+ subpopulations also decreased in the 0.41667 and 0.83333 mg/kg/day groups compared to the control group (Dong, et al. 2009).

Natural killer cell activity was measured by lactate dehydrogenase release from lysed lymphoma cells by active natural killer cells. Natural killer cell activity was significantly decreased in the 0.83333 and 2.08333 mg/kg/day groups and significantly increased in the 0.08333 mg/kg/day group compared to the control group. Spleen cell proliferation (measured ex vivo) was significantly reduced, compared to the control group, in the 0.83333 and 2.08333 mg/kg/day groups. On day 58 (4 days before euthanasia), mice were intraperitoneally injected with sheep red blood cells. Spleen cells were added to sheep red blood cells in agarose and plaques (cleared areas) were counted. The number of plaques were reduced in groups treated with 0.08333 mg/kg/day or more. Elevated corticosterone levels observed in the 0.83333 and 2.08333 mg/kg/day groups indicate that immunosuppression observed was may be due to a stress response rather than the treatment. Based on these experiments, the authors identified a NOAEL of 0.00833 and a LOAEL of 0.08333 mg/kg/day (Dong, et al. 2009).

Keil et al. 2008 designed a study to examine immunotoxicity in pups from maternal PFOS (potassium salt) exposure. Female C57BL/6N mice were bred with male C3H/HeJ mice. The offspring of this pairing were B6C3F1 mice. Pregnant female mice were gavaged with PFOS (0, 0.1, 1.0, or 5.0 mg/kg/day) from gestational day 1 to 17. There were no body, spleen, or thymus weight differences, compared to the control group, at 4 weeks of age for PFOS-exposed male and female pups. Liver weight was significantly lower in 4-week-old female pups in the 0.1 mg/kg/day maternal exposure group, but the male pups in the 5.0 mg/kg/day maternal exposure groups had significantly increased liver weight at four weeks compared to the control group. Kidney weights were significantly lower for 4-week-old female pups in the 5.0 mg/kg/day maternal exposure group compared to the control group. No body, liver, kidney, spleen, or thymus weight differences were identified for any of the pups at 8 weeks. Number of cells in the spleen and thymus were similar for all groups at both 4 and 8 weeks. However, there were six male and female pups per group for the 4-week endpoint, while the number of mice per group ranged from 2 to 7 for the 8-week endpoint (Keil, et al. 2008).

Natural killer cell activity, measured by release of 51Cr from lysed Yac-1 tumor cells, was not altered by PFOS treatment in 4-week-old pups (male and female combined). Reduced natural killer cell activity was identified in 8-week-old female offspring gestationally exposed to 5.0 mg/kg/day and male pups gestationally exposed to 1.0 and 5.0 mg/kg/day. Data from 8-week-old male and female offspring were separated as statistical interactions were identified. Sheep red blood cell-specific IgM antibody production was assessed in the 8-week-old offspring. No differences were identified in the females, but the IgM response was significantly reduced in the males exposed to 5 mg/kg/day. There were no differences among the treatment groups in thymocyte subpopulations in 4-week-old male and female offspring or in splenocyte subpopulations in 8-week-old offspring. No differences were identified in the number of CD3+, CD8+, CD4+, CD4-/CD8-, or CD4+/CD8+ splenocytes in 4-week-old male or female offspring. However, the number of B220 splenocytes (B cells) was significantly reduced in the 5.0 mg/kg/day 4-week-old female offspring. Numbers of CD8+, CD4+/CD8+, CD4-/CD8-, B220 thymocyte subpopulations were not altered by PFOS treatment in 8-week-old offspring. Only the

number of CD3+ and CD4+ thymocytes were significantly reduced in the 8-week-old males gestationally exposed to 5.0 mg/kg/day. No difference in peritoneal macrophage nitric oxide production or differences in number of peritoneal macrophages were identified among the treatment groups (this endpoint was only tested in 8-week-old offspring). The authors note that, as with other studies, male B6C3F1 mice were more sensitive to the effects of PFOS than female mice. Based on the functional immune effects found, the authors identified a NOAEL of 0.1 mg/kg/day and LOAEL of 1.0 mg/kg/day in male offspring. For female offspring, the NOAEL was 1.0 mg/kg/day and the LOAEL was 5.0 mg/kg/day (Keil, et al. 2008).

Sprague-Dawley rats were fed diets containing PFOS (0, 2, 20, 50, and 100 ppm) for 28 days. Male and female rats given the 50 and 100 mg/kg diets had significantly lower body weights at the end of the study compared to controls. Liver weights were significantly increased for male rats given 50 and 100 ppm and female rats given 20, 50, 100 ppm. Spleen and thymus weights were significantly lower in male rats given 100 ppm, however, only thymus weight was significantly lower in female rats given 100 ppm PFOS. Increased apoptotic cells in the thymic cortex and medulla were identified in male rats given 50 and 100 pm, but only in the thymic cortex of female rats given 100 ppm. No histopathological changes were identified in the spleen, mesenteric lymph nodes, small or large intestines (Lefebvre, et al. 2008).

