PETITION TO SET A DRINKING WATER STANDARD MAXIMUM CONTAMINENT LEVEL FOR PFOA NOT TO EXCEED 6 PARTS PER TRILLION

I. PETITIONER INFORMATION

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II. PETITION INFORMATION

A. The petitioner requests the Environmental Quality Board to amend a regulation (citation 25 Pa. Code § 93.9e).

The Delaware Riverkeeper Network (DRN) requests that the Environmental Quality Board (EQB) and the Department of Environmental Protection (Department) set a maximum contaminant level for Perfluorooctanoic Acid (PFOA) not to exceed 6 parts per trillion[1] (ppt).

B. Why is the petitioner requesting this action from the Board? (Describe problems encountered under current regulations and the changes being recommended to address the problems. State factual and legal contentions and include supporting documentation that establishes a clear justification for the requested action.)

Perfluorinated compounds (PFC), like PFOA, are currently unregulated at the federal level, one of thousands of chemicals in use without safe drinking water standards and, in many cases, without any requirement for monitoring for their presence. These unregulated chemicals can enter the environment and show up in water supplies without being detected. Those with

[1] Note: ppt is equivalent to ng/L (nanogram per liter).

toxic properties can expose people to dangerous levels that can result in disease and environmental degradation. The lack of federal regulation of PFCs means that people have been exposed to PFCs in some cases for years without knowing the risks.

There is no federal safe drinking water standard for any PFC and Pennsylvania has not established a standard. Some states have issued guidance levels or adopted maximum contaminant levels; New Jersey is in the process of adopting a safe drinking water standard.

DRN has been working on the problems posed by the presence of perfluorinated compounds in our local environment since 2005 when our staff collected tap water samples in the neighborhoods close to DuPont's Chambers Works facility in Deepwater, New Jersey on the Delaware River. We suspected that there may be a problem because of news reports about a lawsuit that had been brought in West Virginia against DuPont for releasing PFOA into the environment there. Our sampling revealed the presence of PFOA in the drinking water being used by people in the local community near DuPont's Chambers Works plant. We notified the residents and filed the information with New Jersey Department of Environmental Protection (NJDEP), setting off alarm bells and a chain of events that eventually led to NJDEP investigating the occurrence of perfluorinated compounds throughout the state and the issuance of a guidance level of 40 ppt for PFOA in 2007. Since then, after years of scientific study and research, New Jersey Drinking Water Quality Institute (NJDQWI) has recommended that New Jersey adopt a maximum contaminant level (MCL) for PFOA of 14 ppt.

EPA issued short term provisional health advisories for PFOA at 400 ppt and Perfluorooctanesulfonic acid (PFOS), another PFC, at 200 ppt in 2009. These advisory levels were based on short term exposure (5 days to 20 days, approximately) and did not mandate that water suppliers remove PFOA or PFOS from drinking water; it was just an advisory, not a federal enforceable standard. Such a short term advisory is not a valid level to use as a measurement of what is safe for drinking water on an ongoing basis. As a result of the publicity surrounding the disclosure of data from USEPA's Unregulated Contaminant Monitoring Rule 3 (UCMR3) water supply sampling, many locations across the nation took action as a result of the discovery of the presence of PFOA or other PFCs in their water supply. Most turned first to EPA for guidance. After much controversy and confusion at the federal and state levels, in 2016 EPA issued a lifetime PFOA and PFOS health advisory level (HAL) of 70 ppt when found singly or a combined total of 70 ppt when both are found. The HAL, while designed to address long term exposure, is not mandatory and does not require removal of PFOA and PFOS from drinking water. But it is being used by water suppliers and, in some cases, by those responsible for releasing the compounds into the environment, such as military bases, as an enforceable limit for drinking water.

As demonstrated below, this HAL limit has been shown to be ineffective at protecting the public health and a more protective standard not to exceed 6 ppt must be set for PFOA.

1. <u>Legal Standard</u>

The Department has broad authority to protect the drinking water of its citizens from emerging contaminants of concern like PFCs or PFOA. As outlined within this petition, because

PFOA in drinking water creates a substantial health risk to citizens of the Commonwealth, the Department must set a protective maximum contaminant level (MCL) for PFOA not to exceed 6 ppt.

Importantly, the Pennsylvania Constitution recognizes that the people of the Commonwealth have a constitutional right to pure drinking water. Namely, Article I Section 27 of the Pennsylvania Constitution states:

> The people have a right to clean air, pure water, and to the preservation of the natural, scenic, historic and esthetic values of the environment. Pennsylvania's public natural resources are the common property of all the people, including generations yet to come. As trustee of these resources, the Commonwealth shall conserve and maintain them for the benefit of all the people.[2]

Article I Section 27 requires the state government to ensure the preservation of the state's natural resources, including the provision to safe drinking water. This means the state government is responsible for protecting Pennsylvania's environment on behalf of its citizens.

The General Assembly has responded by passing the Pennsylvania Safe Drinking Water Act (SDWA) and declaring that "an adequate supply of safe, pure drinking water is essential to the public health, safety and welfare and that such a supply is an important natural resource in the economic development of the Commonwealth."[3] The SDWA also recognizes that the people of the Commonwealth have a constitutional right to pure drinking water.[4]

The SDWA created a state program to establish drinking water standards and to implement and enforce those standards to ensure the supply of safe drinking water to the public.[5] The Commonwealth also was required to develop a process for implementing plans to provide safe drinking water in times of emergencies and provide public notice of potentially hazardous conditions that may exist in the water supply.[6]

The Pennsylvania Department of Environmental Protection (Department) is tasked with administrating a program to accomplish the objectives of the SDWA.[7] One power and duty of the Department involves the establishment of a maximum contaminant level (MCL) related to drinking water quality standards.[8] MCLs must be no less stringent than those promulgated under the Federal act and regulations.[9] However, the MCLs and treatment technique requirements may be more stringent than those promulgated under the federal act, and the Department has the

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[2] Pa. Const. Art. I, § 27.
[3] 35 Pa. Stat. § 721.2(a)(1).
[4] 35 Pa. Stat. § 721.2(b).
[5] 35 Pa. Stat. § 721.2(b)(1).
[6] 35 Pa. Stat. § 721.2(b)(2) and (b)(3).
[7] 35 Pa. Stat. § 721.5.
[8] 35 Pa. Stat. § 721.4(a).
[9] 35 Pa. Stat. § 721.4(a).

power to adopt an MCL for an unregulated contaminant, like PFOA, "***on a case-by-case basis for a public water system in which an unregulated contaminant creates a health risk to the users of the public water system.***"[10]

Notably, the EQB has clear and expansive authority to adopt "a maximum contaminant levels or treatment technique requirements for any contaminant that a maximum contaminant level or treatment technique requirement has not been promulgated under the national primary and secondary drinking water regulations."[11] The MCL development process is intended to create "standards limiting the concentration of contaminants in public drinking water to protect the consumer from possible short-term and long-term adverse health effects. Contaminants are usually selected for regulation based on potential health risks and their occurrence or potential occurrence in drinking water."[12]

Furthermore, if the Department has reason to believe a contaminant is present in the public water system and creates a health risk to the users of the public water system, the Department "may require a public water supplier to conduct special monitoring for an unregulated contaminant."[13] The Department, and its agents and employees, "may also conduct inspections of public water systems and related activities, whenever a person presents information to the Department which gives the Department reason to believe that a condition exists which may threaten the public health, safety or welfare or the environment."[14]

PFOA has been found in many of the water supply systems in Pennsylvania at alarming levels. Because the consumption of PFOA results in significant adverse health consequences, Pennsylvania must protect its citizens and set an MCL for PFOA not to exceed 6 ppt. A failure to do so would be an abrogation of its duties under the SDWA and the Pennsylvania Constitution.

2. <u>PFOA Background and Health Effects</u>

PFOA is part of a larger group of chemicals referred to as perfluorinated compounds. PFOA is not found naturally in the environment, yet it is ubiquitously present. This is because PFCs were widely used in the production of goods from the 1950's until recently by companies like DuPont and 3M to make products more stain-resistant, waterproof and/or nonstick (e.g. Teflon). In particular, PFOA been used in the following products: cookware, carpets, clothing, fabrics for furniture, paper packaging for food, and other materials. It is also been used in firefighting foams and in a number of industrial processes.[15]

Problematically, PFOA is quite resistant to biodegradation, which contributes to its widespread presence. Blood studies show the presence of PFCs in the blood of 96% of people in

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[10] 25 Pa. Code § 109.203 (emphasis added).
[11] 35 Pa. Stat. § 721.4; see also 25 Pa. Code § 109.203.
[12] Commonwealth of Pennsylvania: Bureau of Water Standards and Facility Regulation, *Citizen's Guide to Volatile Synthetic Organic Chemicals in Drinking Water*, http://pa-montgomerycounty.civicplus.com/DocumentCenter/View/916, (last updated August 2008).
[13] 25 Pa. Code § 109.302(c).
[14] 25 Pa. Code § 109.6(d).
[15] PADEP Fact Sheet available here: http://files.dep.state.pa.us/Water/DrinkingWater/Perfluorinated%20Chemicals/PFC%20Info%20Sheet.pdf

the United States, at approximately 4 ng/mL (nanogram/milliliter). It has even been found in polar bears in the Arctic. Its durable nature also causes the chemical to build up in the human body and is difficult to excrete, the levels in an individual's blood is about 105 times the amount in their drinking water. According to the U.S. Environmental Protection Agency (EPA): "Because PFOA can remain in the body for a long time, drinking water that contains PFOA can, over time, produce concentrations of PFOA in blood serum that are higher than the concentrations of PFOA in the water itself."[16] This magnification of PFOA in the blood can have serious health effects.

The scientific literature and the data gleaned from health studies show that PFCs, and PFOA, are linked to serious disease, including cancers, and detrimental human health conditions.[17] Fetuses, infants, and children are the most vulnerable populations due to negative developmental impacts, which also affect pregnant women, women of child bearing age and women who are breastfeeding. Chief among the new bodies of data and findings available for PFOA are those from the court-ordered C8 Health Panel and the C8 Health Project in West Virginia, related to the Dupont facility there. Among the conclusions of this multi-year study of human subjects, their blood and scientific reports, it was found that PFOA is correlated with Kidney Cancer, Testicular Cancer, Thyroid Disease, High Cholesterol, Pregnancy-Induced Hypertension/Preeclampsia, and Ulcerative Colitis.[18] In addition to the six diseases with probable links, the study also verifies probable links to decreased birth weight and decreased response to vaccines. A report reviewing all of the studies on low birth weight concluded that PFOA does reduce human birth weight. [19]

According to the New Jersey Drinking Water Quality Institute's recent report:

> Human exposure to PFOA has also been associated with increased risk of cancer, including increased risk of kidney and testicular cancer in communities with contaminated drinking water after adjustment for smoking and other relevant factors. These studies accounted for smoking history and other relevant factors. In 2006, the U.S. EPA Science Advisory Board described PFOA as "likely to be carcinogenic to humans." based on the criteria provided in U.S. EPA cancer risk assessment guidance. More recently, the International Agency for Cancer Research concluded that PFOA is possibly carcinogenic to humans. In 2016, the U.S. EPA Office of Water described it as having suggestive evidence of carcinogenic potential.[20]

Sources of exposure to PFOA and/or its precursors include drinking water, food and food packaging, treated fabrics, protective sprays and waxes, cosmetics and personal care products,

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[16] EPA-SAB-06-006 SAB Review of EPA's Draft Risk Assessment of Potential Human Health Effects Associated with PFOA and Its Salts
[17] https://www.epa.gov/sites/production/files/2015-09/ucmr-3-occurrence-data.zip
[18] http://www.c8sciencepanel.org/newsletter10.html
[19] http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4181929/pdf/ehp.1307893.pdf
[20] NJDWQI (2016). Health-based Maximum Contaminant Level Support Document: Perfluorooctanoic Acid (PFOA). New Jersey Drinking Water Quality Institute Health Effects Subcommittee, Public Review Draft, June 27, 2016. Retrieved from http://www.nj.gov/dep/watersupply/pdf/pfoa-hb--mcl-public-review-draftwithappendices.pdf (Enclosed as **Attachment 1**), p. 8.

house dust, and inhalation of indoor and outdoor air.[21] The contribution of ingested drinking water to total exposure from all sources (e.g. diet, consumer products, etc.) is dependent on the concentration of PFOA in the drinking water, and relatively low concentrations in water substantially increases the human body burden.[22]

Furthermore, exposures to PFOA may be higher in young children than in older individuals because of age specific behaviors such as greater drinking water and food consumption on a body weight basis, hand-to-mouth behavior resulting in greater ingestion of house dust, and more time spent on floors where treated carpets are found.[23]

In 2006, the eight major U.S. producers of PFOA voluntarily agreed to reduce emissions and product content of PFOA and related substances, including precursors of PFOA, on a global basis by 95% by 2010 and to work towards elimination of these substances by 2015.[24] According to the U.S. EPA, reports submitted by the participating companies in 2013 and 2014 indicated that they were on track to achieve the goal of phasing out these chemicals by the end of 2015. Nonetheless, PFOA remains ubiquitously present in our environment and presents a serious public health threat as it is linked to serious disease, including cancers, and detrimental human health conditions.[25]

3. PFC Presence in Pennsylvania

PFOA is significantly elevated in many Bucks and Montgomery County water supplies at levels that far exceed EPA's HAL and the more protective 6 ppt standard that we advocate for in this petition. Other locations in Pennsylvania have PFOA water supply contamination as well. Furthermore, if the Commonwealth undertook appropriate study of likely sources of PFOA, it is highly likely that it would identify scores of other locations with similar contamination issues.

a. PFCs in PA: Initial Findings

The PFOS and PFOA levels found in public wells in Bucks County and Montgomery Counties were among the ten highest sampling results in the nation.[26] Sampling done in Warminster, Warrington and Horsham Townships reported that the groundwater that feeds public and private wells for tens of thousands of people in the area was found to be among the worst in the nation, most all in the vicinity of the former Naval Air Station Joint Reserve Base at Willow Grove, the current Horsham Air Guard Station in Horsham and the site of the former Naval Air Warfare Center in Warminster. In Doylestown Township where testing was done for UCMR3 as a smaller representative public water supply, the Municipal Authority had the sixth highest PFOA sample report in the nation but to date the source is not publicly known.[27] Sampling of drinking water in other parts of the state has also revealed contamination problems

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[21] *Id*. at 4.
[22] *Id*. at 5.
[23] *Id*.
[24] *Id*.
[25] *Id*. at 22.
[26] https://www.epa.gov/sites/production/files/2015-09/ucmr-3-occurrence-data.zip
[27] Ibid.

and there could well be more places, as yet undetected, where people are currently drinking contaminated water containing PFCs.

Sampling for the UCMR3 2015 report that revealed the presence of PFCs in Pennsylvania at levels above specific reporting levels (for PFOA UCMR3 reporting level was 20 ppt; for PFOS it was 40 ppt), all in parts per trillion or ppt[28] include:

- Aqua PA, Bristol: PFOA 20 and 26
- Warminster Municipal Authority Well 2: PFOA 34, PFOS 57
- Warminster Municipal Authority Well 5: PFOA 23
- Warminster Municipal Authority Well 9: PFOA 20
- Warminster Municipal Authority Well 10: PFOA 89, PFOS 190
- Warminster Municipal Authority Well 13: PFOA 122, PFOS 160
- Warminster Municipal Authority Well 14: PFOA 25, PFOS 65
- Warminster Municipal Authority Well 26: PFOA 350, PFOS 1090
- Warrington Township Water and Sewer Wells 1, 2, and 6 treatment plant: PFOA 120, PFOS 670
- Warrington Township Water and Sewer Well 3: PFOA 20, PFOS 62
- Warrington Township Water and Sewer Well 9: PFOA 29
- Quakertown Borough Well 13: PFNA 35 and 32
- Doylestown Municipal Utilities Authority Cross Keys: PFOA 210 and 130, PFNA 26
- Ambler Borough Water Department: PFNA 29
- Horsham Water and Sewer Authority Well 10: PFOA 26, PFOS 45
- Horsham Water and Sewer Authority Well 17: PFOA 26, PFOS 97
- Horsham Water and Sewer Authority Well 21: PFOS 140
- Horsham Water and Sewer Authority Well 26: PFOA 290, PFOS 700

[28] DRN is only reporting here results for PFOA, PFOS and PFNA; some wells show presence of other PFCs; all data available at UCMR3 occurrence text file for method 537 at https://www.epa.gov/sites/production/files/2015-09/ucmr-3-occurrence-data-by-method-classification.zip

- Horsham Water and Sewer Authority Well 40: PFOA 63, PFOS 1000

- EMMAUS Borough Public Water: PFNA 22

- United Water PA Airport (PWS ID# PA7220015, Harrisburg): PFOA 38, PFOS 363, PFNA 47

Many of these samples exceed EPA's HAL for PFOA of 70 ppt, and all samples exceed New Jersey's proposed PFOA MCL of 14 ppt and the more protective 6 ppt MCL we are requesting.

b. Current Conditions in Bucks and Montgomery Counties

The former Naval Air Station Joint Reserve Base at Willow Grove, the current Horsham Air Guard Station in Horsham and the site of the former Naval Air Warfare Center in Warminster are the primary sources of PFC contamination in Bucks and Montgomery Counties. The military has been using firefighting foams for decades at these locations.