Male rats had no significant treatment related changes in number of peripheral blood leukocytes or lymphocytes compared to control rats, although there were significant trends toward increased T (CD3+) and T-helper (CD3+/CD4+) and decreased B (CD45RA+) cells with increased exposure to PFOS. Female rats also had significant trends toward increased total leukocytes, lymphocytes, T (CD3+), T-helper (CD3+/CD4+), and T-suppressor/cytotoxic (CD3+/CD8+) cells with increased exposure to PFOS without any significant difference in cell numbers when compared to the control group. There were no significant differences between PFOS-exposed and control serum total IgM, IgA, IgG2c, or IgE in male rats. However, there was a trend toward increasing serum total IgG, IgG2a, and IgG2c levels with higher PFOS exposure in male rats, and there were significantly lower levels of IgG1 in the rats fed 2 and 20 ppm PFOS compared to the control group. Female rats fed 100 ppm PFOS had significantly higher serum total IgM and IgG2c levels than the control group, and there was also a trend for increasing levels of IgM and IgG2c with increasing PFOS exposure. There were no significant differences in serum total IgA, IgG1, IgG2a, IgG2b, or IgE between PFOS-exposed and control female rats. PFOS exposure did not result in any significant differences in splenocyte proliferation (Lefebvre, et al. 2008).

In a measure of T cell dependent antibody response using keyhole limpet hemocyanin (KLH), male rats had a trend of increasing KLH-specific IgG, but female rats had no significant differences. Neither male or female rats exposed to PFOS had any differences in delayed-type hypersensitivity response to KLH. No NOAELs were obtained from this study, but the authors identified a LOAEL of 0.14 mg/kg/day (2 ppm diet) in male rats for reduced serum total IgG1. This effect was not observed in female rats, but a LOAEL of 0.15 mg/kg/day (2 ppm diet) was identified for female rats based on liver weight changes (Lefebvre, et al. 2008).

Adult female B6C3F1 mice were gavaged with PFOS (0, 0.0331, 0.0993, or 9.93 mg/kg/day) for 28 days. Body mass significantly decreased in the 9.93 mg/kg/day mice compared to the control mice over the 28 day exposure. In the 9.93 mg/kg/day group, liver mass was significantly

increased and spleen mass was significantly decreased compared to the control mice. Total number of spleen cells was not different between PFOS-treated and control mice. On day 29, five mice per treatment group (20 mice total) were intraperitoneally injected with lipopolysaccharide (LPS) to induce inflammation. One hour later, the spleen, blood, peritoneal macrophages, and peritoneal lavage fluid were collected (Mollenhauer, et al. 2011).

Serum levels of tumor necrosis factor α (TNF-α) were significantly lower in mice exposed to 0.0331 mg/kg/day compared to control mice and mice exposed to 0.0993 and 9.93 mg/kg/day. Serum IL-6 levels were significantly increased in mice exposed to 0.0331 mg/kg/day compared to control mice. IL-6 levels in the 0.0993 mg/kg/day mice were significantly lower than mice exposed to 0.0331 mg/kg/day. Intracellular cytokines were also measured in splenocytes. PFOS exposure did not affect the number of splenocytes with intracellular IL-1, IL-10, or TNF-α. The number of splenocytes with intracellular IL-6 expression was significantly decreased from mice treated with 0.0993 mg/kg/day compared to control mice. Taken together, the serum and intracellular IL-6 expression displayed a non-linear relationship, with increases in mice treated with 0.0331 mg/kg/day and decreases in mice treated with 0.0993 mg/kg/day (Mollenhauer, et al. 2011).

TNF-α protein levels produced by peritoneal macrophages, cultured for 24 hours, were not altered by PFOS exposure. IL-6 protein levels were significantly increased in peritoneal macrophages, cultured for 24 hours, from mice exposed to 9.93 mg/kg/day compared to control mice. A significant trend of lower TNF-α and IL-6 protein levels from mice with higher PFOS exposure was identified in the peritoneal lavage fluid. Peritoneal macrophages were collected from 20 other mice (five female mice per PFOS group listed above) exposed to LPS for 24 hours ex vivo. TNF-α protein production was significantly increased in peritoneal macrophages from mice exposed to 9.93 mg/kg/day compared to control mice. There were no differences in IL-6 protein production. Both TNF-α and IL-6 protein production were affected in mice exposed to 0.0331 mg/kg/day, the lowest level of PFOS used in the study. As there were both significant increases and decreases of these cytokines, the authors concluded that PFOS might cause a mixture of pro- and anti-inflammatory effects (Mollenhauer, et al. 2011).

Male and female BALB/c mice were gavaged with PFOS (0, 5, and 20 mg/kg/day) for 14 days. Half of the mice (3 groups of 8 male and 8 female mice) were fed a high fat diet (10% more lard and 3% more cholesterol than the regular diet). Mice on the high fat diet weighed more, 6.6 times more for female and 2 times more for male mice, than mice on the regular diet. Male and female control mice on the high fat diet had more ventral fat. Male and female mice exposed to 20 mg/kg/day PFOS had significantly reduced body weight, fat index, and spleen index and significantly increased liver index compared to control mice. (Fat and organ indices were calculated by dividing organ weight by body weight times 100.) PFOS-exposed male mice also had significantly reduced food intake and thymus index compared to control mice. There were no significant changes in the mice on the regular diet exposed to 5 mg/kg/day PFOS. Male and female mice on the high fat diet exposed to 20 mg/kg/day PFOS had significantly reduced body weight, food intake, fat index, thymus index, and spleen index and significantly increased liver index compared to control mice on the high fat diet. Male and female mice exposed to 5 mg/kg/day PFOS on the high fat diet had significantly increased liver index and significantly

reduced fat index. Male mice exposed to 5 mg/kg/day PFOS on the high fat diet also had significantly reduced thymus index (Wang, et al. 2011b).

Mice on the regular diet had changes in the thymus (to the cortex and medulla) in both the 5 and 20 mg/kg/day groups. Similar changes were observed in the mice given the high fat diet. Adipocytes were observed in the lobules of the thymus in mice given the high fat diet and exposed to 20 mg/kg/day PFOS. Changes to the spleen were also observed in PFOS exposed mice on both the regular and high fat diets. The percentage of apoptotic thymocytes, as measured by flow cytometry, was significantly increased in female mice exposed to 20 mg/kg/day and male mice exposed to 5 mg/kg/day, both on the high fat diet (Wang, et al. 2011b).

PPARα and IL-1β mRNA levels were measured in male mice only, due to the level of thymus and spleen atrophy (females appeared to have less). There were no significant changes in PPARα or IL-1 β mRNA levels in the thymus and spleen in male mice on both PFOS-containing diets. The authors concluded that PFOS may interfere with lipid metabolism and can cause atrophy of the spleen and thymus. Additional investigation into the ability of PFOS to damage the immune system was recommended (Wang, et al. 2011b).

Male and female B6C3F1 mice were gavaged with PFOS (potassium salt; 0, 0.166, 1.66, 3.31, 16.6, 33.1, and 166 µg/kg/day) for 28 days. The total administered dose was developed to be within the range of PFOS blood levels found in humans and wildlife. There were no differences in the body or organ weights among the treatment groups. Neither spleen and thymus cell numbers nor cell viability were different in any of the groups. Lymphocyte proliferation, induced with either concanavalin A or LPS, in the PFOS treatment groups was not different from the control group. Natural killer cell activity, measured by chromium release, significantly increased in males given 16.6, 33.1, and 166 µg/kg/day, compared to the control group. No differences were identified in female mice. There were no treatment related differences in serum lysozyme activity in males, but there was a significant increase in 3.31 and 166 µg/kg/day females compared to the control mice (Peden-Adams, et al. 2008).

Sheep red blood cell IgM production, a functional measurement of both B and T cells, was significantly reduced in both male (starting at 1.66 µg/kg/day groups) and female (starting at 16.6 µg/kg/day groups) mice. In a follow-up experiment, to clarify the involvement of B-, T-cells or both, with a T-cell independent antigen (trinitrophenyl [TNP]-LPS), female mice were exposed to 0.334 mg/kg/day orally for 21 days. Serum levels of TNP-specific IgM production was significantly reduced compared to the control group, indicating that PFOS may affect B cell function. Both spleen and thymus subpopulations were measured. In male mice, starting in the 3.31 µg/kg/day group, CD4-/CD8+ and CD4-/CD8- splenocytes were significantly increased while CD4+/CD8+ and CD4+/CD8- splenocytes were significantly reduced compared to the control group. In female mice given 3.31, 16.6, or 166 µg/kg/day, CD4-/CD8+ splenocytes were significantly reduced compared to the control group. Female mice given 3.31 and 16.6 µg/kg/day had significantly reduced numbers of CD4+/CD8- splenocytes compared to the control group. PFOS treatment did not alter thymocyte subpopulations in male mice. For female mice, thymic CD4-/CD8+ cells were significantly reduced in mice given 33.1 and 166 µg/kg/day compared to the control group (Peden-Adams, et al. 2008).