The Naval Air Warfare Center in Warminster, Bucks County was designated for closure in 1995 by the Defense Base Closure and Realignment Commission (BRAC) program and now operates under BRAC as the Naval Air Development Center. It is classified as a CERCLA National Priority List (NPL) "Superfund" site due to contamination of area groundwater, primarily trichloroethylene (TCE), tetrachloroethylene (PCE) and carbon tetrachloride documented in 1989.[29] A treatment system is operating on the site that removes Volatile Organic Compounds from the groundwater under BRAC's supervision. The site is 824 acres and is located in Warminster Township, Ivyland Borough and Northampton Township. The area has used groundwater for both public and private water supplies. A groundwater analysis is being conducted by the Navy to assess where the PFC pollution plume is and where it is going, according to the Willie Lin of BRAC, but no results are available publicly at this time.[30]

The Horsham Air Guard Station opened in 2011 after the 2006 base closure of the adjacent former Willow Grove Naval Air Station Joint Reserve Base. It is located on approximately 207 acres in Horsham Township, Montgomery County. In the 1990s, the Willow Grove Naval Air Station Joint Reserve Base investigated groundwater contamination from volatile organic compounds (VOC's) in the aquifer; cleanup was carried out of underground storage tank leaks. The site was classified as a Superfund site in 1995. Site remediation needs and the impact of proposed site changes regarding water resources, air quality hazardous materials and solid and hazardous waste, utilities and other affected environmental aspects are still being assessed.[31]

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[29] https://cumulis.epa.gov/supercpad/cursites/csitinfo.cfm?id=0302466
[30] Statement of Willie Lee, US NAVY BRAC as per Tracy Carluccio, DRN, at Northampton Township Board of Supervisors meeting, 1.25.2017.
[31] http://horshamlibrary.org/docview.aspx?docid=28289

PFCs were discovered at these bases during the 2012 Five Year Superfund Review of the Naval Air Warfare Center in Warminster, according to the EPA.[32] The public subsequently found out about PFOA and PFOS in their drinking water through the UCMR3 reporting from 2013 to 2015. The Navy shut down two Warminster water wells as early as 2014 because of PFCs.

At the commencement of 2017, 22 public drinking water wells and 230 private drinking water wells have been shut down by a variety of agencies because they exceed the 70 ppt EPA HAL in Warminster, Warrington and Horsham Townships. As testing is completed in more places, more wells report contamination by PFOA and/or PFOS, some at concentrations that exceed the EPA HAL and some that are under that level.

Since the UCMR3 sampling, additional water testing in the region around the military bases has revealed PFOA and PFOS contamination in more locations. The most recent count is seventeen communities in Bucks and Montgomery Counties where some level of PFOA/PFOS has been discovered. The seventeen communities in Bucks and Montgomery Counties with water wells that have been found to contain some concentration of PFOA and/or PFOS at levels leading to the shutdown of the well or at levels lower than the EPA HAL include:

- Horsham
- Warminster
- Warrington
- Abington
- Bristol Township
- Bristol Borough
- Buckingham Township
- Doylestown Borough
- Northampton Township
- East Rockhill Township
- Upper Southampton Township
- Doylestown Twp
- Ivyland
- Plumstead Township
- Upper Dublin
- Warwick
- Hatboro

Some communities in the region are provided drinking water by municipal authorities, some are served by regional water companies, and some people have private individually owned wells. Water systems proximate to the military bases are testing the water they provide for PFOA and PFOS even though there is no maximum contaminant level in force, presumably under PADEP guidance. For instance, Aqua America serves multiple municipalities in Bucks and Montgomery Counties and has set up a testing protocol and a web page, making data available to the public.[33] The company has found PFCs in some of their water sources and their data shows that concentrations change in their wells over time. PFOA and PFOS has also been found in surface water the company uses, most notably high concentrations of PFOA and PFOS at their Neshaminy Creek intake.[34] Aqua America is using the EPA HAL to measure if the water

[32] Ibid.
[33] http://www.waterfacts.com/
[34] http://www.theintell.com/news/horsham-pfos/pfcs-increasing-in-neshaminy-creek-widespread-in-northampton-wells/article_1813c26a-7f4f-11e6-8aaa-1fe2604163a6.html

is safe.[35] In some instances, water companies and municipal authorities are blending water that contains some level of PFCs with cleaner water to dilute the concentration to below the EPA HAL.

The Navy is employing the EPA HAL as if it were a safe drinking water level and only taking action if PFOA and PFOS concentrations exceed 70 ppt. The Navy is replacing or treating water supplies in some municipalities that have contaminated wells and are connecting some private wells with systems that meet the EPA HAL. The Navy has committed publicly to pay for the replacement or treatment of all water supplies that exceed the EPA HAL (70 ppt) if the Navy concludes that the water was contaminated by their facility. So far, $19 million has been committed. However, this does not cover the cost of remediating all contamination and sampling has not been done of all water supplies that could have been contaminated.

Action by the Navy has not addressed all of the contamination issues; indeed actions have varied greatly from municipality to municipality. For instance, the Navy has sampled some but not all private water wells in the region. Where the Navy's sampling of private wells show an exceedance of the EPA HAL, the Navy is supplying bottled water while permanent connections are being arranged. However, where sampling has not been done by the Navy, the private well owner is on their own to test their water and, if there are PFCs, the Navy will not commit to taking any action until they are ready and will not commit to reimbursing homeowners who install treatment systems.[36] This leaves private well owners left to buy point-of-entry treatment systems on their own at a cost of thousands of dollars.

The delivery of contamination-free water is not uniform and some municipalities have set different policies that are resulting in different outcomes in terms of the presence of PFCs. For instance, Warminster, Warrington and Horsham Townships are employing plans to remove PFOA and PFOS to a concentration of "non-detect" due to zero tolerance for any PFCs in their water supplies. The Navy's use of the EPA HAL does not align with the municipalities' plans and excludes many water wells and systems that contain PFC contamination from being acted on by the Navy. Municipalities do not have the funds to address all of the contamination. The municipalities' "non-detect" policy is being adopted by municipal officials and supported by some state and federal representatives from the region seeking protection of residents from exposure to PFOA and PFOS due to concern for an increased risk of developing disease and adverse health impacts, especially for vulnerable populations such as infants, children, and women of childbearing age. It is being carried out despite an expected substantial rate hike for customers in some municipalities such as Horsham.[37] Warminster Township has estimated their costs could go as high as $26 million.[38]

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[35] Ibid.

[36] Statement of Willie Lee, US NAVY BRAC as per Tracy Carluccio, DRN, at Northampton Township Board of Supervisors meeting, 1.25.2017.

[37] http://www.theintell.com/news/horsham-pfos/horsham-residents-to-pay-surcharge-for-additional-north-wales-water/article_e129c946-712c-11e6-8c8c-bf8764ec9b59.html

[38] http://www.theintell.com/news/horsham-pfos/state-rep-kathy-watson-hints-at-upcoming-pfc-bill/article_7c904d36-cdf5-11e6-bcd1-37acef07f06e.html

In addition, the Horsham Air Guard Station is acting independently from the Navy BRAC, with uneven results. For instance, it was stated by State representative Kathy Watson that military officials said that Horsham Air Guard Station is still contributing PFC contaminated flow to the Little Neshaminy Creek and Park Creek.[39] Both creeks flow to the Neshaminy Creek, the source of drinking water for downstream residents, including Aqua America that wheels water to several locations not obviously local to the military bases.[40] The result of the lack of a comprehensive plan for addressing the problem has led to some residents receiving more protection than others and the lack of a uniform and thorough military response shouldering the cleanup costs has meant that much of the burden of increased costs is being carried by the public and some communities have a greater burden than others.

In Horsham Township, for instance, granulated activated carbon (GAC) treatment to remove PFCs is being installed and water that has any level of PFOA or PFOS is being replaced with water that has no detection of the compounds for both public and private water users.[41] A $10 million grant will come from the Pennsylvania Infrastructure Investment Authority to help defray some of the costs of attaining PFC-free water for township residents.[42] The total costs, however, are not yet tallied. Yet, other communities have not adopted a "non-detect" policy and are only applying the EPA HAL as a trigger for action. This is resulting in uneven protection for residents in the region.

Many water systems may have PFOA and PFOS in the water they provide to consumers but they do not know it because the water is not being sampled or is not being tested to or reported at a low enough level to find where it is occurring. This is especially a problem for areas where there may have been releases of PFCs to the environment from unknown sources. This is the case in Doylestown Township, Bucks County. Doylestown Township was included in the UCMR3 sampling from 2013-2015, which is when the contamination was first discovered but no action was taken until the EPA HAL was issued.

After the EPA issued its HAL, the Doylestown Township Cross Keys well was shut down by the Township's Municipal Authority in May 2016.[43] The pollution source is unknown but the well is located near the Doylestown Airport and a site that handled waste for a laboratory. See more information on this occurrence later in this report.

c. PFCs in Other Pennsylvania Locations

Elsewhere in Pennsylvania, the Susquehanna Area Regional Airport Authority (SARAA) sells water to Suez[44], formerly known as United Water Pennsylvania. SARAA shut down three wells in June 2014 after being notified of "elevated" levels of PFOS. In 2014, EPA's provisional

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[39] Ibid.
[40] http://www.theintell.com/news/horsham-pfos/pfcs-increasing-in-neshaminy-creek-widespread-in-northampton-wells/article_1813c26a-7f4f-11e6-8aaa-1fe2604163a6.html
[41] https://www.horshamwater-sewer.com/news/short-term-plan-progress
[42] http://www.theintell.com/news/horsham-pfos/rep-todd-stephens-confirms-million-for-pfc-clean-up/article_ddec8bfc-e34f-11e6-835c-f7387628e06d.html
[43] http://bit.ly/2kCtcje
[44] http://www.mysuezwater.com/about-us

health advisory was 200 ppt and the SARAA sampling showed the concentration to be from 400 to 1100 ppt, which triggered the shutdown. The areas that get their drinking water from those wells were reportedly Lower Swatara Township and Highspire. That connection served about 2,700 customers, including Penn State's Capitol Campus, and may also have provided water to Harrisburg International Airport (HIA) and commercial customers north of the Airport but the precise number of customers that have been exposed to the PFC-contaminated water is unclear. As per EPA's UCMR3 Report, other PFCs were found in at least one well at the Airport: PFOA at 38 ppt and PFNA at 47 ppt. Those results indicate the EPA HAL combined concentration for PFOA and PFOS of 70 ppt issued in 2016 was exceeded based on the 2014-2015 data available. The 2015 UCMR3 sampling documented a PFOS concentration of 363 ppt.

In response to the UCMR3 sampling results, PADEP and SARAA changed pumping regimes to use water that was below the provisional HAL for PFOS in 2014 (200 ppt) and United Water closed the interconnection with SARAA.[45] A plan for long-term treatment to remove PFCs is in process, as reported by EPA.[46]

The source of the PFC contamination is not settled but the EPA Superfund Report for Middletown Air Field sheds light on the history of groundwater contamination at the Air Field, operated by the United States Air Force (USAF).[47] The Harrisburg International Airport (HIA) occupies part of the Middletown Air Field. In 1983 when groundwater contamination by chlorinated solvents was discovered in wells at HIA, the Pennsylvania Department of Transportation owned the airport. The state of Pennsylvania, represented by PADEP, and the USAF were involved in the cleanup of the Superfund site in the 1980's and 1990's. The site was removed from the Superfund National Priorities List in 1997. Groundwater continued to be supplied through water wells at HIA through United Water, now Suez, until the three wells were shut down and pumping changes went into effect in 2014.

Since 2014, sampling of some private wells in the Harrisburg area has been conducted under the federal Hazardous Sites Cleanup Act of 1988.[48] Currently, in instances when the wells' PFC level exceeded the EPA HAL, alternative drinking water supplies have been provided, according to PADEP's website.[49] Sampling was done in Middletown Borough as well but the data for these and other wells is not available publicly except that EPA states that the concentrations did not exceed the EPA HAL.[50]

Other locations where PFCs found in exceedance of the EPA HAL are being investigated in Pennsylvania, according to PADEP are:

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[45] https://cumulis.epa.gov/supercpad/cursites/dsp_ssppSiteData1.cfm?id=0301295
[46] Ibid.
[47] Ibid.
[48] http://www.dep.pa.gov/Citizens/My-Water/drinking_water/Perfluorinated%20Chemicals%20%e2%80%93PFOA%20and%20PFOS%20%e2%80%93%20in%20Pennsylvania/Pages/DEP-Program-Involvement.aspx
[49] ibid.
[50] ibid.

- Ridge Run Road, Bucks County - parts of East Rockhill and West Rockhill Townships, and Perkasie Borough. One public water supply well exceeded the HAL; one was just under the HAL. Private water well sampling is underway for a one mile radius.[51] A survey and letter to residents within the radius was sent by PADEP in November 2016.[52] The contamination source is not publicly identified.[53]
- Easton Road, Bucks County – parts of Doylestown, Plumstead and Buckingham Townships. The Doylestown Municipal Utilities Authority "Cross Keys" public water supply well was documented to contain combined concentrations of PFOA and PFOS that exceeded the EPA HAL as part of the UCMR3 sampling. Concentrations of PFOA at 210 and 130 ppt and PFNA at 26 ppt were reported in the 2015 UCMR3 sampling results.[54] PADEP took no action to shut down the well until EPA issued the lifetime HAL in 2016; the well was then shut down by the Water Authority and since then water from approximately 280 private wells within 1 mile of the contaminated well has been sampled.[55] PADEP reports that bottled water is being provided to any resident whose water exceeds the EPA HAL.[56] The contamination source has not yet been identified.[57] Round One sampling results show that most private wells sampled had the presence of PFCs with three wells above the combined EPA HAL for PFOA and PFOS of 70 ppt and many wells above 10 ppt.[58,59] PADEP announced they will conduct a second round of sampling in an expanded area and a third round to re-sample properties with results that exceeded 40 ppt.[60]

In addition to these sites under investigation in Pennsylvania, there are other sites that are likely sources of PFCs, particularly PFOA and PFOS. These sites include: military facilities, firefighting and aviation testing sites; fire departments where foam was stored, used and/or tested; aqueous firefighting foam manufacturers, testers, and suppliers; airports; wastewater treatment facilities and their discharge points; sewage sludge and dredge spoils application sites; and manufacturing sites that manufactured or used PFCs in their process. Some of these sites

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[51] http://files.dep.state.pa.us/Water/DrinkingWater/Perfluorinated%20Chemicals/Ridge-Run-Map.jpg
[52] http://files.dep.state.pa.us/Water/DrinkingWater/Perfluorinated%20Chemicals/RidgeRunWellSurvey.pdf
[53] http://www.ahs.dep.pa.gov/NewsRoomPublic/articleviewer.aspx?id=21105&typeid=1
[54] https://www.epa.gov/sites/production/files/2015-09/ucmr-3-occurrence-data.zip
[55] http://files.dep.state.pa.us/RegionalResources/SERO/SEROPortalFiles/Community%20Info/EastonRoadPFC/Site%20area%20radius%20with%20TWPs.pdf
[56] http://files.dep.state.pa.us/RegionalResources/SERO/SEROPortalFiles/Community%20Info/EastonRoadPFC/Easton%20Road%20Information%20Sheet_final.pdf
[57] http://files.dep.state.pa.us/Water/DrinkingWater/Perfluorinated%20Chemicals/Easton%20Rd%20PFC%20FAQ_final%20(1)%20%20.docx
[58] http://files.dep.state.pa.us/Water/DrinkingWater/Perfluorinated%20Chemicals/EastonRoadPFCSite/Sampling%20Summary%20-%20Round%201.pdf
[59] http://files.dep.state.pa.us/Water/DrinkingWater/Perfluorinated%20Chemicals/EastonRoadPFCSite/Sampling%20Map%20-%20Round%201.pdf
[60] http://files.dep.state.pa.us/Water/DrinkingWater/Perfluorinated%20Chemicals/EastonRoadPFCSite/rls-DEP-SERO-Easton%20Road%20update%2012062016.pdf

can be researched through Department of Defense; USEPA regulatory and reporting programs; EPA Superfund (C.E.R.C.L.A.) sites; the Hazardous Sites Cleanup Program; and Resource Conservation and Recovery Act sites. Sampling at these sites is essential to provide an informed and accurate assessment of the scope of the PFC contamination problem in Pennsylvania. The cost of sampling should be borne by the Department of Defense in locations that could have been affected by a military facility or activity and can be paid for through existing programs that are reimbursed by responsible parties. Sampling cannot wait. It is crucial that people know if their drinking water is contaminated by the presence of PFCs.

By way of illustration, military facilities where firefighting foam was used or training for firefighting was carried out include several sites in Pennsylvania that have the potential to cause groundwater contamination by PFCs, particularly PFOA and PFOS. These sites could enormously increase the locations where PFC-contaminated drinking water is discovered within Pennyslvania. These include:

- **Letterkenny Army Depot (originally Letterkenny Ordnance Depot)**, the Center of Industrial and Technical Excellence (CITE) for Air Defense and Tactical Missile Systems under the U.S. Army Aviation and Missile Command encompassing 18,000 acres in Letterkenny Township and extending into Greene Township and Hamilton Township, all in Franklin County.
- **Fort Indiana Gap** under the U.S. Army in Lebanon and Dauphin Counties is still an active National Guard Training Center and is the headquarters for the Pennsylvania National Guard and Pennsylvania Department of Military and Veterans Affairs.
- **NORTH PENN USARC** owned by the U.S. Army, near Worcester, Montgomery County is a private airport and heliport where there was a fire training area burn area.
- **Tobyhanna Army Depot,** located in Coolbaugh Township, Monroe County, under the command of the Department of Defense was a military equipment and firefighting training center, today specializing in electronic systems and intelligence for all branches of the Armed Forces.
- **Philadelphia Naval Shipyard** in Philadelphia was a centrally important shipyard under the U.S. Navy for more than 200 years on the Delaware River. Closed in the 1990's, it was used as a fire training site in addition to an active ship yard.
- **Defense Logistics Agency Susquehanna** is located in New Cumberland and Mechanicsburg, and is the Department of Defense's largest distribution processing facility. Firefighting testing and products are handled there.

The inventory of aqueous firefighting foam that contains PFOA and PFOS in the United States is estimated at about 9.9 million gallons, rounded off to the nearest tenth of a million gallons. These are located at military bases, aviation facilities, merchant ships, fire departments, oil refineries, petro-chemical facilities, and other locations. These stockpiles are geographically scattered and all are a potential source of PFC release to the environment.

Examples of a widely dispersed potential source of PFCs are fire departments and firefighting schools. There are over 27,000 fire departments in the U.S. and hundreds in Pennsylvania.[61] Firefighting foam is an essential tool used routinely by these stations. Aqueous firefighting foam containing PFCs has not been phased out yet, especially because the shelf life of this foam is about 20 years. The proper disposal of foams and the containment of foam when used to put out a fire are not regulated in a manner that prevents inadvertent release into the environment. When used, foams end up in the air and/or on land and in water.