The authors identified suppression of SRBC-specific antibody production as the most sensitive endpoint tested. The LOAEL for male mice was 1.66 µg/kg/day and for female mice was 16.6 µg/kg/day. NOAELs were 0.166 µg/kg/day for male and 3.31 µg/kg/day for female mice. Serum PFOS levels were measured in the control, NOAEL and LOAEL groups. The mean (± standard deviation) serum PFOS for control mice was 12.1(± 4.64) ng/g for male and 16.8 (±4.31) ppb for female mice. Serum PFOS levels that corresponded to the NOAELs were 17.8 (±4.24, for males) and 123 (±18.7, for females) ppb. Serum PFOS levels for mice exposed to the LOAELs were 91.5 (± 22.2) for males and 666 (±108) ppb for females. Male and female PFOS serum levels were similar in matching dose groups, so the authors noted that the differences between the male and female mice are not likely related to differences in bioaccumulation or elimination. Instead, based on other published studies, the authors speculate that the differences may be due to PFOS-induced endocrine modulation (Peden-Adams, et al. 2008).

Male C57BL/6 mice were exposed to PFOS in diet for 10 (0.004% PFOS diet) or 28 days (0.0001% PFOS diet). After PFOS exposure, half of the mice were injected with concanavalin A and sacrificed 24 hours later. Concanavalin A causes immune-mediated liver damage and was used to determine if PFOS alters the immune system function in the liver (Qazi, et al. 2013).

Mice given the 0.004% PFOS for 10 days were exposed to 6 ±1.3 mg/kg/day. There was no difference in body weight gain or mass of the thymus, spleen or fat pad between control and PFOS-exposed mice. PFOS exposed mice did have increased relative liver weight, but no increase in serum levels of alanine aminotransferase (ALT) or aspartate aminotransferase (AST). Hypertrophy of the centrilobular hepatocytes was observed in mice exposed to PFOS. Control mice injected with concanavalin A had elevated serum ALT and AST. Serum AST and ALT levels were significantly higher in mice also exposed to PFOS. PFOS, alone, significantly reduced TNF-α, IFN-γ, and IL-4 protein levels in supernatant from liver homogenates. Concanavalin A treated mice had significantly different levels of TNF-α, IFN-γ (both increased), and IL-4 (decreased) compared to untreated mice. Mice treated with PFOS and concanavalin A had significantly different levels of TNF-α (reduced compared to untreated and only concanavalin A treated mice), IFN-γ (increased compared to untreated and reduced compared to concanavalin A only), and IL-4 (reduced compared to untreated and only concanavalin A treated mice). DNA fragmentation, an indicator of apoptosis, was measured in supernatant from liver homogenates. Mice given concanavalin A had increased DNA fragmentation compared to untreated or PFOS alone treated mice. Mice exposed to both PFOS and concanavalin A had slightly elevated levels, but not significantly so, of DNA fragmentation compared to mice given concanavalin A alone. PFOS exposure increased concanavalin A -induced liver damage while suppressing cytokine protein expression. Based on the results, the 10-day, higher dose PFOS exposure followed by concanavalin A treatment caused severe hepatic necrosis (Qazi, et al. 2013).

Mice given the 0.0001% PFOS diet for 28 days were exposed to 0.144±0.004 mg/kg/day. There was no difference in body weight gain or mass of the thymus, spleen or fat pad between control and PFOS-exposed mice. Control mice injected with concanavalin A had elevated serum ALT and AST. Serum AST and ALT levels were significantly higher in mice also exposed to PFOS. TNF-α, IFN-γ, and IL-4 protein levels in supernatant from liver homogenates were not significantly different between untreated and PFOS-treated mice. Concanavalin A treated mice

had significantly different levels of TNF-α and IFN-γ (both increased), but not IL-4 compared to untreated mice. Mice treated with PFOS and concanavalin A had significantly different levels of TNF-α, IFN-γ, and IL-4 (all increased) compared to untreated mice, but not compared to mice given only concanavalin A. Mice given concanavalin A had increased DNA fragmentation, measured in supernatant from liver homogenates, compared to untreated or PFOS-alone treated mice. Mice exposed to both PFOS and concanavalin A had significantly elevated levels of DNA fragmentation compared to mice given concanavalin A alone. PFOS exposure increased concanavalin A -induced liver damage without altering cytokine protein expression. Based on the result, the 28-day lower dose PFOS exposure followed by concanavalin A treatment caused increased apoptosis. The authors concluded that dietary exposure to PFOS may sensitize liver cells to additional insults that may worsen liver damage during acute inflammation (Qazi, et al. 2013).

Male B6C3F1 mice were fed approximately 0.25 mg PFOS/kg/day for 28 days. The purpose of this study was to determine if PFOS doses that result in human equivalent serum levels affect the immune system. Mice exposed to PFOS had significantly decreased body weight and significantly increased liver mass and serum corticosterone levels compared to the control group. There were no significant differences in food intake, serum corticosterone levels, and epididymal fat pad, thymus, or spleen masses. Although thymocyte (immature, mature T, and natural killer cells) and splenocyte (macrophages, granulocytes, B, T, natural killer, and plasma cells) subpopulations were counted, no significant differences were identified between the control and PFOS-exposed mice. Serum PFOS levels were measured and control mice had a mean of 0.0409 µg/mL while PFOS-exposed mice had a mean of 11.6 µg/mL (Qazi, et al. 2010).

In order to investigate T-cell dependent antibody production, control and PFOS-exposed mice were injected with sheep red blood cells on day 23 of the 28-day exposure. PFOS exposure did not alter T-cell dependent antibody production. No differences in percentage of plasma cells in the spleen or number of splenocytes producing SRBC-specific IgM were identified. These results were confirmed with a haemagglutination assay. No differences were identified in serum levels of anti-SRBC IgM between control and PFOS-exposed mice. PFOS exposure did not alter T-cell independent antibody production. No differences in serum anti-TNP IgM levels were identified. The authors proposed that dietary administration of PFOS may produce less toxicity than orally gavaged PFOS (Qazi, et al. 2010).

In summary, a NOAEL for nervous system effects in male Crl:CD (SD) rat pup was identified as 0.3 mg/kg/day (Butenhoff, et al. 2009). The pups were exposed from gestational day 0 to postnatal day 20. Mice (B6C3F1) also exposed during gestation, from day 1 to 17, have a similar NOAEL of 0.1 mg/kg/day based on immune system effects (Keil, et al. 2008). Other NOAELs based on immune system effects have been around an order of magnitude lower. In studies using male C57BL/6 mice exposed for 60 day, NOAELs of 0.0167 and 0.00833 mg/kg/day have been identified (Dong, et al. 2011, Dong, et al. 2009). Additional investigation into the immune system effects of PFOS is needed, including studies that examine the relevance of the immune endpoints tested for humans.

Differences in rats and mice when measuring immune system related changes in response to PFOA treatment were highlighted in a review by DeWitt et al. (2009). No immune-related

changes where identified at PFOA doses that caused systemic toxicity in rats, whereas in mice, immune-related effects were identified at doses that caused systemic toxicity. In B6C3F1 mouse pups exposed from gestational day 0 to 17, a maternal PFOS dose of 1.0 (male) and 5.0 (female) mg/kg/day suppressed natural killer cell function. PFOS also affected T cell-dependent antibody synthesis, measured by sheep red blood cell plaque-forming assay, in B6C3F1 mice. This occurred without signs of stress or toxicity. The review authors note that mice (C57BL/6 females or B6C3F1 males) are the most sensitive laboratory species, of those tested, to PFOA or PFOS induced immune effects. (DeWitt, et al. 2009)

Male C57BL/6 mice were fed PFOA- (0%, 0.02%) or PFOS (tetrabutylammonium salt; 0%, 0.001%, 0.005%, 0.02%, 0.05%, 0.1%, 0.25%, 0.5%, 1.0%)-containing chow for 10 days. The 0.02% PFOS diet was equivalent to about 40 mg/kg/day based on food intake. The purpose of the study was to determine if PFOS exposure resulted in immune system effects similar to those observed with PFOA exposure. Mice fed more than 0.02% PFOS lost greater than 20% of their initial body weight and had lethargy and poor grooming. These animals were withdrawn from the study. Mice fed 0.02% PFOS had a significantly reduction in body weight compared to control mice. Mean serum PFOS levels were 0.0287 µg/mL for the 0%, 50.8 µg/mL for the 0.001%, 96.7 µg/mL for the 0.005%, and 340 µg/mL for the 0.02% group. Mean serum PFOA levels were 0.0191 for the 0% and 152 µg/mL for the 0.02% PFOA group. Liver weights were significantly increased in all PFOS and PFOA treatment groups compared to control mice (Qazi, et al. 2009b).

Thymus, spleen, and fat (epididymal) weight were significantly reduced in mice fed 0.02% PFOS or PFOA compared to control mice. Total numbers of thymocytes and splenocytes were significantly reduced in mice fed 0.02% PFOS or PFOA compared to control mice. Mice fed 0.02% PFOS or PFOA also had significantly reduced subpopulations of thymocytes (CD8+, CD4+, CD4+/CD8+, and CD4-/CD8-) and splenocytes (NK1.1+, CD8+, CD4+, CD19+) compared to control mice. Mice treated with PFOS or PFOA had alterations in the thymus and spleen tissue structures when compared to control mice (Qazi, et al. 2009b).

Male wild-type and PPAR-α null 129/Sv mice were also fed 0%, 0.005%, and 0.02% PFOS to examine the role of PPAR-α in the endpoints measured. PPAR-α null mice fed PFOS had significantly increased liver weight and significantly decreased spleen weight (0.02% PFOS only) compared to untreated PPAR-α null mice. Unlike the wild-type mice, no significant changes were observed with epididymal fat weight or thymus weight. Similar to the wild-type mice, PPAR-α null mice fed 0.02% PFOS had a significant reduction in number of total thymocytes and almost all thymocyte subpopulations measured (CD8+, CD4+, CD4+/CD8+ subpopulations were significantly lower, but not CD4-/CD8-). While wild-type mice fed 0.02% PFOS had significant reductions in total number of splenocytes, the observed reduction in PPAR-α null mice did not reach statistical significance. Spleen or thymus cell numbers in wild-type or PPAR-α null mice fed 0.005% PFOS were not reduced. PPAR-α null mice given 0.005% PFOS did have higher, though not significantly, cell numbers than PPAR-α null mice with no PFOS exposure (Qazi, et al. 2009b).