Another example of geographically dispersed locations where PFCs may have been released is airports. There are more than 140 active or decommissioned airports in the Commonwealth. Airports are a known source of PFC contamination in New Jersey and other locations. Foam manufacturers are also locations where groundwater should be sampled. For instance, National Foam in West Chester, PA is a large aqueous firefighting foam manufacturer and supplier. [62]

Pennsylvania should sample all locations where PFCs may have been released to the environment to discover the occurrence in the state. Pennsylvania is a large state with many locations where this is likely to have occurred. PFCs, resistant to being broken down in the environment and extremely durable, are carried into waterways and percolate into groundwater from the soil, carrying toxic properties that persist indefinitely. To protect human health and the environment, it is crucial that surface water, groundwater and groundwater wells, as well as soils and other media where releases could have occurred, be sampled to discover if PFCs are present as the first step towards the adoption of a maximum contaminant level for PFOA in the Pennsylvania. The Pennsylvania Safe Drinking Water Act compels the EQB to act to protect the public from these highly toxic compounds that are, at present, unregulated. Once it is known how widespread these contaminants are in the state, it will become clear that a MCL is immediately necessary for PFCs statewide, starting with PFOA.

4. <u>A MCL for PFOA not to exceed 6 ppt must be set in Pennsylvania</u>

The New Jersey Drinking Water Quality Institute (NJDWQI) has recently recommended that the NJDEP adopt a MCL for PFOA at 14 ppt. However, a recent study by the Cambridge Environmental Consulting (CEC) demonstrates that the NJDWQI has overlooked recent relevant studies, failed to account for children in their analysis, and recommends that a MCL of 1 ppt is feasible and most protective of human health, but at minimum a MCL for PFOA should not exceed 6 ppt.[63]

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[61] https://en.wikipedia.org/wiki/List_of_Pennsylvania_fire_departments
[62] http://nationalfoam.com/
[63] Oliaei, F.Z., & Kriens, D.L. (2016). Proposed Health-Based Maximum Contaminant Level (MCL) for Perfluorooctanoic Acid (PFOA) in Drinking Water. Technical Analyses of New Jersey Drinking Water Quality Institute, Cambridge Environmental Consulting, November 18, 2016. Retrieved from http://www.delawareriverkeeper.org/sites/default/files/cvr%20ltr%20PFOA%20mcl%20cmnt11.19.combinedpdf.pdf (Enclosed as **Attachment 2**).

a. Summary of the NJDWQI Report (14ppt PFOA MCL)

On March 21, 2014, NJDEP Commissioner Bob Martin requested that a MCL be developed for PFOA in drinking water in New Jersey.[64] The NJDWQI issued a public review draft of a MCL for PFOA of 14 ppt dated June 27, 2016 on September 12th.[65] A 60 day comment period followed from September 22 – November 21, 2016. On February 16, 2017, the NJDWQI members unanimously approved its recommendation of 14 ppt.[66] On March 15, 2017 the NJDWQI transmitted to NJDEP with its Basis and Background document its recommendation of a health-based Maximum Contaminant Level for PFOA of 14 ppt.[67] The MCL was developed with guidance from the 2005 USEPA draft risk assessment for PFOA and also considered were the conclusions of the USEPA Science Advisory Board in 2006.[68]

The NJDWQI deviated from some of USEPA's conclusions because the 2005 USEPA draft risk assessment problematically did not develop a cancer slope factor or Reference Dose (Rfd) for PFOA, and it did not address the relationship between human body burden and drinking water concentration, as measured by blood serum level.[69] Comparisons between effect levels in human exposures and animal studies were made by the NJDWQI on the basis of serum levels rather than external dose because the half-life of PFOA is much longer in humans (several years) than in the animal species used in the toxicological studies (several hours to 30 days).[70]

Seven health endpoints were evaluated comprehensively by the NJDWQI in the development of the MCL. These included: liver enzymes, liver disease, serum cholesterol/lipids, thyroid function, thyroid disease, uric acid, and antibody concentrations following vaccination. Some of the factors considered in selection of these endpoints were the consistency and extent of the data, evidence for reverse causality, and whether the effect has been observed at exposures relevant to potential drinking water exposures.[71] In total, 54 epidemiological studies from the United States, Canada, and several Asian and European countries were utilized.[72]

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[64] NJDWQI (2017). Maximum Contaminant Level Recommendation for Perfluorooctanoic Acid in Drinking Water, Basis and Background. New Jersey Drinking Water Quality Institute, March 15, 2017. http://www.nj.gov/dep/watersupply/pdf/pfoa-recommend.pdf (Enclosed as **Attachment 3**) at 2.

[65] See **Attachment 1** (NJDWQI (2016). Health-based Maximum Contaminant Level Support Document: Perfluorooctanoic Acid (PFOA). New Jersey Drinking Water Quality Institute Health Effects Subcommittee, Public Review Draft, June 27, 2016. Retrieved from http://www.nj.gov/dep/watersupply/pdf/pfoa-hb--mcl-public-review-draftwithappendices.pdf at 23).

[66] http://www.nj.gov/dep/watersupply/pdf/minutes170216.pdf

[67] See **Attachment 3** (NJDWQI (2017). Maximum Contaminant Level Recommendation for Perfluorooctanoic Acid in Drinking Water, Basis and Background. New Jersey Drinking Water Quality Institute, March 15, 2017. http://www.nj.gov/dep/watersupply/pdf/pfoa-recommend.pdf)

[68] See **Attachment 1** (NJDWQI (2016). Health-based Maximum Contaminant Level Support Document: Perfluorooctanoic Acid (PFOA). New Jersey Drinking Water Quality Institute Health Effects Subcommittee, Public Review Draft, June 27, 2016. Retrieved from http://www.nj.gov/dep/watersupply/pdf/pfoa-hb--mcl-public-review-draftwithappendices.pdf at 23).

[69] *Id*. at 23.

[70] *Id*. at 23.

[71] *Id*. at 60.

[72] *Id*. at 60.

The NJDWQI also used data from animal studies in developing its MCL.[73] In humans, it has been estimated that as much as 55% of PFOA exposure comes from drinking water.[74] The range of health-based drinking water concentrations for the seven endpoints assessed was 40-260 ppt, and multiple concentrations fell within a similar range (40, 50, 60, 70, and 80 ppt).[75] The most sensitive endpoints were hematological (blood) effects and decreased body weight in adult female rats in a chronic dietary study, which resulted in a drinking water concentration of 40 ppt.[76]

The health-based MCL developed by the NJDWQI was intended to be protective for lifetime (chronic) exposure through drinking water.[77] It was based on well-established and sensitive animal toxicology endpoints that are considered relevant to humans based on mode of action data.[78] Delayed mammary gland development from exposure around birth is the most sensitive systemic endpoint for PFOA. However, the Health Effects Subcommittee decided not to recommend a Health-based MCL with the RfD for delayed mammary gland development as its primary basis because it believed the use of this endpoint as the basis for human health criteria is a currently developing topic.[79] Therefore, the NJDWQI did not calculate an MCL based on delayed mammary gland development.

Instead, increased liver weight was the primary endpoint for the NJDWQI's Heath-based MCL.[80] Increased relative liver weight is a well-established effect of PFOA that is more sensitive than most developmental/reproductive effects and other toxicological effects such as immune system toxicity. A Relative Source Contribution (RSC) factor that accounts for non-drinking water sources including water, air, soil, food, and consumer products was used in the development of health-based drinking water concentrations based on non-carcinogenic effects. In addition to its use by the NJDWQI, an RSC is used by the USEPA for Maximum Contaminant Level Goals and by other states in development of similar health-based drinking water values. The RSC is intended to prevent total exposure from all sources from exceeding the RfD.[81]

The Health Effects Subcommittee concluded that there are insufficient data to develop a chemical-specific RSC for PFOA.[82] There are no New Jersey-specific biomonitoring data for PFOA, and its frequent occurrence in public water supplies suggests that New Jersey residents may also have higher exposure from non-drinking sources than the general population in the U.S.[83] The exposure factors used to develop the Health-based MCL are based on body weight and an adult drinking water consumption rate. Exposures to infants, both those who consume

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[73] *Id*. at 203.
[74] *Id*. at 41.
[75] *Id*. at 24.
[76] *Id*. at 24.
[77] *Id*. at 203.
[78] *Id*. at 204.
[79] *Id*. at 205.
[80] *Id*. at 210.
[81] *Id*. at 215.
[82] *Id*. at 215.
[83] *Id*. at 215.

formula prepared with contaminated drinking water and those who are breastfed, are much higher than in older individuals and therefore a default RSC of 20% was used.[84]

For carcinogenic effects, dose-response modeling was based on administered PFOA dose to rats (mg/kg/day) instead of internal dose (serum PFOA level) since serum PFOA levels were not measured in the study.[85] As per the 2005 USEPA guidelines for carcinogen risk assessment, converting the doses from rats to humans was made based on drug interaction differences between species instead of through the default adjustment based on body weight.[86] In calculating the MCL, the half-lives used for this adjustment were 7 days for male rats and 840 days for humans.[87] The human lifetime cancer risk was one in one million (1×10^{-6}) and default drinking water assumptions were 2 L/day with a body weight of 70 kg.[88] Using these values, the NJDWQI recommended a MCL of 14 ppt for both increased liver weight and carcinogenic risk.[89]

Ongoing exposure to the recommended health-based MCL of 14 ppt is expected to increase blood serum PFOA levels by about 1.6 ng/ml with average daily water consumption and 2.8 ng/ml with upper percentile daily water consumption in adults on average.[90] The proposed Health-based MCL includes an uncertainty factor to protect for more sensitive developmental effects. It is unknown whether it is sufficiently protective for more subtle effects that may occur later in life that may result from low exposures during the developmental period.[91] The chronic studies did not assess effects such as carcinogenicity which might result from exposures during the critical developmental stages that are identified to be sensitive periods for PFOA toxicity.[92]

There are also uncertainties about whether the human relevance of effects seen in animals is applicable to all risk assessments based on animal data.[93] Finally, the toxicity of PFOA and other PFCs may also be additive because the modes of action and target organs are typically similar for PFOA and other PFCs such as PFNA. Although PFOA and other PFCs, including PFNA, are known to co-occur in some NJ public water supplies, the potential for additive toxicity between these compounds was not considered in development of the Health-based MCL.[14] For these reasons, the NJDWQI recommended MCL may not be protective enough.

b. <u>The MCL for PFOA must be set not to exceed 6 ppt</u>

The Cambridge Environmental Consulting (CEC) prepared a technical analysis of the NJDWQI Health-based Maximum Contaminant Level Support Document for PFOA that was summarized above. CEC has concluded that the proposed drinking water MCL of 14 ppt for PFOA that is based on increased relative liver weight is not adequately protective of all

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[84] *Id.* at 216.
[85] *Id.* at 219.
[86] Id. at 219.
[87] *Id.* at 220.
[88] Id. at 220.
[89] Id. at 220.
[90] *Id.* at 221.
[91] *Id.* at 221.
[92] *Id.* at 221.
[93] *Id.* at 221.

population segments.[94] Instead, CEC has recommended that the proposed MCL for PFOA should be lowered to 1 ppt, or alternatively, should be no higher than 6 ppt.[95]

CEC's recommendation of a MCL of 1 ppt is consistent with the values found pursuant to the immunotoxic epidemiologic study and/or animal studies showing adverse developmental effects. However, if these values are excluded, the CEC has identified that the PFOA MCL should be no greater than 6 ppt to assure protection of children.[96]

In particular, CEC disagrees with the NJDWQI's conclusion that the "review of epidemiologic studies provides evidence of consistent findings among studies of decreased antibody concentrations following vaccination and PFOA. However, while there is epidemiologic evidence of temporality, evidence of an exposure-response is limited."[97] Rather, CEC identifies that there is strong, significant epidemiologic evidence that includes quantitative data to enable derivation of a benchmark dose level (BMDL) and such data should be taken into account in derivation of the MCL.[98] CEC cites to a study by Grandjean and Budtz-Jørgensen that represents the greatest sensitivity to PFOA so far studied, un-confounded by exposure to other chemical contaminants.[99] The NJDWQI report does not refer to this study (although it does refer to an unrelated 2012 study by the same authors). Based on the acceptable dose level identified by Grandjean and Budtz-Jørgensen, CEC calculated that the MCL for PFOA should be 0.5 ppt.

CEC also disagreed with NJDWQI's decision to use increased liver weight as its primary endpoint when delayed mammary gland development is the more sensitive endpoint. The NJDWQI's reasoning to exclude the mammary gland endpoint (lack of precedent for delayed mammary gland development), does not explain why NJDWQI arbitrarily applied an additional 10 uncertainty factor to an unrelated endpoint (increased liver weight that forms the basis for their MCL derivation) as compensation.[100] Because adequate toxicity data already exists for the more sensitive delayed mammary gland development endpoint, this endpoint must be used when calculating a MCL.[101] Taking delayed mammary gland development into account, CEC proposed that the MCL for PFOA be 1 ppt.[102]

CEC also disagrees with NJDWQI's use of adult default exposure values because it omits protection for the population's most vulnerable exposure group, children. Children have a greater

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[94] See **Attachment 2** (Oliaei, F.Z., & Kriens, D.L. (2016). Proposed Health-Based Maximum Contaminant Level (MCL) for Perfluorooctanoic Acid (PFOA) in Drinking Water. Technical Analyses of New Jersey Drinking Water Quality Institute, Cambridge Environmental Consulting, November 18, 2016. Retrieved from http://www.delawareriverkeeper.org/sites/default/files/cvr%20ltr%20PFOA%20mcl%20cmnt11.19.combinedpdf.pdf)
[95] Id. at 3.
[96] *Id.* at. 3.
[97] *Id*. at 5.
[98] *Id*. at 5
[99] *Id*. at 5-6.
[100] *Id*. at 7.
[101] *Id*. at 7.
[102] *Id*. at 7.

rate of food and drinking water consumption based on body weight than adults do.[103] Calculation of an MCL using adult default values results in a reference dose (RfD) to children (age group 1-6) that significantly surpasses that deemed allowable by NJDWQI based on the increased liver weight toxicity endpoint.[104] Although the MCL should be based on human immunotoxicity and/or the delayed mammary gland development shown in test animals, CEC assert that, at a minimum, MCL calculations using increased liver weight as an endpoint should be based on children exposure values for drinking water intakes and body weight.[105] Using children group ages 1-6, the consultants conclude that an MCL of 5.65 ppt (rounded to 6 ppt) be promulgated.[106]

In conclusion, absent lowering the proposed PFOA MCL to 1 ppt, the MCL should be no higher than 6 ppt because (i) animal studies show significant delayed mammary gland development are appropriate and sufficient to use in the MCL determination and the NJDQWI failed to use this endpoint, (ii) substantial epidemiological evidence (e.g. study by Grandjean and Budtz-Jørgensen) show a significant association between PFOA and suppression of antibody responses in children, (iii) children exposure values mandate heightened protection, and (iv) toxic effects from PFOA exposures in early childhood may persist into adulthood and could result in more profound disease in later life.[107]

5. <u>The challenges the PA Department of Environmental Protection identifies with setting a MCL for PFOA do not outweigh the significant public health risk of continued exposure to contaminated drinking water supplies</u>

The Department identifies a number of perceived challenges with setting a state MCL for PFOA on their website.[108] Those include (a) lack of state funding and resources; (b) lack of data evaluating whether PFOA contamination is a statewide problem; and (c) lack of funding and resources to develop the science in support of a PFOA MCL.[109] Provided these challenges are resolved, the Department believes these additional steps must be complete prior to setting a MCL: (d) evaluate whether a PFOA MCL is technically feasible; (e) conduct a cost/benefit analysis of the proposed MCL; and (f) develop the necessary justification for proposing a standard that is more stringent than what the EPA has set.[110]

Importantly, all of these challenges can be resolved if the Commonwealth recognizes the serious health consequences of PFOA drinking water contamination and prioritizes committing the resources to set a MCL. This is particularly true for the first three challenges identified by the Department. Regarding the lack of data evaluating whether PFOA contamination is a statewide problem, as discussed at II.B.3. above, there is already ample data showing that PFOA

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[103] *Id*. at 8.
[104] *Id*. at 8.
[105] *Id*. at 8.
[106] *Id*. at 8.
[107] *Id*. 9-10.
[108] See http://www.dep.pa.gov/Citizens/My-Water/drinking_water/Perfluorinated%20Chemicals%20%e2%80%93PFOA%20and%20PFOS%20%e2%80%93%20in%20Pennsylvania/Pages/Establishing-a-State-MCL.aspx
[109] *Id*.
[110] *Id*.

presents a significant health risk in Bucks and Montgomery Counties' drinking water sources, effecting, at a minimum, over 70,000 residents. The Department need only engage in a targeted review of other similarly situated facilities statewide that are likely sources of PFOA and PFOS. These sites include: military facilities, firefighting and aviation testing sites; fire departments where foam was stored, used and/or tested; aqueous firefighting foam manufacturers, testers, and suppliers; airports; wastewater treatment facilities and their discharge points; sewage sludge and dredge spoils application sites; and manufacturing sites that manufactured or used PFCs in their process. Finally, the Department's claim that it lacks funding and resources to develop the science in support of a PFOA MCL fails to account for the NJDWQI's Draft Reports from its Health Effects, Treatment, and Testing Subcommittees which have provided the Department a substantial head start with its research.

Like the three challenges identified by the Department, the three additional next steps are also achievable. Much of the work involved in evaluating whether a PFOA MCL is technically feasible has been performed by NJDWQI subcommittees and DRN's consultants. The NJDWQI Treatment Subcommittee evaluated the treatment technologies for PFOA removal and found that granulated activated carbon was an effective removal technology.[111] Further research by DRN's experts Cambridge Environmental Consulting conclude that while granulated activated carbon has been highly effective in removing PFCs, the best available and economically achievable technology to remove PFOA from dilute aqueous streams at public water supplies is reverse osmosis.[112] Additionally, the NJDWQI Testing Subcommittee has recommended a practical quantification limit (PQL) of 6 ng/L for PFOA.[113] DRN's experts Cambridge Environmental Consulting have reviewed the PQL recommendation from the NJDWQI Testing Subcommittee and conclude that by using the method detection limit (MDL) approach a PQL of 3.0 ppt is achievable and by using the minimum reporting level (MRL) approach to determine a PQL for PFOA, a MRL of 2.0 ppt is achievable.[114]

The additional step requiring the Department to conduct a cost/benefit study of the proposed MCL for PFOA can be accomplished by the Department recognizing the imminent health consequences of PFOA in the public drinking water supply and prioritizing this work. The Department need only remember that the General Assembly has entrusted it to protect the drinking water supply of the citizens of Pennsylvania and that the Pennsylvania Constitution provides that each citizen has a right to clean and safe drinking water.