Male C57BL/6 mice were fed 0%, 0.02% PFOS, or 0.02% PFOA for 10 days. The purpose of this study was to determine if PFOS or PFOA affects innate immune cells. As shown previously,

mice fed 0.02% PFOS or PFOA had reduced food intake, increased liver weights, and decreased body, thymus, spleen, and epididymal fat weights (Qazi, et al. 2009b). Both PFOS and PFOA exposure resulted in reduced numbers of circulating total white blood cells and lymphocytes. Only PFOA exposure resulted in significantly lower numbers of circulating neutrophils (Qazi, et al. 2009a).

Number of total cells and number of macrophages (CD11b+) were assessed from the peritoneal cavity, bone marrow, and spleen. While PFOA or PFOS treatment did not change the number of macrophages in the peritoneal cavity, the percentage of macrophages increased compared to the control mice. For PFOS exposed mice, number and percentage of macrophages decreased in the bone marrow. In the mice exposed to PFOA, number of total cells and number and percentage of macrophages decreased compared to the control group. PFOS exposure resulted in a reduction in the number of total cells, but no change to number or percentage of macrophages, in the spleen compared to the control mice. In contrast, PFOA exposure resulted in a reduction of number of total cells and percentage of macrophages in the spleen (which may be due to the reduction of total cells) (Qazi, et al. 2009a).

Peritoneal cavity, bone marrow, and spleen cells were isolated and cultured for 18 hours then cultured with LPS for another 18 hours. TNF-α and IL-6 protein levels were measured in culture supernatant both before and after LPS treatment. PFOS and PFOA alone resulted in significant increases in TNF-α and IL-6 protein produced by peritoneal cavity cells. In mice also treated with LPS, TNF-α and IL-6 protein also significantly increased in mice exposed to PFOS or PFOA compared to control mice. In cultures of bone marrow cells, PFOA or PFOS exposure significantly increased TNF-α and IL-6 protein levels with LPS treatment. Without LPS treatment, only PFOA-exposed mice had a significant increase in TNF-α protein levels. Spleen cell cultures from PFOS- or PFOA-exposed mice produced significantly less TNF-α protein levels compared to the control mice, but no differences were found for IL-6 protein levels. LPS treatment resulted in a significant reduction, for PFOS exposed cells, and a significant increase, for PFOA-exposed cells, of TNF-α protein levels in spleen cell cultures. Only LPS treated PFOA-exposed spleen cells had significantly increased levels of IL-6 protein production (Qazi, et al. 2009a).

Mice were injected with LPS two hours before sacrifice on the 10^{th} day and cells from the peritoneal cavity, bone marrow, and spleen were isolated. TNF-α and IL-6 protein levels were measured in culture supernatants. TNF-α protein levels were significantly increased in peritoneal cavity and bone marrow cell cultures and significantly decreased in spleen cultures in mice exposed to PFOS or PFOA and injected with LPS. IL-6 protein levels were significantly increased in bone marrow cell cultures and significantly decreased in spleen cultures in mice exposed to PFOS or PFOA and injected with LPS. In mice exposed to PFOA, but not PFOS, IL-6 protein levels were significantly increased after LPS injection in peritoneal cavity cell cultures. Serum levels of TNF-α and IL-6 proteins were also measured after 10 days of PFOS or PFOA exposure with or without LPS injection 2 hours prior to blood collection. Without LPS injection, serum IL-6 protein levels significantly increased with PFOS or PFOA exposure, while TNF-α protein levels were similar to the control group. With LPS injection, both serum TNF-α and IL-6 protein levels significantly increased in PFOA- but not PFOS-exposed mice. None of the above results were obtained when mice were fed with diets containing 0.001% PFOA or PFOS. The

authors concluded that additional investigation into PFOS- or PFOA-induced immune system effects is needed (Qazi, et al. 2009a).

Male C57BL/6 mice were gavaged with 0, 5, 20, or 40 mg/kg/day PFOS (potassium salt) for seven days. Body weight, spleen mass, and thymus mass were significantly reduced in the mice given 20 or 40 mg/kg/day. Food intake was decreased in those groups as well. Liver mass was significantly increased in all groups given PFOS (5, 20, or 40 mg/kg/day) while kidney mass was unchanged. Serum corticosterone, measured at the end of the 7 day exposure, was significantly increased in the mice given 20 or 40 mg/kg/day compared to the control group (Zheng, et al. 2009).