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[111] See NJDWQI Treatment Subcommittee Draft Report, Addendum to Appendix C: Recommendation on Perfluorinated Compound Treatment Options for Drinking Water (August 2016), p. 3. The draft report can be found here: http://www.nj.gov/dep/watersupply/pdf/addendum-public-review.pdf and is enclosed as **Attachment 4**.

[112] See **Attachment 2**, p. 7 (Cambridge Environmental Consulting, Technical Analysis of New Jersey Drinking Water Quality Institute – Recommendation of Perfluorinated Compound Treatment Options for Drinking Water (Nov. 18, 2016)).

[113] NJDWQI Testing Subcommittee Draft Report on the Development of a Practical Quantification Level for Perfluorooctanoic Acid (PFOA) in Drinking Water, 8/29/2016, p. 19. The NJDWQI Testing Subcommittee Draft Report is enclosed as **Attachment 5** and can be found here: http://www.nj.gov/dep/watersupply/pdf/testing-subcompql-pfoa-8.29.16KA.pdf.

[114] See **Attachment 2**. (Cambridge Environmental Consulting, Technical Analysis of New Jersey Drinking Water Quality Institute – Development of a Practical Quantitation Level for Perfluorooctanoic Acid (PFOA) in Drinking Water (Nov. 18, 2016).

C. Describe the types of persons, businesses and organizations likely to be impacted by this proposal.

All users of treated water, including residents, workers, businesses, agricultural animals and pets, and manufacturing such as consumer and food products, will benefit from the treatment of drinking water to a safe standard that protects human health. Infants, fetuses, women of childbearing age and children, known to be highly vulnerable populations to harm from PFOA exposure, will especially benefit from the use of safe drinking water. The dependency of these populations on adult decisionmaking put them at additional risk of exposure. Benefits include greater protection from disease that is correlated with exposure to PFOA and the multiple benefits of the removal of other potentially dangerous contaminants that are filtered out by the employed treatment technology, specifically through the use of recommended activated carbon filtration. As stated in the New Jersey Drinking Water Quality Institute's Report on Perfluorinated Compound Treatment Options for Drinking Water:

> Activated carbon is commonly used to adsorb contaminants found in water. It is used to remove synthetic organic chemicals, natural organic compounds, and other compounds affecting taste and odor in drinking water treatment.[115]

Persons who have been exposed to concentrations of PFOA in their drinking water for a period of time will benefit by having the compound removed so their body can excrete the compound over time. PFOA does not break down in the human body, accumulating and staying in the blood for years. The only way to reduce or eliminate its presence in the body is to stop exposure and allow for the slow process of natural elimination to take its course.

Treatment of water to remove PFOA will benefit groundwater and the environment because it will filter out the compound, allowing the residue to be disposed of in a safe manner. Since PFOA does not biodegrade, it persists in the environment indefinitely as a toxin. Groundwater, soil, vegetation, and other environmental media contain PFOA, allowing it to migrate to fish and fishlife. Delaware River Estuary surface water and fish flesh in the Delaware River Estuary contain concentrations of PFOA and other PFCs.[116] The treatment and removal of PFOA from drinking water will reduce the concentrations and distribution of PFOA, reducing the exposure to wildlife and to humans who consume fish, reducing the population's intake of PFOA-contaminated food.

Increased property values are also expected by improving the quality of available drinking water to communities that are now suffering depressed home and land values due to known drinking water contamination. Improved water quality can also increase the property values of nearby communities. According to Kauffman's report on the Socioeconomic Value of the Delaware River Basin:

> Several studies along rivers, estuaries, and coasts throughout the United States indicate that improved water quality can increase shoreline property values by 6% to 25% (Table

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[115] http://www.nj.gov/dep/watersupply/pdf/pfna-pfc-treatment.pdf, p. 3.
[116] http://www.nj.gov/drbc/library/documents/contaminants-of-emerging-concernAug2013rev.pdf and http://www.nj.gov/drbc/library/documents/contaminants-of-emerging-concernAug2013rev.pdf

17). The EPA (1973) estimated that improved water quality can raise property values by up to 18% next to the water, 8% at 1000 feet from the water, 4% at 2000 feet from the water, and 1.5% at 3000 feet from the water. Leggett, et al. (2000) estimated that improved bacteria levels to meet state water quality standards along the western shore of the Chesapeake Bay in Maryland raised shoreline property values by 6%. The Brookings Institution (2007) projected that investments of $26 billion to restore the Great Lakes would increase shoreline property values by up to 10%. For this analysis, shoreline property values within 2000 feet of the waterways are estimated to increase by an average of 8% due to improved water quality in the Delaware Estuary.[117]

Finally, the federal and state Safe Drinking Water Act requires the provision of safe water to customers. Municipal, County and State government units and their water supply facilities will benefit from the treatment and removal of PFOA from drinking water by delivering on government's duty to supply safe drinking water under federal and state Safe Drinking Water Acts. The trust, reliability and service that community members and commerce require from elected officials and government agencies is supported when those entities fulfill their responsibility under these statutes and is eroded when contaminated water is consumed by the public and businesses, regardless of intentionality.

D. Does the action requested in the petition concern a matter currently in litigation? If yes, please explain.

No, to our knowledge, the action requested in the petition does not concern a matter currently in litigation.

E. For stream redesignation petitions, the following information must be included for the petition to be considered complete. Attach supporting material as necessary.

DRN's petition is not a petition for stream redesignation.

[117] Kauffman, G. (October 11, 2011). Socioeconomic Value of the Delaware River Basin in Delaware, New Jersey, New York, and Pennsylvania, p. 50. Enclosed as **Attachment 6**.

PUBLIC REVIEW DRAFT

HEALTH-BASED MAXIMUM CONTAMINANT LEVEL SUPPORT DOCUMENT: PERFLUOROOCTANOIC ACID (PFOA)

New Jersey Drinking Water Quality Institute
Health Effects Subcommittee
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ABBREVIATIONS

ALP — alkaline phosphatase
ALT /SGPT — alanine aminotransferase
APFO — ammonium perfluorooctanoate, the ammonium salt of PFOA
AST /SGOT — aspartate aminotransferase
BMD — Benchmark Dose
BMDL — lower 95% confidence limit on the Benchmark Dose
BMR — Benchmark Response
CAR — constitutive androstane receptor
C8 — a synonym for PFOA
DWQI — New Jersey Drinking Water Quality Institute
FTOH — fluorotelomer alcohol
GD — gestational day
GFR — glomerular filtration rate
GGT — gamma-glutamyl transferase
GM — geometric mean
H & E — hematoxylin and eosin
HDL — high-density lipid cholesterol
IARC — International Agency for Cancer Research
IRIS — USEPA Integrated Risk Information System
LDL — low-density lipid cholesterol
LOAEL — Lowest Observed Adverse Effect Level
MCL — Maximum Contaminant Level
NHANES — National Health and Nutrition Examination Survey
NJDEP — New Jersey Department of Environmental Protection
NJDOH — New Jersey Department of Health
NOAEL — No Observed Adverse Effect Level
NTP — National Toxicology Program
OR — odds ratio
PAPs —polyfluoroalky phosphoric acid diesters
PCO — palmitoyl CoA oxidase
PFC — perfluorinated compound
PFOA — perfluorooctanoic acid
PFOS — perfluorooctane sulfonate
PFNA — perfluorononanoic acid
PND — postnatal day
POD — Point of Departure
PPAR — peroxisome proliferator activated receptor
PTFE – polytetrafluoroethylene
PWS – public water supplies
PXR — pregnane X receptor
RfD — Reference Dose
RL — Reporting Level
RSC — Relative Source Contribution
SAB — Science Advisory Board
SDWA — Safe Drinking Water Act

SMR — standardized mortality ratio
TSH — thyroid stimulating hormone
TT3 — total triiodothyronine
TT4 - total thyroxine
UCMR3 — Unregulated Contaminant Monitoring Rule 3
UF — uncertainty factor
VLDL — very low-density lipid cholesterol
USEPA — United States Environmental Protection Agency
WY — Wyeth 14,643 (4-Chloro-6-[2,3-xylidino]-2-pyrimidinylthio)acetic acid), a model PPAR-alpha activating compound

ABSTRACT

A Health-based Maximum Contaminant Level (Health-based MCL) for perfluorooctanoic acid (PFOA, C8) was developed using a risk assessment approach intended to protect for chronic (lifetime) drinking water exposure. A public health-protective approach in developing a Health-based MCL based on animal toxicology data is supported by associations of PFOA with a number of health effects in the general population and communities with drinking water exposure, as well as PFOA's biological persistence and bioaccumulation from drinking water in humans. PFOA was described as "likely to be carcinogenic to humans" by the USEPA Science Advisory Board, "possibly carcinogenic to humans" by the International Agency for Research on Cancer (IARC), and as having "suggestive evidence of carcinogenic potential" by the USEPA Office of Water. Both non-carcinogenic and carcinogenic effects were evaluated for Health-based MCL development. Delayed mammary gland development and increased liver weight were the most sensitive non-carcinogenic endpoints with data needed for dose-response modeling. For each of these endpoints, benchmark dose modeling of serum PFOA levels from mouse studies was performed and appropriate uncertainty factors were applied to develop a Target Human Serum Level (analogous to a Reference Dose but on a serum level basis). A clearance factor (1.4×10^{-4} L/kg/day) which relates serum PFOA concentrations to human PFOA doses was applied to the Target Human Serum Levels to develop Reference Doses. For delayed mammary gland development, the Target Human Serum Level is 0.8 ng/ml, which is below the median serum PFOA level in the U.S. general population. The Reference Dose for this endpoint is 0.11 ng/kg/day. Because the use of delayed mammary gland development as the basis for quantitative risk assessment is a currently developing topic, a Health-based MCL using this endpoint as its primary basis was not recommended. However, it was concluded that an uncertainty factor for sensitive endpoints is needed to protect for this and other effects that occur at similarly low doses. A Health-Based MCL protective for increased relative liver weight was derived based on a study in which male mice were exposed to PFOA for 14 days. For increased relative liver weight, the Target Human Serum Level is 14.5 ng/ml and the Reference Dose is 2 ng/kg/day. This Target Human Serum Level and Reference Dose incorporate uncertainty factors to protect sensitive human subpopulations, to account for toxicodynamic differences between human and experimental animals, and to protect for more sensitive endpoints that occur from developmental exposures (delayed mammary gland development, persistent hepatic toxicity, and others). Default values for drinking water exposure assumptions (2 L/day water consumption; 70 kg body weight) and Relative Source Contribution factor (20%) were used to develop a Health-based MCL of 14 ng/L was based on the Reference Dose for increased relative liver weight. A cancer slope factor of 0.021 $(mg/kg/day)^{-1}$ was developed based on increased incidence of testicular tumors in a chronic rat study. This slope factor was used to develop a Health-based MCL protective for cancer effects at the 1×10^{-6} (one in one million) lifetime cancer risk level of 14 ng/L, identical to the Health-based MCL based on non-cancer endpoints. The recommended Health-based MCL is therefore 14 ng/L (0.014 µg/L).

EXECUTIVE SUMMARY

Perfluorooctanoic acid (PFOA, C8) is a member of the group of substances called perfluorinated compounds (PFCs), chemicals that contain a totally fluorinated carbon chain which varies in length and a functional group such as carboxylic or sulfonic acid. PFCs are part of a larger group of chemicals called poly- and perfluoroalkyl substances (PFAS).

The New Jersey Drinking Water Quality Institute (DWQI) voted to pursue development of a Maximum Contaminant Level (MCL) recommendation for PFOA on January 27, 2009 based on its potential health effects and its occurrence in New Jersey public water supplies (PWS). The Health Effects Subcommittee began its evaluation of PFOA during 2009-2010, but a Health-based MCL recommendation was not finalized at that time. A review of PFOA as an emerging drinking water contaminant was subsequently published by several current and former Subcommittee members in 2012 (Post et al., 2012). On March 21, 2014, New Jersey DEP Commissioner Bob Martin requested that the DWQI recommend an MCL for PFOA and two other perfluorinated compounds, perfluorononanoic acid (PFNA, C9) and perfluorooctane sulfonic acid (PFOS). The Subcommittee's evaluation and recommended Health-based MCL for PFOA are presented in this document.

As is the case for Health-based MCLs recommended by the DWQI in general, the recommended Health-based MCL for PFOA is based on the goals specified in the 1984 Amendments to the New Jersey Safe Drinking Water Act (SDWA) at N.J.S.A. 58:12A-20. This statute specifies a one in one million (10^{-6}) risk of cancer from lifetime exposure to carcinogens, and that no "adverse physiological effects" are expected to result from lifetime ingestion for non-carcinogenic effects. Human health risk assessment approaches used by the DWQI to develop Health-based MCLs generally follow USEPA risk assessment guidance.

Manufacturing and Use

Because carbon-fluorine bonds are among the strongest found in organic chemistry, PFOA and other PFCs are extremely stable and resistant to chemical reactions. PFOA has been produced for use in commercial products and industrial processes for over 60 years. Its unique surfactant properties and resistance to chemical and thermal degradation make it useful in many applications including water-, soil-, and stain-resistant coatings, fire-fighting foams, and industrial uses. Large amounts of PFOA were used industrially as a processing aid (emulsifier) in the production of fluoropolymers and fluoroelastomers for use as non-stick coatings.

Because of concerns about its ubiquitous presence in environmental media (including wildlife) and human blood serum worldwide, its persistent and bioaccumulative nature, and its potential health effects, the eight major U.S. producers of PFOA entered into a voluntary agreement with USEPA in 2006 to reduce emissions and product content of PFOA and its precursors by 95% by 2010 and to work towards eliminating them by 2015. However, other manufacturers and users of PFOA that are not participants in the voluntary agreement with USEPA continue to emit large

amounts of PFOA to the environment, particularly overseas. Although the production and use of PFOA and its precursors has been phased out by major U.S. manufacturers, environmental contamination and resulting human exposure to PFOA are anticipated to continue for the foreseeable future due to its persistence, formation from precursor compounds, and continued production by other manufacturers.

Environmental Fate and Transport

Because of the extreme stability of their carbon−fluorine bonds, PFOA and other PFCs are extremely resistant to degradation in the environment and thus persist indefinitely. PFOA and other PFCs are found in many environmental media and in wildlife worldwide including in remote polar regions. PFOA is much less bioaccumulative in fish than PFOS or perfluorinated carboxylates with more than eight carbons, and PFOA concentrations in wildlife are generally lower than for these other PFCs. PFOA and other PFCs can be taken up into plants from contaminated soil or irrigation water. In general, PFOA and other longer chain PFCs are preferentially taken up into the root and shoot parts of the plant.

PFOA and some other PFCs are distinctive from other persistent and bioaccumulative organic compounds because of their importance as drinking water contaminants. PFOA does not bind well to soil, migrates readily from soil to ground water, and is highly water-soluble. These properties of PFOA differ from those of other well-known persistent and bioaccumulative organic pollutants such as polychlorinated dioxins and polychlorinated biphenyls (PCBs) that have a high affinity for soil and sediments but low water solubility.

PFOA that is released into the environment can contaminate surface water and groundwater used as drinking water sources. Environmental sources include industrial discharge to soil, air, and water; release of aqueous firefighting foams; disposal in landfills; wastewater treatment plant discharge; street and storm water runoff; and land application of biosolids, industrial solid waste, and wastewater. PFOA also enters the environment through the breakdown of precursor compounds such as the fluorotelomer alcohol 8:2 FTOH and larger molecules that can release 8:2 FTOH. These precursor compounds are used industrially and in consumer products. They are converted to PFOA by microbes in soil, sludge, and wastewater and through atmospheric chemical reactions.

As is the case for other ground water contaminants, PFOA can reach drinking water wells via migration of a ground water plume. Unlike many other environmental contaminants, PFOA emitted to air from industrial facilities can also contaminate distant groundwater wells through air transport, followed by deposition from air onto soil, and migration through the soil to groundwater.

Occurrence in Drinking Water

PFOA and other PFCs are not effectively removed from drinking water by standard treatment processes but can be removed from drinking water by granular activated carbon (GAC) or reverse osmosis. Therefore, unless specific treatment for removal of PFCs is in place, concentrations of PFOA detected in raw drinking water can be considered to be representative of concentrations in finished drinking water.

The occurrence of PFOA and other PFCs in public water supplies (PWS) has been evaluated more extensively in New Jersey than in most or all other states. More than 1,000 samples from 80 NJ PWS were analyzed with relatively low Reporting Levels (RLs; generally $\leq$ 5 ng/L) in 2006-2016. PFOA was the most frequently detected PFC and was found in samples from approximately 60% of the 80 NJ PWS tested. In the 2013-2015 USEPA Unregulated Contaminant Monitoring Rule 3 (UCMR3) survey of all large (>10,000 users) and a subset of smaller PWS in the U.S., PFOA was detected more than five times more frequently in New Jersey PWS (10.5%) than nationally (1.9%). The RL in UCMR3 was 20 ng/L, much higher than the RLs for most other NJ PWS monitoring. PFOA has also been detected in NJ private wells near sources of industrial discharge.

Human Biomonitoring

PFOA and other PFCs are found ubiquitously in the blood serum of the general population in the U.S. and worldwide. The most recent (2011-2012) National Health and Nutrition Examination Survey (NHANES), a representative sample survey of the U.S. general population conducted by the U.S. Centers for Disease Control and Prevention (CDC), determined the geometric mean and 95th percentile serum PFOA concentrations as 2.08 and 5.68 ng/ml, respectively. Serum PFOA levels in the U.S. general population have declined since the first NHANES monitoring in 1999-2000 when the geometric mean and 95th percentile values were 5.21 and 11.9 ng/ml. In communities exposed through contaminated drinking water, serum PFOA levels are elevated compared to the general population. Exposures to industrially-exposed workers or others with occupational exposure are much higher than in the general population. Serum PFOA concentrations of greater than 100,000 ng/ml (100 ppm) have been reported in industrially exposed workers, although levels in most workers were lower.