Number of cells in the spleen and thymus were significantly reduced in the mice given 20 or 40 mg/kg/day compared to the control group. Certain subpopulations in the spleen and thymus significantly changed after PFOS exposure. Numbers of CD4+, CD8+, and B220+ in the spleen were significantly reduced in the mice given 20 or 40 mg/kg/day compared to the control group. Numbers of CD4-/CD8- cells in the spleen were significantly reduced in only the mice given 40 mg/kg/day. Numbers of CD4+, CD8+, CD4+/CD8+, and CD4-/CD8- in the thymus were significantly reduced in mice given 20 or 40 mg/kg/day compared to the control group (Zheng, et al. 2009).

Splenic natural killer cell activity was measured using a lactate dehydrogenase release assay. Mice given 20 or 40 mg/kg/day had significantly reduced natural killer cell activity compared to control mice. Splenic cell proliferation was measured using a viability assay (the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide [MTT] assay). Splenic T cell proliferation was significantly reduced in all PFOS treatment groups (5, 20, and 40 mg/kg/day) compared to the control mice. Splenic B cell activity was significantly reduced in mice given 20 or 40 mg/kg/day compared to the control group (Zheng, et al. 2009).

Plaque-forming cell response (antibodies against sheep red blood cells) was measured in splenocytes. Sheep red blood cell-specific IgM was significantly reduced in all groups given PFOS (5, 20, and 40 mg/kg/day) compared to the control group. The authors note that, while some immune system effects in the mice given 20 and 40 mg/kg/day could be due to stress (increased serum corticosterone in the 20 and 40 mg/kg/day groups), T cell proliferation and antibodies to sheep red blood cells were significantly reduced in the mice given 5 mg/kg/day. The authors recommended additional studies to investigate the immunotoxic effects of PFOS. (Zheng, et al. 2009)

Male C57BL/6 mice were gavaged with 0, 5, or 20 mg/kg/day PFOS for seven days. There were significant reductions in body weight, food intake, splenic and thymic indicies for the mice given 20 mg/kg/day. Spleen and thymus indices were calculated by dividing organ weight (in grams) by body weight (in grams), then multiplying by 100. Both PFOS exposure groups (5 and 20 mg/kg/day) had significantly increased hepatic indicies compared to the control group. PFOS exposure did not alter kidney mass. Serum corticosterone levels were significantly increased in the mice given 20 mg/kg/day compared to the control group (Zheng, et al. 2011).

Splenocytes were isolated and cultured for 48 hours. Supernatants were collected and cytokines were measured. IFNγ protein levels were significantly reduced in splenocyte cultures from mice given 20 mg/kg/day, while IL-4 protein levels were significantly increased in cultures from mice given 5 or 20 mg/kg day compared to cultures from control mice. Mice given 20 mg/kg/day had a reduced number of cells with IL-2 protein production compared to the control group, but there were no differences in number of cells with IL-10 protein production (Zheng, et al. 2011).

Serum levels of total (non-specific) IgM were significantly reduced in mice given 5 or 20 mg/kg/day PFOS compared to the control. Serum levels of total (non-specific) IgG were significantly higher only in mice given the 5 mg/kg/day compared to the control group. As serum corticosterone levels were significantly increased in mice given 20 mg/kg/day, immune effects seen at this dose may have been due to an increase in the mice's stress response. However, immune system effects were observed in mice given 5 mg/kg/day, a dose that did not alter serum corticosterone levels. The authors noted that lower doses are needed to further investigate immune system effects from PFOS exposure (Zheng, et al. 2011).

In vitro studies

Human whole blood was used to assess the effects of PFOS and PFOA on immune parameters. Plasma levels of PFOA, PFOS, PFHxS, PFHpS, PFNA, and PFDA were measured. Every PFC was detected in plasma from all eleven donors. The mean levels of PFOS and PFOA were 4.04 and 3.3 ppb, respectively. Neither PFOS nor PFOA, added to diluted whole blood cultures at 1, 10, or 100 µg/mL, altered basal levels of TNF-α or IL-6 compared to control groups. After pretreatment with lipopolysaccharide (LPS) for 4 or 24 hours, 100 µg/mL PFOS significantly reduced the levels of TNF-α protein produced. Levels of TNF-α induced by LPS-treated cultures significantly correlated to levels of PFOA measured in the plasma. Levels of IL-6 induced by LPS-treated cultures significantly correlated to levels of PFOS and PFOA measured in the plasma. PFOA (100 µg/mL) caused an increase in vitamin D-induced differentiation of HL-60 (a human promonocytic line) to monocytes. Exposure to PFOS (100 µg/mL) significantly reduced natural killer cell activity in peripheral blood mononuclear cells isolated from the donors. The authors noted that the number of donors was small, and that with larger sample numbers, effects at lower levels of PFOS or PFOA could possibly be detectable (Brieger, et al. 2011).