Sources of Exposure

Sources of exposure to PFOA and/or its precursors include drinking water, food and food packaging, treated fabrics, protective sprays and waxes, cosmetics and personal care products, house dust, and inhalation of indoor and outdoor air. Most studies predict that food and food packaging are the predominant exposure sources, and several studies suggest that PFOA and its precursors in indoor air and/or house dust can be a major exposure source. It should be noted that migration of PFOA from polytetrafluoroethylene (PTFE)-coated non-stick cookware into food is not considered to be a significant source of exposure. The contribution of ingested drinking water to total exposure from all sources (e.g. diet, consumer products, etc.) is dependent

on the concentration of PFOA in the drinking water, and relatively low concentrations in water substantially increase human body burden. Inhalation from showering, bathing, laundry, and dishwashing, and dermal absorption during showering, bathing, or swimming, are not expected to be significant sources of exposure from contaminated drinking water.

Exposures to PFOA may be higher in young children than in older individuals because of age-specific behaviors such as greater drinking water and food consumption on a body weight basis, hand-to-mouth behavior resulting in greater ingestion of house dust, and more time spent on floors where treated carpets are found.

Toxicokinetics

PFOA is well absorbed orally, and it was also absorbed dermally and by inhalation in toxicological studies. It is water soluble and distributes primarily to the liver and serum, and, to a lesser degree, to the kidney. Unlike most other bioaccumulative organic compounds, it does not distribute to fat. In the serum, PFOA is almost totally bound to albumin and other proteins. Since it is chemically non-reactive, it is not metabolized. The rate of excretion is largely dependent on the extent of secretion and reabsorption by organic anion transporters in the kidney. The excretion rate varies widely among species, and in some cases between males and females of the same species.

PFOA's half-life in humans is several years and is similar in males and females. Because of its long half-life, it remains in the human body for many years after exposures cease. PFOA is persistent in both male and female mice and in male rats, with half-lives of days to weeks. However, PFOA is rapidly excreted in female rats (half-life of 2-4 hours); thus, this species is not an ideal model for studying potential human developmental effects. Because of the large variation in half-lives, the internal dose resulting from a given administered dose varies widely among species and, in some cases, genders of the same species. For this reason, interspecies (e.g. animal-to-human) comparisons are made on the basis of internal dose, as indicated by serum level, rather than administered dose.

Relationship between drinking water exposure and human serum levels

Data from communities with contaminated drinking water indicate that ongoing human exposure to PFOA in drinking water increases serum levels, on average, by at least 100 times the drinking water concentration. A human clearance factor for PFOA of 1.4×10^{-4} L/kg/day was developed by USEPA researchers (Lorber and Egeghy, 2011) to relate serum PFOA concentration to administered dose. Assuming an average U.S. daily water consumption rate, the clearance factor predicts a serum:drinking water ratio of 114:1, consistent with the ratios that have been observed in exposed communities.

Continued exposure to even low drinking water concentrations results in substantially increased serum PFOA levels. Based on the clearance factor, each 10 ng/L in drinking water is predicted

to increase serum PFOA by 1.1 ng/ml with an average water consumption rate, and 2.0 ng/ml with an upper percentile water consumption rate. These increases in serum PFOA from drinking water can be compared to the most recent NHANES geometric mean, 2.08 ng/ml, and 95th percentile, 5.68 ng/ml, serum PFOA concentrations. Increases in serum PFOA levels predicted from average and upper percentile drinking water consumption at various drinking water PFOA concentrations are shown in Figure E-1.

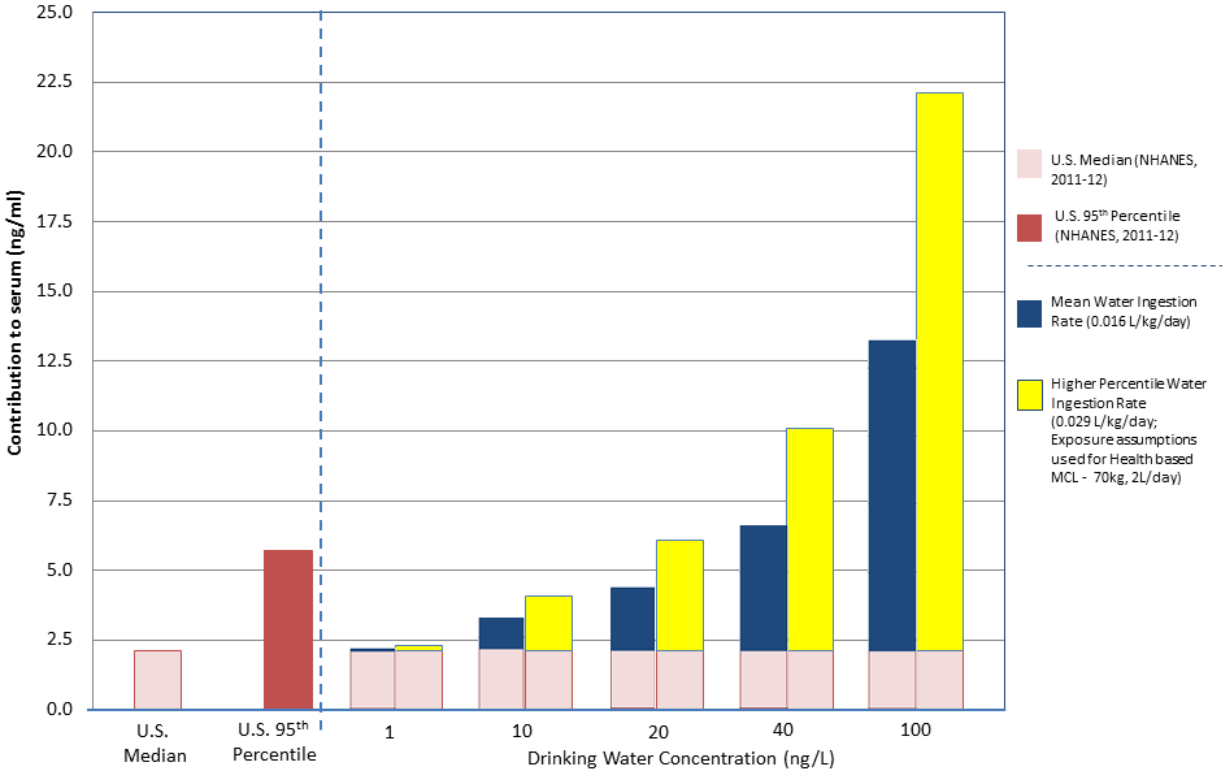


Figure E-1. Increases in serum PFOA concentrations predicted from mean and upper percentile consumption of drinking water with various concentrations of PFOA, as compared to U.S median and 95th percentile serum PFOA levels (NHANES, 2011-12).

<u>Exposures to infants</u>

In humans, PFOA has been measured in amniotic fluid, maternal serum, umbilical cord blood, and breast milk. Serum PFOA concentrations in infants at birth are similar to those in maternal serum. Both breast-fed infants whose mothers ingest contaminated drinking water and infants fed with formula prepared with contaminated drinking water receive much greater exposures to PFOA than older individuals who consume drinking water with the same PFOA concentration. PFOA exposure in breast-fed infants is greatest during the first few months of life because both PFOA concentrations in breast milk and the rate of fluid consumption are highest then. As a result, serum PFOA concentrations in breast-fed infants increase several fold from levels at birth within the first few months of life (Figure E-2). Exposures to infants who consume formula prepared with contaminated water are also highest during this time period. While serum PFOA levels peak during the first year of life, they remain elevated for several years. These elevated

exposures during infancy and early childhood are of particular concern because early life effects are sensitive endpoints for the toxicity of PFOA.

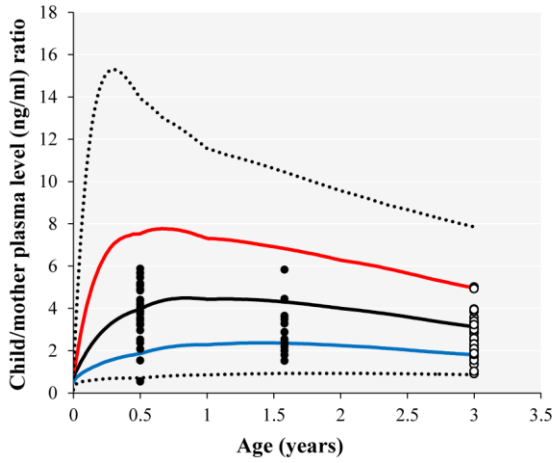


Figure E-2. From Verner et al. (2016a). Modeling simulation of the ratio of PFOA in blood plasma in breast fed infants/children to plasma concentration in mother.
Black line - 50th percentile. Blue line - 5th percentile. Red line - 95th percentile. Dotted lines - minimum and maximum values.

Health Effects

Because the scientific database related to health effects of PFOA is very large, the Subcommittee focused its evaluation on specific endpoints from human and animal studies. Relevant studies were identified through literature searches of the PubMed database, from earlier evaluations of PFOA by the Health Effects Subcommittee, and through backwards searching.

Epidemiology

The choice of endpoints selected for comprehensive review from epidemiology studies was largely based on knowledge gained from previous evaluations by the Subcommittee. Health endpoints evaluated comprehensively were serum cholesterol/lipids, liver enzymes/bilirubin and liver disease, uric acid, thyroid function and thyroid disease, and antibody concentrations following vaccination. In total, 54 epidemiological studies were evaluated in depth, including studies from the general population, communities with drinking water exposures including most notably the C8 Health Study - a large study of about 70,000 Ohio and West Virginia residents exposed to a wide range of PFOA concentrations (>50 ng/L to over 3000 ng/L) in drinking water, and occupationally exposed workers. Recent comprehensive reviews by other authoritative scientific groups were evaluated for two additional critical endpoints, fetal growth following developmental exposure and cancer.

Of the endpoints that were evaluated comprehensively, the evidence for associations with PFOA was strongest for increases in serum levels of cholesterol, the liver enzyme ALT, and uric acid.

PFOA was associated with clinically defined hypercholesterolemia in a community exposed through drinking water. The epidemiological evidence supports multiple criteria for a causal relationship between PFOA and both serum cholesterol and ALT. Notably, the steepest dose-response for associations with these endpoints was within the range of serum PFOA concentrations found in the general population and communities with drinking water exposures, with a much flatter curve at higher serum concentrations.

For some other endpoints that were comprehensively reviewed, limited evidence of an association with PFOA was found. Although there is consistent evidence of decreased antibody concentrations following vaccination, most of the vaccine types were evaluated in only one or two studies and there is limited evidence of exposure-response. Other endpoints with limited evidence of an association include LDL, the liver enzymes GGT and AST, bilirubin, liver disease, and thyroid disease. There was limited or no evidence of association of PFOA with TSH and thyroid hormones, and no evidence for association with HDL or the liver enzyme ALP.

A systematic review using the Navigation Guide methodology concluded that there is "sufficient" human evidence, the strongest descriptor for strength of evidence, that developmental exposure to PFOA reduces fetal growth (e.g. birth weight) in humans (Johnson et al., 2015). The Health Effects Subcommittee found that the basis for this conclusion is reasonable and supportable. Maternal glomerular filtration rate (GFR) was evaluated as a potential confounding factor for this effect, and it was concluded that decreased GFR does not account for the major portion of the decrease in fetal growth associated with PFOA.

PFOA was associated with increased incidence of testicular and kidney cancer in communities with drinking water exposure. These studies accounted for smoking history and other relevant factors. The USEPA SAB (2006) described PFOA as "likely to be carcinogenic to humans." based on the criteria provided in USEPA (2005b) cancer risk assessment guidance. More recently, IARC (2015) concluded that PFOA is possibly carcinogenic to humans, and the USEPA Office of Water (2016a) described it as having suggestive evidence of carcinogenic potential.

Although the magnitude of change for some of the parameters associated with PFOA was relatively small, they are of public health concern because population-level changes of this magnitude will result in a shift in the overall distribution of values such that the number of individuals with clinically abnormal values is increased. Additionally, small changes in a clinical biomarker may be an indicator of other effects that were not evaluated. For example, relatively small decreases in birth weight may be an indication of changes in other more subtle developmental parameters which were not assessed.

In summary, associations of PFOA with numerous health endpoints have been found in human populations with evidence supporting criteria for causality for some endpoints. These health endpoints include non-carcinogenic effects in the general population, and both non-carcinogenic

effects and cancer in communities with drinking water exposure. The epidemiologic data for PFOA are notable because of the consistency between results among human epidemiologic studies in different populations, the concordance with toxicological findings from experimental animals, the use of serum concentrations as a measure of internal exposure, the potential clinical importance of the endpoints for which associations are observed, and the observation of associations within the exposure range of the general population. These features of the epidemiologic data distinguish PFOA from most other organic drinking water contaminants and justify concerns about exposures to PFOA through drinking water. Although there is evidence to support causality for some epidemiological endpoints, the human data have limitations and therefore are not used as the quantitative basis for the Health-based MCL. Instead, the potential Health-based MCLs developed below are based on sensitive and well established animal toxicology endpoints that are considered relevant to humans based on mode of action data.

Toxicology

The toxicological database for PFOA includes evaluation of numerous effects in non-human primates and rodents. The Health Effects Subcommittee's review focused on endpoints that were identified as sensitive and potentially appropriate for use in risk assessment. The effects selected for detailed review were hepatic toxicity, developmental effects, immune system toxicity, and carcinogenicity. As discussed above, effects relevant to these endpoints have been associated with PFOA in human epidemiological studies. Additionally, information is presented on general toxicity in non-human primates, as well as thyroid, neurobehavioral, and male reproductive effects.

The non-human primate studies have limitations that preclude their consideration as the basis for risk assessment. These include very small numbers of animals, severe toxicity at the lowest dose, loss of animals during the study due to toxicity and/or mortality, and lack of dose-response for key endpoints (e.g. increased liver weight).

Increased liver weight is a sensitive toxicological endpoint for PFOA which has been observed in many studies in both non-human primates and rodents. Increased liver weight can co-occur with and/or progress to more severe hepatic effects including hepatocellular necrosis, fatty liver, increased serum liver enzymes, and hyperplastic nodules. Recent studies show that developmental exposure to low doses of PFOA in mice causes cellular changes indicative of liver toxicity that persist until adulthood.

Reproductive or developmental effects of PFOA have not been studied in non-human primates. The mouse is an appropriate species for evaluating effects on reproduction and development since the female mouse excretes PFOA slowly, as do humans. In contrast, rats and rabbits are not ideal models for studying these effects because they excrete PFOA very quickly, with a half-life of a few hours. Effects from developmental exposures in mice include full litter resorptions,

decreased postnatal survival and growth, delayed development, accelerated sexual maturation in males, persistent liver toxicity (noted above), and delayed mammary gland development. PFOA also causes reproductive toxicity in male mice.

Delayed mammary gland development and persistent liver toxicity after perinatal (prenatal and/or neonatal) exposure are sensitive endpoints which occur in mice at lower doses of PFOA than other developmental effects. Delayed mammary gland development has been reported in nine separate studies presented in five publications, while only one study which has several general problematic issues did not find this effect. Gestational and/or lactational exposures to PFOA caused delayed mammary gland development in pregnant dams and/or female offspring in two strains of mice. Histological changes in the mammary gland of exposed offspring occurred in a dose-related fashion, persisted until adulthood, and were considered permanent. However, available toxicological information is not sufficient to make conclusions about the effects of PFOA on lactational function. Maternal PFOA exposure has been associated with shorter duration of breastfeeding in humans, and there is no information indicating that the histological changes observed in mice are not relevant to humans.

Additional studies evaluated effects of peripubertal (around the time of puberty) exposure on mammary gland development in mice. These studies cannot be directly compared to studies of perinatal exposure because effects on mammary gland development differ depending on the lifestage when exposure occurs. Additionally, interpretation of the peripubertal studies is problematic because each PFOA dose level was used in only one study in each of the strains of mice evaluated, such that dose-response interpretations can only be made by combining data from different studies.

PFOA suppressed the immune system in studies of rhesus monkeys and mice. Decreased bone marrow cellularity and lymphoid atrophy occurred in monkeys, while effects in mice included decreased spleen and thymus weights, decreased thymocyte and splenocyte counts, decreased immunoglobulin response, and changes in total numbers and/or specific populations of lymphocytes. Immune system effects were not observed in two rat studies which included doses higher than those which generally caused these effects in mice.

Review of the toxicological data indicates that increased liver weight is an endpoint that is as sensitive or more sensitive than immune system toxicity or reproductive/developmental effects, with the exception of delayed mammary gland development.

PFOA caused tumors of the liver, pancreatic acinar cells, and testicular Leydig cells in male rats. Since PFOA is rapidly excreted by female rats, chronic studies in another species in which PFOA is persistent in both sexes, such as the mouse, would provide important information specific to females. A recent study suggests that prenatal exposure to PFOA in mice caused an

increased incidence of liver tumors. However, this study was not designed as a carcinogenicity bioassay and does not provide definitive information on this issue. Additional research on carcinogenicity later in life after developmental exposures to PFOA is needed.

Mode of Action

The mode(s) of action of PFOA have not been fully characterized. Based on the information reviewed by the Health Effects Subcommittee, the toxicological effects of PFOA are generally considered relevant to humans for the purposes of risk assessment.

PFOA is not chemically reactive. Thus, it is not metabolized to reactive intermediates and does not covalently bind to nucleic acids and proteins. Consistent with these properties, available data indicate that it is not genotoxic.

Activation of nuclear receptors and role of PPAR-alpha

Effects of PFOA occur through multiple modes of action including activation of receptors that control the expression of genes involved in many biological pathways. Much attention has been focused on the potential human relevance of effects that occur through activation of the nuclear receptor, peroxisome proliferator-activated receptor-alpha (PPAR-alpha). This question arises because many PPAR-alpha activating compounds cause rodent liver tumors; the human relevance of these tumors is subject to debate due to lower levels and/or differences in intrinsic activity of PPAR-alpha in human liver. However, the uncertainty about human relevance does not necessarily apply to PPAR-alpha mediated effects other than liver tumors. Both human and mouse PPAR-alpha are activated by PFOA *in vitro*, and the results do not clearly indicate that human PPAR-alpha is less sensitive than rodent PPAR-alpha in these *in vitro* systems.

Hepatic effects

Studies of non-human primates, standard strains of rats and mice, PPAR-alpha null mice, and humanized PPAR-alpha mice support the conclusion that hepatic effects of PFOA are relevant to humans for the purposes of risk assessment. As noted above, PFOA is associated with increased liver enzymes in human epidemiological studies.

In a subchronic study of cynomolgus monkeys, a species in which human relevance of hepatic effects is not in question, PFOA caused increased liver weight and peroxisomal proliferating activity similar in magnitude to that seen in rats, demonstrating that hepatic PPAR-alpha activity in response to PFOA is not limited to rodents. In this study, several animals exhibited notably increased liver weight, highly elevated serum liver enzymes, and/or severe hepatic toxicity.

Observations in standard strains of laboratory rodents indicate that PFOA causes PPAR-alpha independent hepatic effects in rodents with normal PPAR-alpha function. In these strains, increased relative liver weight caused by PFOA did not directly correspond with hepatic

peroxisome proliferating activity. Additionally, PFOA caused fatty liver in these standard strains, although PPAR-alpha activation decreases hepatic lipids. Finally, developmental exposure to PFOA caused abnormal mitochondria in livers of a standard mouse strain, with no evidence of peroxisome proliferation.

PFOA caused decreased serum lipids, typically associated with PPAR-alpha activation, in rodents, while increased serum lipids are associated with PFOA exposure in humans. Recent studies suggest that these differences may be related to the low fat diet generally used in laboratory rodent studies versus the higher fat content of a typical Westernized human diet, rather than solely to interspecies differences.

Studies comparing wild type (with normal PPAR-alpha) and PPAR-alpha null (lacking PPAR-alpha) mice provide further evidence that hepatic effects occur through both PPAR-alpha dependent and independent pathways. PFOA caused similar increases in liver weight in wild type and PPAR-alpha null strains. Increased liver enzymes and histopathological changes, particularly damage to the bile duct, also occurred in PFOA-treated PPAR-alpha null mice. Additionally, developmental exposures to PPAR-alpha null mice caused persistent histopathological changes in the liver.

Studies of strains of mice which express human PPAR-alpha in the liver (humanized PPAR-alpha mice) indicate that PFOA causes hepatic effects through activation of human PPAR-alpha. In humanized PPAR-alpha mice, PFOA caused increased liver weight similar to that in wild type mice, activation of hepatic genes associated with PPAR-alpha, and histopathological changes in the liver. Fetal liver weight was increased similarly in wild type and humanized PPAR-alpha mice after *in utero* exposure, and expression of genes associated with PPAR-alpha in fetal liver was increased to a greater degree in humanized PPAR-alpha mice than in wild type mice.

<u>Immune system effects</u>

PFOA suppresses the immune system in both non-human primates and mice. As noted above, decreased response to vaccinations has been associated with PFOA in human epidemiological studies. Data from mouse studies indicate that these effects on the immune system occur through both PPAR-alpha dependent and independent modes of action. Both PPAR-alpha dependent and independent effects on the immune system are considered relevant to humans for the purposes of risk assessment.

<u>Developmental and reproductive effects</u>

As noted above, decreased fetal growth is associated with PFOA in human epidemiological studies. Developmental effects of PFOA in rodents appear to occur primarily through PPAR-alpha dependent mechanisms, while some reproductive effects such as full litter resorptions appear to be PPAR-alpha independent. PPAR-alpha and other PPARs are present in human fetal

tissues and are expected to have important roles in reproduction and development. Therefore, PPAR-alpha mediated effects of PFOA on development are considered relevant to humans for the purposes of risk assessment. Toxicity to the placenta may play a role in PFOA's developmental effects such as fetal growth retardation; more research is needed on this question.

Delayed mammary gland development after developmental exposure is a sensitive endpoint for PFOA toxicity in mice. The rodent is considered a good model for human mammary gland development, and there is no mode of action evidence suggesting that the effects of PFOA on this endpoint are not relevant to humans.

PFOA also causes male reproductive toxicity in mice, and there is no mode of action information to suggest that these effects are not relevant to humans.

Carcinogenicity

As noted above, PFOA has been associated with increased incidence of kidney and testicular cancer in communities exposed through drinking water after adjustment for smoking and other relevant factors. The USEPA Science Advisory Board (2006) concluded that the liver tumors caused by PFOA in rats are potentially relevant to humans, based on similarities in hepatic effects of PFOA in monkeys and rodents and the limited evidence available at the time on hepatic effects of PFOA in PPAR-alpha null mice. Subsequent studies in PPAR-alpha null mice have provided substantial additional relevant data. Importantly, hepatic cell proliferation, a causal event for tumor formation, is increased similarly by PFOA in wild type and PPAR-alpha null mice. Although a carcinogenicity bioassay of PFOA has not been conducted in PPAR-alpha null mice, a recent study suggests that developmental exposures to PFOA may cause hepatic tumors in adulthood in this strain. Finally, studies in rainbow trout, a species used as a model for human liver cancer because it lacks PPAR-alpha, suggest that PFOA causes liver tumors through an estrogenic mode of action.

The mode of action for the testicular and pancreatic tumors caused by PFOA in rats has not been established. Therefore, they are considered relevant to humans for the purposes of risk assessment.

Additional modes of action

A number of other modes of action for PFOA have been suggested including effects on intercellular gap junction communication, effects on mitochondria, changes in expression of microRNAs (miRNAs), and effects related to transporter proteins such as organic anion transporters (OATs) and multidrug resistance-associated proteins (MRPs).

Development of Recommended Health-based MCL

Health-based MCLs developed by the DWQI are intended to be protective for chronic (lifetime) exposure through drinking water. The 1984 Amendments to the New Jersey Safe Drinking Water Act (N.J.S.A. 58:12A-20) stipulate that Health-based MCLs be based on a one in one million lifetime cancer risk level for carcinogens and no adverse effects from lifetime ingestion for non-carcinogens. PFOA was described as "likely to be carcinogenic to humans" by the USEPA Science Advisory Board and "possibly carcinogenic to humans" by the International Agency for Research on Cancer (IARC), and as having "suggestive evidence of carcinogenic potential" by the USEPA Office of Water. As such, the Health Effects Subcommittee evaluated both non-carcinogenic and carcinogenic effects using approaches consistent with USEPA risk assessments guidance and previous risk assessments developed by NJDEP and the DWQI.

Both the human epidemiology data and the animal toxicology data were considered as part of the overall weight of evidence for the potential human health effects of PFOA. As discussed above, PFOA is associated with non-carcinogenic effects in the general population, and with both non-carcinogenic effects and cancer in communities with drinking water exposure. Although the data for some endpoints support multiple criteria for causality, the human epidemiology data have limitations and are therefore not used as the quantitative basis for the Health-based MCL. As such, the recommended Health-based MCL is based on sensitive and well established animal toxicology endpoints that are considered relevant to humans. Notwithstanding, the human data suggest that continued human exposure to even relatively low concentrations of PFOA in drinking water results in elevated body burdens that increase the risk of health effects, indicating a need for caution about exposures from drinking water. Therefore, the human epidemiological data support the use of a public health-protective approach in developing a Health-based MCL recommendation based on animal toxicology data.

Health-based MCL based on non-carcinogenic effects

Delayed mammary gland development and increased relative liver weight were identified as the most sensitive non-carcinogenic endpoints with data appropriate for dose-response modeling, and it was concluded that these endpoints are relevant to humans for the purposes of risk assessment. Benchmark dose (BMD) modeling of serum PFOA data from toxicological studies was performed to determine the BMDLs (lower 95% confidence limit on the doses corresponding to a minimal response) for the serum concentrations that are used as the points of departure (PODs) for these endpoints. Only studies that provide serum PFOA data were considered for dose-response modeling for these effects, since measured serum levels are associated with less uncertainty than serum level estimates from pharmacokinetic modeling or interspecies extrapolations based on half-life differences.

Effects on mammary gland development

Delayed mammary gland development is the most sensitive systemic endpoint with data appropriate for dose-response modeling, and a Reference Dose (RfD) was developed for this endpoint. To the knowledge of the Health Effects Subcommittee, this endpoint has not previously been used as the primary basis for health-based drinking water concentrations or other human health criteria. Because the use of delayed mammary gland development as the basis for quantitative risk assessment is a currently developing topic, a Health-based MCL with this RfD as its primary basis was not recommended. However, it was concluded that an additional uncertainty factor (UF) should be incorporated into the RfD based on increased liver weight (the endpoint used as the basis for the recommended Health-based MCL - see below) to protect for mammary gland effects, persistent liver toxicity, and other effects from developmental exposures at doses far below those that cause increased relative liver weight.

A study of exposure to pregnant mice on days 10-17 of gestation (Macon et al., 2011) is the only developmental exposure study of mammary gland development that provides serum PFOA data appropriate for dose-response modeling. Of the multiple time points assessed in this study, delays in mammary gland development were most evident on postnatal day (PND 21). Of the several endpoints related to mammary gland development that were evaluated, decreases in mammary gland developmental score and number of terminal end buds were selected for dose-response modeling because they showed a statistically significant dose-related decrease at PND 21. BMD modeling was based on serum levels at PND 1, since they were higher at this time than at later time points. The serum concentration BMDLs for a 10% change in decreased developmental score and decreased number of terminal end buds were 24.9 and 22.9 ng/ml, respectively.

A total UF of 30, including UFs of 10 for intra-human variability and 3 for animal-to-human toxicodynamic differences, was applied to the serum level BMDL for decreased number of terminal end buds, 22.9 ng/ml, to derive a Target Human Serum Level of 0.8 ng/ml. The typical UF of 3 for toxicokinetic variability between species is not included because the risk assessment is based on comparison of internal dose (serum levels) rather than administered dose. The Target Human Serum Level is analogous to a RfD in terms of internal, rather than administered, dose. This Target Human Serum Level for delayed mammary gland development of 0.8 ng/ml is below the median serum PFOA level in the U.S. general population (2.1 ng/ml). The clearance factor mentioned above, 1.4×10^{-4} L/kg/day, was applied to the Target Human Serum Level, 0.8 ng/ml, to calculate an RfD of 0.11 ng/kg/day.

Hepatic effects

Increased relative liver weight is a well-established effect of PFOA which is more sensitive than most other toxicological effects such as immune system toxicity and most

reproductive/developmental effects (Table 12 of Animal Toxicology section). The recommended Health-based MCL for non-carcinogenic effects is based on this endpoint.

A study of male mice exposed to branched/linear PFOA for 14 days (Loveless et al., 2006) that showed a dose-related increase in relative liver weight was selected for dose-response modeling. This isomeric mixture is relevant to environmental contamination and human exposure, and it was used in almost all toxicological studies of PFOA. Because review of studies of increased relative liver weight indicated that the magnitude of this effect does not increase with exposure durations longer than 14 days, this study was considered to be of sufficient duration for use as the basis for a Health-based MCL. BMD modeling of the serum PFOA data from the study determined a serum level BMDL for a 10% increase in relative liver weight of 4350 ng/ml.

A total UF of 300 was applied to the serum level BMDL of 4350 ng/ml to derive a Target Human Serum Level of 14.5 ng/ml. This UF includes UFs of 10 for intra-human variability, 3 for animal-to-human toxicodynamic differences, and 10 to protect more sensitive toxicological effects. These more sensitive effects, including delayed mammary gland development and hepatic toxicity after developmental exposures, occurred at doses 100-fold lower than the Lowest Observed Adverse Effect Level (LOAEL) for increased liver weight. Although the study duration was only 14 days and the Health-based MCL is intended to protect for chronic exposure, a UF for less-than-chronic duration of exposure was not applied because increased liver weight does not appear to increase in magnitude when exposures continue beyond two weeks. The clearance factor mentioned above, 1.4×10^{-4} L/kg/day, was applied to the Target Human Serum Level, 14.5 ng/ml, to calculate an RfD of 2 ng/kg/day.

Relative Source Contribution factor

A Relative Source Contribution (RSC) factor that accounts for non-drinking water sources including food, soil, air, water, and consumer products is used by USEPA, NJDEP, and the DWQI in the development of health-based drinking water concentrations based on non-carcinogenic effects. The default value for the RSC is 20%, meaning that 20% of total exposure is assumed to come from drinking water and 80% from non-drinking water sources. If supported by available data, a higher chemical-specific value (up to 80%) can be used (i.e. the percent exposure from non-drinking water sources is lower than the default assumption of 80%). The Health Effects Subcommittee concluded that there are insufficient data to develop a chemical-specific RSC for PFOA. USEPA UCMR3 monitoring shows that PFOA occurs (at concentrations greater than 20 ng/L) more frequently in PWS located throughout New Jersey (10.5%) than nationwide (1.9%).

There are no New Jersey-specific biomonitoring data for PFOA, and the more frequent occurrence in NJ PWS suggests that New Jersey residents may also have higher exposures from non-drinking sources, such as contaminated soils, house dust, or other environmental media,

than the U.S. general population. Additionally, the default RSC of 20%, while not explicitly intended for this purpose, also partially accounts for the greater exposures to infants who are breast-fed or consume formula prepared with contaminated drinking water, as compared to older individuals. These higher exposures during infancy must be considered because short term exposures to infants are relevant to the effects of concern (delayed mammary gland development and increased relative liver weight). Therefore, the default RSC of 20% was used to develop the Health-based MCL.

Health-based MCL based on non-carcinognic effects

The Health-based MCL protective for increased liver weight, based on the RfD of 2 ng/kg/day, standard drinking water exposure assumptions (2 L/day water consumption; 70 kg body weight), and a 20% RSC is 14 ng/L (0.014 µg/L).

Health-based MCL based on carcinogenic effects

Testicular tumor data from the chronic dietary exposure rat study (Butenhoff et al., 2012) are the only tumor data appropriate for dose-response modeling and were used to develop a cancer potency factor. The BMDL for 5% tumor incidence is 2.36 mg/kg/day, and the corresponding cancer potency factor is 0.021 $(mg/kg/day)^{-1}$. The dose in rats corresponding to a 1×10^{-6} risk level, 4.8×10^{-5} mg/kg/day, was converted to the human equivalent dose of 4×10^{-7} mg/kg/day (0.4 ng/kg/day) using a pharmacokinetic adjustment based on the ratio of half-lives in the two species. Using default drinking water assumptions (2 L/day water consumption; 70 kg body weight), the Health-based MCL at the 1×10^{-6} lifetime cancer risk level is 14 ng/L. This value is identical to the Health-based MCL based on non-cancer endpoints developed above.

Potential for additive toxicity with other PFCs

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

The recommended Health-based MCL is 14 ng/L (0.014 µg/L).

INTRODUCTION

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

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

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

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

To support the development of an MCL recommendation by the DWQI, the Health Effects Subcommittee has developed a draft Health-based Maximum Contaminant Level for PFOA. As specified in the 1984 Amendments to the NJ SDWA, this Health-based MCL is intended to be protective for chronic (lifetime) drinking water exposure.

Timeline of Health Effects Subcommittee's evaluation of PFOA

On January 27, 2009, the DWQI voted unanimously to add PFOA to its work plan for development of an MCL recommendation (DWQI, 2009b). On September 10, 2010, the Health Effects Subcommittee reported to the DWQI Testing and Treatment Subcommittees that it had made progress in its evaluation PFOA. Although no decision on a Health-based MCL recommendation had been made, the Subcommittee provided a memo stating that it was

considering potential values for a PFOA Health-based MCLs within the range of 10 ng/L to 40 ng/L, or as low as reasonably achievable (DWQI, 2010).

In 2012, several current and former members of the Health Effects Subcommittee published a comprehensive review of PFOA as an emerging drinking water contaminant in a peer-reviewed journal (Post et al., 2012). This publication was based in part on the literature review and evaluation conducted by the Health Effects Subcommittee in 2009-2010.

On March 21, 2014, New Jersey DEP Commissioner Bob Martin requested that the DWQI recommend MCLs for three perfluorinated compounds: perfluorononanoic acid (PFNA, C9), PFOA, and perfluorooctane sulfonic acid (PFOS). The Health Effects Subcommittee commenced its evaluation of PFOA after completing its work on PFNA, for which the DWQI recommended an MCL on July 1, 2015 (DWQI, 2015a).

Document development process

The Subcommittee began its current evaluation of PFOA by formulating an approach for the review of relevant information and document development. Because the scientific database related to health effects of PFOA is very large, the Subcommittee chose to focus on specific endpoints from human and animal studies for hazard identification and/or dose-response. Criteria for selection of the human and animal endpoints that were reviewed in depth are discussed in the Epidemiology and Toxicology sections.

The Health Effects Subcommittee conducted a literature search of the PubMed database through April 2015 using relevant search terms which are provided in Appendix 1. The U.S. National Library of Medicine's Toxline database was searched using similar keywords as the PubMed search strings. The Toxline search yielded a significant number of non-peer reviewed documents including articles, policy papers, and grant proposals, and ultimately Toxline did not identify additional peer-reviewed literature for inclusion in the Subcommittee's review. Studies evaluated by the Subcommittee also included relevant citations from the earlier Subcommittee evaluations of PFOA mentioned above, as well as backward searching. PubMed is searched on a monthly basis by the NJDEP Environmental Research Library, and an ongoing title review of these searches was conducted to identify any additional studies for inclusion.

The original PubMed search identified 2,016 references. All of these references were screened by title, abstract, and/or full text. Title and abstract review was used to sort studies into inclusion categories for consideration for detailed evaluation related to hazard identification and/or dose-response evaluation using EndNote (Appendix 1). Studies were excluded if they were "Unrelated" (did not assess PFOA, proposals, or reviews), or "Non-health"' (studies of a analytical methods, environmental occurrence, sources of human or wildlife exposure, and other topics not directly related to health effects). Some studies categorized as "Non-health" are cited

in relevant sections of the document, when appropriate. Remaining studies were identified as either "*in vitro*", "Experimental Animal", or "Human". Further categorization of included studies is described in more detail in the Epidemiology and Toxicology sections. The number of records retrieved and number of studies sorted into inclusion/exclusion categories are also provided in Appendix 1. Following identification of studies to be reviewed in depth, data were extracted from included studies into individual study tables and/or summary tables, as described in the Epidemiology and Toxicology sections. Individual study tables for the Epidemiology section are provided in Appendix 4 and for the Toxicology section in Appendix 5.

Some sections of the document that provide background information but do not directly impact development of the Health-based MCL (e.g. Environmental Sources, Fate, and Occurrence) are based on updates of the Subcommittee's previous evaluations of PFOA.