PFCs (PFOS or PFOA) were added to THP-1 cells, a human monocyte line, and diluted whole blood cultures. Levels of LPS induced cytokines were reduced in the presence of the PFOS and PFOA in whole blood cultures and THP-1 cells. However, PFOS and PFOA differentially affected both the whole blood cultures and the THP-1 cells. Levels of PFOS and PFOA used in these experiments were not cytotoxic. Diluted whole blood cultures were co-treated with LPS and PFOS or PFOA. In the diluted whole blood cultures, both PFOS (0.1, 1, and 10 µg/mL) and PFOA (1 and 10 µg/mL) treatment reduced TNF-α protein levels. IL-6 protein levels were only reduced in cells treated with PFOS (0.1, 1, and 10 µg/mL), while IL-8 protein levels were not reduced by either PFOS or PFOA. In phytohemagglutinin (PHA)-treated whole blood cultures, PFOS (0.1, 1, and 10 µg/mL) reduced the levels of IL-4, IL-10, and IFN-γ protein. Only in the highest PFOA treatment group (10 µg/mL) were levels of IL-4 and IL-10 proteins reduced (Corsini, et al. 2011).

In THP-1 cells, both LPS-induced TNF-α and IL-8 protein levels were reduced after treatment with PFOA (for TNF-α, the 10 and 100 µg/mL groups; for IL-8, only the 100 µg/mL group). PFOS (at 1, 10, and 100 µg/mL) reduced levels of LPS-induced TNF-α and IL-8 protein. Treatment with both PFOS and PFOA reduced LPS-induced TNF-α and IL-8 mRNA levels. Both PFCs interfered with LPS-induced NF-κB (a transcription factor) activation in THP-1 cells. However, PFOS prevented IκB degradation, possibly preventing activation of NF-κB, and reduced NF-κB p65 binding to DNA. LPS-induced TNF-α and IL-8 protein levels reduced by PFOA, but not PFOS, required PPARα activity in THP-1 cells (Corsini, et al. 2011).

Corsini et al. (2012) examined the effects of PFBS, PFOA, PFOS, PFOSA, PFDA, and a fluorotelomer on LPS-induced cytokines in diluted whole blood cultures and THP-1 cells. LPS-induced TNF-α protein was reduced in the whole blood cultures and THP-1 cells by PFBS, PFOSA, PFOS, PFOA, PFDA, and the fluorotelomer. The amount of the reduction of TNF-α protein was different in whole blood from males and females for some of the PFCs. PFOS, PFBS, and PFDA were able to prevent IκB degradation in THP-1 cells. All of the PFCs tested were able to prevent LPS-induced NF-κB p65 phosphorylation at a specific amino acid (necessary for optimal NF-κB-driven transcription). When the role of PPARα was investigated, only PFOA was found to activate PPARα in THP-1 cells (Corsini, et al. 2012).

The neurotoxicity of PFOS, PFOA, PFOSA, and PFBS were examined in a rat cell line. PC-12 cells are from a male rat adrenal gland tumor and can be differentiated into neuronal cells. Undifferentiated PC-12 cells had a significant reduction in DNA synthesis after 24 hours of exposure to PFOS (10, 50, 100, 250 µM), PFOA (250 µM), or PFOSA (10, 50, 100, 250 µM). DNA content, an index of cell number, was significantly reduced in undifferentiated cells treated with 250 µM PFOSA for 24 hours. Lipid peroxidation, a measure of oxidative stress, was significantly increased by PFOS (10, 50, 100, 250 µM), PFOA (10 µM), or PFOSA (10, 50, 100, 250 µM) in undifferentiated cells treated for 24 hours. Only undifferentiated cells treated with PFOA (100, 250 µM) or PFOSA (250 µM) for 24 hours had significantly decreased cell viability (Slotkin, et al. 2008).

Differentiated PC-12 cells were treated with the PFCs. DNA content was significantly reduced in cells treated with PFOA (250 µM), or PFOSA (100, 250 µM) after six days of exposure. Cell size, as measured by total protein/DNA content, was significantly increased in cells treated with PFBS (50, 100, 250 µM) for six days. Lipid peroxidation was significantly increased in differentiated cells treated with PFOS (50, 100, 250 µM), PFOA (10, 250 µM), PFOSA (50, 100, 250 µM), or PFBS (50, 100, 250 µM) for four days. Cell viability was significantly reduced in differentiated cells treated with PFOS (250 µM) or PFOSA (250 µM) for four days. PFOS (10, 50, 100, 250 µM) or PFOA (10 µM) treatment significantly reduced the ratio of activity of two neurotransmitters after six days of exposure. PFOSA treatment (100 µM) significantly increased the ratio after six days of exposure. PFBS treatment did not alter the ratio, but significantly reduced the activity of each neurotransmitter. These results indicate that PFCs are able to affect the type of neuronal cell that the PC-12 cells differentiated into as well as affecting their viability (Slotkin, et al. 2008).