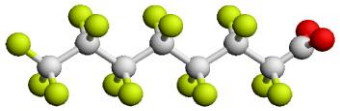
In 2014, the DWQI posted a request for public input regarding data or technical information about the toxicology, epidemiology, toxicokinetics, or other health effects topics related to PFOA that should be considered in developing an MCL. The DWQI received one submission on PFOA, and relevant comments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

BACKGROUND INFORMATION

PFOA is a member of a class of anthropogenic chemicals called perfluorinated chemicals (PFCs) with structures consisting of a totally fluorinated carbon chain of varying length and a charged functional group, such as carboxylate or sulfonate (Lindstrom et al., 2011a). PFCs are members of a larger class of compounds, poly- and perfluoroalkyl substances (PFAS) which also includes fluorinated compounds with structures that differ from PFCs (Buck et al., 2011). The eight-carbon PFCs, PFOA and PFOS, were the most intensively investigated compounds in earlier studies, while current research focuses on a wider range of PFAS.

Physical and Chemical Properties (PubChem, 2016)

Chemical Name:	Perfluorooctanoic acid
Synonyms:	PFOA, C8
CAS #:	335-67-1
Chemical Formula:	$C_8HF_{15}O_2$
Chemical Structure:	$CF_3(CF_2)_6COOH$



Molecular Weight:	414.07
Physical State:	Solid
Melting Point:	54.3 ^oC

Boiling Point:	189 – 192.4 ^{o}C
Vapor Pressure:	0.017 mm Hg at 20 ^{o}C
Density:	1.8 g/cm^3 at 20 ^{o}C
Water Solubility:	9.5 g/L at 25 ^{o}C
Log octanol/water partition coefficient:	Not applicable (see below).
Taste Threshold (water):	No data
Odor Threshold (water):	No data
Odor Threshold (air):	No data

PFOA is a fully fluorinated carboxylic acid. Because carbon-fluorine bonds are among the strongest found in organic chemistry due to fluorine's electronegativity, PFOA and other PFCs are extremely stable and resistant to chemical reactions. PFOA is resistant to biodegradation, direct photolysis, atmospheric photooxidation, and hydrolysis, and is not known to degrade in the environment (Vaalgamaa et al., 2011).

PFOA contains a long perfluorocarbon tail that is both hydrophobic and oleophobic (repels both water and oil) and a charged end (the carboxylate group) that is hydrophilic. Because it forms a separate layer when mixed with hydrocarbons and water, measurement of the octanol:water partition coefficient is not practical (Prevedouros et al., 2006).

PFOA has been manufactured as salts such as ammonium perfluorooctanoate (APFO) or sodium perfluorooctanoate (NaPFOA) which dissociate in water. The PK_a of PFOA is 2.8. At the pH range found in drinking water (6.5-8.5) and within the body, PFOA is present almost totally in the non-volatile anionic form, the perfluorooctanoate anion (Goss, 2008; Rayne and Forest, 2010).

Production and Use

PFOA and other PFCs have been produced for use in commercial products and in industrial processes for over 60 years. Because of their unique surfactant properties and their resistance to chemical and thermal degradation, they have been used in many applications including water-, soil-, and stain-resistant coating for fabrics used in clothing, upholstery, and carpets, oil-resistant coatings for food contact paper, aviation hydraulic fluids, fire-fighting foams, paints, adhesives, waxes, and polishes, and other products. They are used industrially as surfactants, emulsifiers, wetting agents, additives, and coatings. PFOA is used as a processing aid (emulsifier) in the production of fluoropolymers such as polytetrafluoroethylene (PTFE) and fluoroelastomers used as non-stick coatings on cookware, membranes for waterproof/breathable clothing, electrical wire casing, fire and chemical resistant tubing, and plumbing thread seal tape (Lau et al., 2007; Buck et al., 2011; Lindstrom et al., 2011a; Post et al., 2012).

PFOA has been produced by two different manufacturing methods, electrochemical fluorination (ECF) and telomerization. The ECF process was primarily used from 1947 to 2002. In this process, 1-heptanecarbonyl fluoride is dissolved in anhydrous hydrogen fluoride, and an electrical current is passed through the solution causing all hydrogen atoms on the carbon backbone to be replaced with fluorine atoms. This process produces a mixture of isomeric forms including branched, linear, and cyclic isomers of various chain lengths (Prevedouros et al., 2006; Buck et al, 2011; Lindstrom et al., 2011a).

The second process, telomerization, has been primarily used since 2002. This process involves reacting pentafluoroiodoethane with tetrafluoroethane in the molar ratio that gives the desired chain length. The product of this reaction is then oxidized to form the carboxylic acid. This process produces straight chain (linear) PFOA (Prevedouros et al., 2006; Buck et al, 2011; Lindstrom et al., 2011a).

Historically, PFOA and PFOS were the two PFCs produced in the greatest amounts. PFOS was principally manufactured by the 3M Company, which completed its phase-out of production of this chemical in 2002. In 2006, the eight major U.S. producers of PFOA voluntarily agreed to reduce emissions and product content of PFOA and related substances, including precursors of PFOA, on a global basis by 95% by 2010 and to work towards elimination of these substances by 2015 (USEPA, 2016b). According to USEPA, reports submitted by the participating companies in 2013 and 2014 indicated that they were on track to achieve the goal of phasing out these chemicals by the end of 2015. However, other manufacturers and users of PFOA that are not participants in the voluntary agreement with USEPA continue to emit large amounts of PFOA to the environment, particularly in nations overseas including China, India, Russia, and Poland (USEPA, 2009; Lindstrom et al., 2011; OECD, 2015).

In 2009, the USEPA Office of Pollution Prevention and Toxics (OPPT) developed action plans for several groups of chemicals of concern including PFCs (USEPA, 2009a). According to the USEPA Action Plan, concerns about PFOA and other PFCs include their worldwide presence in the environment, wildlife, and humans; their persistence in the environment and bioaccumulative potential in humans and wildlife; and the significant adverse effects observed in wildlife and laboratory animals. USEPA stated that "given the long half-life of these chemicals in humans (years), it can reasonably be anticipated that continued exposure could increase body burdens to levels that would result in adverse outcomes."

USEPA (2009a) stated that PFOA and other long-chain PFCs are of concern for children's health, based on studies in laboratory animals that have demonstrated developmental toxicity, including neonatal mortality. They stated that: "Children's exposures are greater than adults due to increased intakes of food, water, and air per pound of body weight, as well as child-specific exposure pathways such as breast milk consumption, mouthing and ingestion of non-food items,

and increased contact with the floor. Biomonitoring studies have found PFCs in cord blood and breast milk, and have reported that children have higher levels of some PFCs compared to adults. Thus, given the pervasive exposure to PFCs, the persistence of PFCs in the environment, and studies finding deleterious health effects, USEPA will examine the potential risks to fetuses and children."

USEPA (2009a) stated that it intended to propose actions to address the potential risks from long-chain PFCs in 2012 under the Toxic Substances Control Act (TSCA). USEPA stated that potential actions could include banning or restricting their manufacture (including import), processing, and use, depending on the findings of more detailed analysis of information on these compounds.

In 2013, the European Chemical Agency (ECHA) Member State Committee unanimously agreed that PFOA should be classified as a Substance of Very High Concern (SVHC) because it has potential to cause reproductive toxicity and is persistent, bioaccumulative, and toxic (ECHA, 2013. ECHA (2015) is currently considering restrictions on the manufacture, marketing and use of PFOA, its salts and PFOA-related substances, as well as of articles and mixtures containing these substances.

GUIDANCE AND STANDARDS DEVELOPED BY NEW JERSEY, OTHER STATES, AND USEPA

New Jersey Health-based Drinking Water Guidance

New Jersey DEP developed chronic (lifetime) drinking water guidance for PFOA in drinking water of 40 ng/L in 2007 (NJDEP, 2007). The basis for the NJDEP guidance was subsequently published in a peer-reviewed journal (Post et al., 2009a).

The New Jersey guidance is based on the NOAELs (No Observed Adverse Effects Levels) and LOAELs from toxicology studies identified in the draft USEPA (2005a) PFOA risk assessment and considered the conclusions of the USEPA Science Advisory Board (2006) review of this draft risk assessment. The draft USEPA (2005a) risk assessment compared PFOA exposures prevalent within the U.S. general population with NOAELs and LOAELs for various life stages identified in toxicology studies. As such, the USEPA (2005a) draft risk assessment did not develop a Reference Dose or a cancer slope factor for PFOA, and it did not address the relationship between drinking water concentration and human body burden, as measured by serum level.

Because the half-life of PFOA is much longer in humans (several years) than in the animal species used in the toxicological studies (several hours to 30 days), a given external dose (mg/kg/day) results in a much greater internal dose (as indicated by serum level) in humans than in animals. Therefore, comparisons between effect levels in animal studies and human exposures

were made on the basis of serum levels rather than external dose. This approach was recommended by USEPA (2005a) and the USEPA Science Advisory Board (2006).

Target Human Serum Levels (analogous to RfDs, but on a serum level basis) were derived by applying UFs to the measured or modeled serum levels at the NOAELs or LOAELs identified by USEPA (2005a). The default RSC of 20% was applied to the Target Human Serum Levels to account for contributions to serum PFOA from non-drinking water exposures. The default RSC value is used when the relative contributions of drinking water versus non-drinking water sources are not fully characterized, as is the case for PFOA.

USEPA (2005a) classified PFOA as having "suggestive evidence of carcinogenic potential", whereas the USEPA Science Advisory Board (2006) disagreed and recommended a classification of "likely to be carcinogenic to humans". For the cancer end point, the serum level resulting in a one in one million (10^{-6}) risk level was estimated by linear extrapolation from the modeled serum level in animals at a dose resulting in an approximate 10% tumor incidence.

The mean ratio of approximately 100:1 between serum PFOA levels and drinking PFOA water concentrations in exposed communities was used to determine the drinking water concentrations that are expected to result in a given increase in serum PFOA level (Post et al., 2009a). Data supporting a ratio of 100:1 or greater is discussed in the Toxicokinetics section below. Because this approach is based on the observed relationship between serum and drinking water concentrations, assumptions for body weight, volume of water ingested daily, or half-life of PFOA in humans or experimental animals were not explicitly used in the calculation of the health-based drinking water concentrations.

The range of health-based drinking water concentrations for the seven endpoints assessed was 0.04-0.26 μg/L, and several of the concentrations fell within a similar range (0.04, 0.05, 0.06, 0.07, and 0.08 μg/L). The most sensitive endpoints, resulting in a drinking water concentration of 40 ng/L, were decreased body weight and hematological effects in the adult female rat in a chronic dietary study (Sibinski, 1987). This value was determined to be protective for carcinogenic effects, as the drinking water concentration at the 10^{-6} cancer risk level was estimated as 60 ng/L.

It should be noted that a large body of health effects information, including toxicology studies reporting sensitive developmental effects in mice and epidemiology studies reporting associations of PFOA with numerous health effects, has become available subsequent to the USEPA (2005a) risk assessment that served as the basis for the New Jersey guidance. These data were therefore not considered in the development of the NJDEP (2007) guidance, and they are considered in the development of the recommended Health-based MCL presented in this document.

USEPA Drinking Water Health Advisory

In May 2016, the USEPA Office of Water finalized a drinking water Health Advisory for PFOA of 70 ng/L (USEPA, 2016a). This Health Advisory is intended to apply to both lifetime exposure and short term exposure. It replaces the earlier the USEPA Office of Water (2009b) Provisional Health Advisory for PFOA of 400 ng/L, developed in 2009, which was stated to be intended to protect for "short-term exposure" (defined by the USEPA Integrated Risk Information System (IRIS) as up to 30 days; USEPA, 2011a).

USEPA (2016c) also finalized a Health Advisory for PFOS of 70 ng/L, and USEPA (2016d) states that the total concentration of PFOA and PFOS in drinking water should not exceed 70 ng/L.

A detailed discussion of the basis for the USEPA (2016a) Health Advisory for PFOA and a comparison with the recommended DWQI Health-based MCL are provided in Appendix 2. In summary, the USEPA Health Advisory is based on a Reference Dose (RfD) of 20 ng/kg/day. The RfD is based on delayed ossification and accelerated puberty in male offspring in a mouse developmental toxicology study (Lau et al., 2006). The default Relative Source Contribution factor of 20% was used to account for non-drinking water exposures. The USEPA Health Advisory uses a drinking water consumption rate of 0.054 L/kg/day, based on the 90th percentile for lactating women, which is higher than the default consumption rate of based on adult exposure factors.

Figure 1 shows the predicted increases in serum PFOA levels from ongoing exposure in drinking water at the USEPA Health Advisory (70 ng/L), the NJDEP (2007) guidance (40 ng/L), and the Health-based MCL (14 ng/L) recommended in this document. Predictions based on both average (0.016 L/kg/day) and upper percentile (0.029 L/kg/day) drinking water ingestion rates are shown. A clearance factor developed by USEPA scientists (Lorber and Egeghy, 2011) to relate human PFOA exposures to human serum PFOA levels was used to predict the increases in serum PFOA from exposures to these level in drinking water. With average water consumption, ongoing exposure to 70 ng/L (the USEPA Health Advisory) is predicted to increase serum PFOA by 8.0 ng/ml, a 4.8-fold increase from the U.S. general population (NHANES) median of 2.1 ng/ml (CDC, 2015). With upper percentile water consumption, the increase in serum PFOA level from 70 ng/L is predicted as 14 ng/ml, a 7.7-fold increase from the general population (NHANES) median.

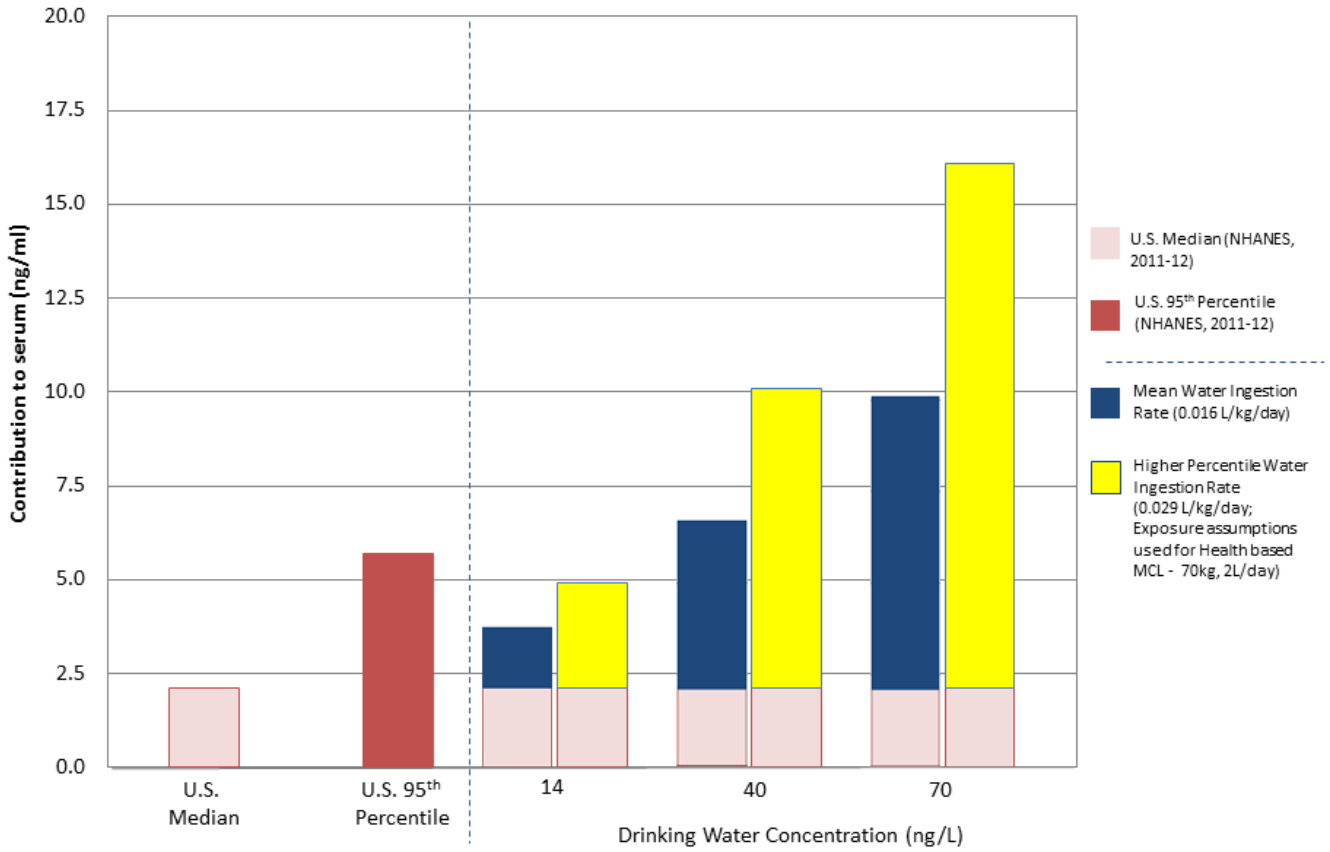


Figure 1. Increases in serum PFOA concentrations predicted from consumption of drinking water with various concentrations of PFOA (14 ng/L – Recommended Health-based MCL; 40 ng/L – NJDEP guidance (2007); 70 ng/L – USEPA Lifetime Health Advisory).

Guidance and standards of other states

Several other states (e.g. Minnesota, Maine, and North Carolina) developed standards or guidance for PFOA in drinking water or groundwater prior to 2016. Because many states have stated that their earlier PFOA values will be updated or are under review in light of the finalization of the USEPA (2016a) Health Advisory, the basis for these values is not presented here.

In 2016, Vermont developed a drinking water health advisory (VT DOH, 2016) and an Interim Ground Water Enforcement Standard (VT DEC, 2016) for PFOA of 20 ng/L. These Vermont values are based on the Reference Dose (RfD) of 2 x 10^{-5} mg/kg/day from the draft USEPA (2014) PFOA Health Advisory (which is the same as the RfD in the final USEPA [2016a] PFOA Health Advisory), drinking water exposure assumptions for a child less than 1 year of age (instead of default adult exposure assumptions), and the default Relative Source Contribution (RSC) factor of 20%.

ENVIRONMENTAL FATE, TRANSPORT, AND OCCURRENCE

Environmental Fate and Transport

Because of the extreme stability of their carbon−fluorine bonds, PFOA and other PFCs are extremely resistant to degradation in the environment and thus persist indefinitely (Buck et al., 2011; Lindstrom et al., 2011a). As discussed above, the production and use of PFOA and its precursors has been phased out by major U.S. manufacturers. However, environmental contamination and resulting human exposure to PFOA are anticipated to continue for the foreseeable future due to its environmental persistence, formation from precursor compounds, and continued production by other manufacturers.

PFOA and other PFCs are found in many environmental media (e.g. drinking water, surface water, groundwater, air, sludge, soils, sediments, outdoor and indoor dust, and ice caps) in locations around the world including remote polar regions (Lau et al., 2007). PFCs are also found in wildlife (fish, birds, mammals) including in remote polar regions. However, the bioconcentration factor for PFOA is lower than for PFOS or longer chain perfluorocarboxylates such as PFNA (Martin et al., 2003; Conder et al., 2008), and concentrations of PFOA in wildlife in remote locations are generally lower than for these other compounds (Butt et al., 2010).

Two major pathways have been proposed for long-range transport of PFOA and other PFCs to remote locations worldwide, including the Arctic (Figure 2; Lau et al., 2007, 2013; Butt et al., 2010). The relative contributions of each of these pathways are not known. The first pathway involves the atmospheric transport of volatile precursors such as 8:2 fluorotelomer alcohol (8:2 FTOH), followed by oxidation of the precursors to PFOA and other PFCs which are then deposited onto the land or the water. The second pathway involves long-range aqueous transport of emitted perfluorinated carboxylates such as PFOA in their anionic forms to remote locations by currents on the ocean's surface.

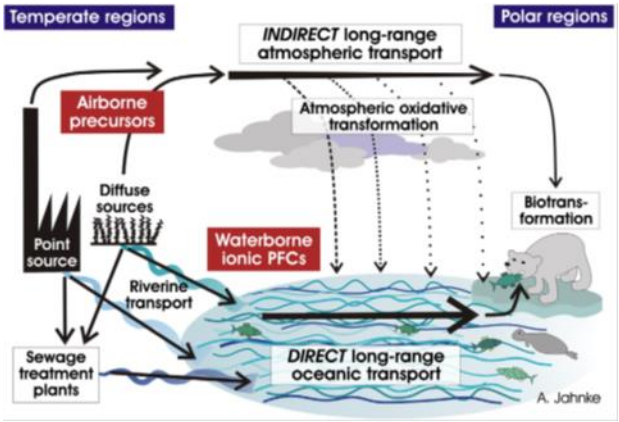


Figure 2. Major transport pathways of PFCs to the Arctic (and other remote locations), by Annika Jahnke (Butt et al., 2010)

Fate and Transport Relevant to Drinking Water Contamination

PFOA and some other PFCs are distinct from other persistent and bioaccumulative organic compounds because of their importance as drinking water contaminants. PFOA exists predominantly as an anion under environmental conditions, does not bind well to soil, migrates readily from soil to groundwater, and is highly water-soluble (Davis et al., 2007). These properties of PFOA differ from those of other persistent and bioaccumulative organic pollutants such as polychlorinated dioxins and furans, PCBs, and pesticides like chlordane and DDT. These other compounds are generally not significant as drinking water contaminants because they have high octanol/water partition coefficients. Thus, they have a high affinity for soil and sediments but low water solubility (Post et al., 2011).

PFOA that is released to the environment can contaminate surface water and groundwater used as sources of drinking water. Sources of PFOA in the environment include discharge to air and water from industrial facilities where it is made or used (Davis et al., 2007); release of aqueous firefighting foams, particularly at military sites, airports, and fire fighter training facilities (Moody et al., 2003; Backe et al., 2013); disposal in landfills (Eggen et al., 2010); discharge from wastewater treatment plants treating domestic and/or industrial waste (Sinclair and Kannan, 2006); street runoff (Murakami et al., 2009); storm water runoff (Kim and Kannan, 2007); land application of biosolids (sludge) from wastewater treatment plants treating industrial waste (Clarke and Smith, 2011; Lindstrom et al., 2011b; Sepulvado et al., 2011); land application of wastewater from industrial sources (Konwick et al., 2008); and use of contaminated industrial waste as a soil amendment (Skutlarek et al., 2006; Hölzer et al., 2008).

Environmental transport pathways that can result in surface water and groundwater contamination by PFOA after release from an industrial source are shown in Figure 3 (Davis et al., 2007) and were reviewed by Lau et al. (2007) and Butt et al. (2010).

As is the case for other groundwater contaminants, PFOA can reach drinking water wells via the well-established pathway of migration of a groundwater plume that has been contaminated either directly from surface spills or by contaminated surface water mixing with groundwater drawn in by pumping wells. Unlike many other environmental contaminants, PFOA can also reach groundwater from air emissions from nearby industrial facilities, followed by deposition from air onto soil, and migration through the soil to groundwater (Davis et al., 2007).

In West Virginia and Ohio, drinking water wells as far as 20 miles away were contaminated with PFOA by releases from an industrial facility where it was used as a processing aid in fluoropolymer production. Groundwater contamination occurred via soil deposition of PFOA that had been emitted into the air followed by migration to groundwater, and, to some extent, recharge of the groundwater aquifer with contaminated surface water from the Ohio River (Steenland et al., 2009a; Shin et al., 2011). PFOA was detected in public water supply wells in

this vicinity at levels up to > 4000 ng/L (DuPont and URS Diamond Corporate Remediation Group, 2008) and in private wells at up to >13,000 ng/L (Hoffman et al., 2011). In New Jersey, PFOA was detected at up to 190 ng/L in shallow unconfined wells of a public water supply located near an industrial source (Post et al., 2009a), and at > 40 ng/L, with a maximum above 400 ng/L, in 59 of 104 private wells within a radius of slightly more than 2 miles of this facility (DuPont, 2009); contamination of the distant wells was likely due to air deposition (Post et al., 2012).

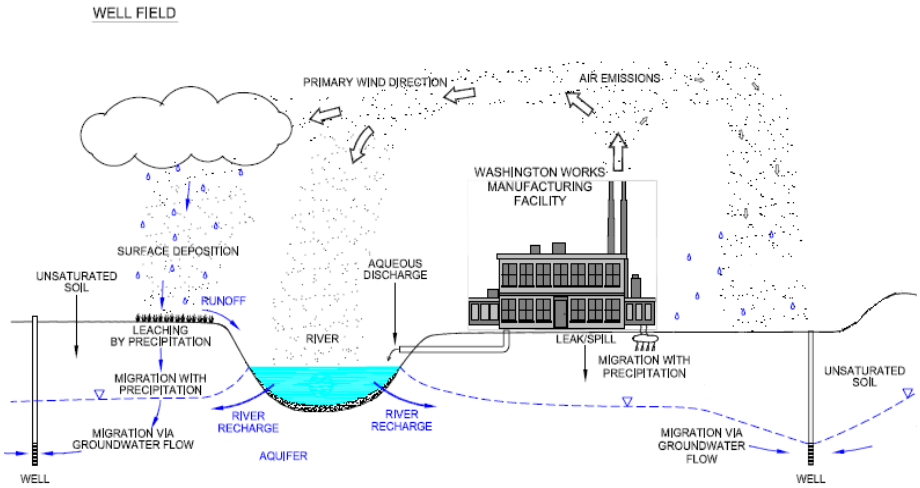


Figure 3. APFO (PFOA) transport near discharge source (Davis et al., 2007)

<u>Formation from precursor compounds</u>

An additional source of PFOA in the environment is the breakdown of precursor compounds such as the fluorotelomer alcohol, 8:2 FTOH [$F_3(CF_2)_7CH_2\,CH_2OH$], used industrially and in consumer products (Butt et al., 2010; Buck et al., 2010; Butt et al., 2014).

$$\text{8:2 FTOH } [CF_3(CF_2)_7CH_2\,CH_2OH] \rightarrow \text{PFOA } [CF_3(CF_2)_6COOH]$$

Larger molecules such as polyfluoroalkyl phosphoric acid diesters (diPAPs) (e.g. diPAPs 8:2; Figure 4) are found in greaseproof food contact papers, wastewater treatment plant sludge, and paper fibers from paper mills (D'eon et al., 2009). These larger molecules release 8:2 FTOH that can degrade to PFOA.

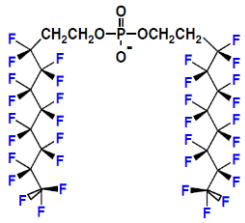


Figure 4. Structure of diPAPs 8:2

PFOA is formed from these precursor compounds through biodegradation in soil, sludge, and wastewater (Sinclair and Kannan, 2006; Lee et al., 2010) as well as through chemical reactions in the atmosphere (Figure 2). PFOA and other PFCs have been found at higher concentrations in effluent than influent at wastewater treatment plants. This increase is believed to result from the biodegradation of telomer alcohols and other precursors from domestic and industrial sources within the wastewater treatment plant (Sinclair and Kannan, 2006; Lee and Mabury, 2011). Fluoroacrylate polymers, used in commercial products, may also degrade in soil to release FTOH which can degrade to PFCs such as PFOA (Russell et al., 2008; Washington et al., 2009). Since PFOA, once formed, does not degrade appreciably, environmental PFOA levels are increased by conversion of even a small fraction of the precursors to the terminal breakdown product, PFOA.

Occurrence in drinking water

PFOA and other PFCs occur in raw and finished drinking water from both groundwater and surface water sources in New Jersey, other parts of the United States, and nations around the world (reviewed by Mak et al., 2009; Post et al., 2012; Post et al., 2013). PFOA and other PFCs are found in drinking water impacted by discharges from industrial facilities, release of aqueous firefighting foam, and other known sources of contamination, as well as where the source is unknown (Post et al., 2012).

PFOA has been detected at high frequency in some river basins that are important sources of drinking water. For example, it was detected (>1 ng/L) in 82.3% of samples from 80 locations throughout the Cape Fear River (North Carolina) drainage basin, population 1.7 million, at a median of 12.6 ng/L and a maximum of 287 ng/L (Nakayama et al., 2007). In the Upper Mississippi River drainage basin in the Midwestern U.S., population 30 million, it was detected (>1 ng/L) in 73% of 88 locations with a median of 2.07 ng/L and a maximum of 125 ng/L. Elevated levels at certain sites were attributed to point sources in this study (Nakayama et al., 2010). In the Tennessee River in Alabama, PFOA levels were 395±128 ng/L in samples from the 35 river miles downstream of the site of discharge from a fluorochemical manufacturing facility, with the highest levels (521-598 ng/L) in the 6 river miles furthest downstream (Hansen et al., 2002). In Germany, PFOA and other PFCs in organic material applied to agricultural land contaminated the Moehne and Ruhr Rivers, important sources of drinking water. PFOA was detected at up to 33,900 ng/L in a creek near the site of contamination upstream of these rivers, and at up to 519 ng/L in drinking water from the Moehne River (Skutlarek et al., 2006).

PFOA and other PFCs are not effectively removed from drinking water by standard treatment processes such as coagulation/flocculation, sand filtration, sedimentation, medium-pressure ozonation, chloramination, and chlorination. However, PFOA can be removed from drinking water by granular activated carbon (GAC) or reverse osmosis (Rumsby et al., 2009, Bartell et al., 2010a, Tagaki et al., 2011; Eschauzier et al., 2012; Appleman et al., 2014; DWQI, 2015b). Therefore, unless specific treatment for removal of PFCs is in place, concentrations of PFOA and

other PFCs detected in raw drinking water can be considered to be representative of concentrations in finished drinking water (Post et al., 2013).

Occurrence in New Jersey drinking water

Considerable information is available on the occurrence of PFOA and other PFCs in New Jersey public water systems (PWS). This includes data from 53 PWS from two NJDEP occurrence studies of PFCs, substantial additional data submitted to NJDEP by PWS and other parties, and data from the nationwide USEPA Unregulated Contaminant Monitoring Rule 3 (UCMR3) survey. For the two NJDEP occurrence studies and most of the additional data submitted to NJDEP, analysis of samples was performed by certified laboratories with Reporting Levels (RLs) that were generally 4-5 ng/L or lower. To the knowledge of the Health Effects Subcommittee, statewide drinking water studies of PFOA with sensitive RLs such as these have not been conducted in states other than New Jersey. In contrast, the RL for PFOA in USEPA UCMR3 is much higher (20 ng/L) than the RLs in the other NJ PWS monitoring data.

NJDEP studies of occurrence in New Jersey public water systems

Following detection of PFOA in a New Jersey PWS at up to 190 ng/L in a groundwater source and up to 64 ng/L in tap water, two statewide studies of the occurrence of PFOA and other PFCs in drinking water were conducted by NJDEP. The 2006 study tested 23 PWS for PFOA and PFOS, and the 2009-10 study tested 33 additional PWS for PFOA, PFOS, and eight other PFCs (NJDEP, 2007b; NJDEP, 2014; Post et al., 2009a; Post et al., 2013).

The 2006 NJDEP study included 29 samples of raw and/or finished water from 23 NJ PWS including 14 with groundwater sources, 8 with surface water sources, and one using both groundwater and surface water. In the 4 PWS where both raw and finished water were analyzed, PFOA concentrations were similar in both samples. Of the PWS in this study, PFOA was detected in 15 of 23 systems (65%) at or above the RL (4 ng/L), and in 3 of 23 systems below the RL. PFOA was detected above the RL (9 of 13) at up to 33 ng/L, or below the RL (1 of 13), in 10 of 13 groundwater samples (77%) from unconfined or semiconfined aquifers, but was not detected in the two groundwater samples from confined aquifers. Additionally, PFOA was detected above the RL (7 of 9; 78%) at up to 39 ng/L, or below the RL (2 of 9; 22%), in samples from all 9 PWS using surface water sources. In this study, PFOS was detected (>4 ng/L) in 30% of the PWS, less frequently than PFOA (NJDEP, 2007; Post et al., 2009a).

The 2009-2010 NJDEP study tested raw water from 30 PWS for PFOA, PFOS, and 8 other PFCs. The sites for this study were chosen for geographic diversity, representing 19 of NJ's 21 counties. The study included 18 PWS with groundwater sources (17 unconfined, one confined) and 12 PWS with surface water sources. One or more PFC was detected (>5 ng/L) at 21 sites (70%), with the number of individual compounds detected varying from one (in 8 samples) to a maximum of 8 in one sample. PFOA was the most commonly detected PFC (17 of 30 samples;

57%), including 6 of 18 of groundwater samples (33%) and 11 of 12 of surface water samples (92%). When PFOA was detected, other PFCs were often but not always found in the same sample. PFOA was found at the highest maximum concentration of any of the PFCs analyzed in the study, 100 ng/L. This highest detection was in a PWS intake from a river, and the likely source was subsequently identified as discharge from an upstream facility that made and used products containing PFOA and other PFCs (Post et al., 2013; NJDEP, 2014).

NJDEP database of PFCs in New Jersey Public Water Systems

The NJDEP Division of Science, Research, and Environmental Health maintains an internal database of PFC results from NJ PWS including the two NJDEP occurrence studies, additional raw and finished water data submitted to NJDEP by PWS and other parties, and detections from UCMR3 data. As of January 2016, the database included 1035 samples (423 raw water, 549 finished water, and 63 distribution system) from 282 sampling locations in 80 PWS (including 72 PWS with data from NJDEP studies and/or submitted to NJDEP, and 8 additional PWS with PFC detections in UCMR3). Of these samples, 374 were analyzed for only PFOA and PFOS, and 661 were analyzed for a broader suite of PFCs.

PFOA was the most frequently detected PFC in NJ PWS. It was detected at some level in 65% of 72 PWS included in the NJDEP database (excluding UCMR3 data; Table 1). The highest detection in finished water was 100 ng/L, and concentrations exceeding 40 ng/L were reported in at least one finished water sample from 12 of 72 PWS (17%). It was also detected at >20 ng/L in UCMR3 monitoring in finished water from six additional PWS that are not otherwise included in the database, including two PWS that had levels above 40 ng/L.

Table 1. PFOA concentrations in raw or finished water from PWS included in NJDEP database*

PFOA Concentration (ng/L)	Number of PWS	% of PWS
ND**	25	35%
RL - <10**	15	21%
10 - <20**	10	14%
20 - <40	10	14%
>40	12	17%

*Data shown are highest concentration found in raw or finished water from the PWS. Levels in finished water from some water supplies included may be lower because several raw water sources are blended in the treatment plant.

**Reporting levels (RLs) vary among samples and range from 2.5 - 20 ng/L. Therefore, the percentage of PWS with RL-<10, and 10 - <20, may actually be higher than shown.

Comparison of NJ occurrence to nationwide UCMR3 data and studies from other nations

Data on PFOA in PWS in New Jersey and nationwide is available through the USEPA UCMR3. Under UCMR3, nationwide monitoring of finished water for 30 unregulated contaminants, including PFOA and 5 other PFCs, was conducted in 2013−2015 by all U.S. large PWS (serving

more than 10,000 people) and 800 representative smaller PWS (serving less than 10,000 people) (USEPA, 2012b). UCMR3 data therefore provide useful information on occurrence of PFCs in NJ in comparison to the rest of the United States. However, comparison of the UCMR3 PFC data with other New Jersey PFC occurrence data is complicated by the fact that the UCMR3 RLs for PFOA (20 ng/L) and other PFCs are much higher than the RLs for other PFC data in the NJDEP database (generally $\leq$ 5 ng/L).

UCMR3 monitoring in New Jersey includes all 165 large community PWS, 13 of about 435 small community PWS, and 8 of about 700 non-transient non-community water systems. A comparison of national versus New Jersey PFC data from UCMR3 reported through January 2016 is shown in Table 2 (data obtained from USEPA, 2016f). PFOA was detected ($\geq$ 20 ng/L) in PWS at locations throughout the state, and was detected more than five times more frequently in New Jersey PWS (10.53%) than nationally (1.93%). PFNA was also detected much more frequently in NJ (2.34%) than nationally (0.22%). However, PFNA was detected only in the vicinity of a likely industrial source located in Gloucester County (DWQI, 2015c) but not in other parts of New Jersey. The occurrence of the other PFCs included in UCMR3 (PFHpA, PFOS, PFHxS, PFBS) was similar or slightly higher in New Jersey compared to nationally.

Table 2. New Jersey versus national UCMR3 PFC occurrence data as of January 2016

*Compound**	*Reporting Level (RL) (ng/L)*	*New Jersey*			*United States (other than NJ)*		
		Number of PWS	*Number above RL*	*Percent above RL*	*Number of PWS*	*Number above RL*	*Percent above RL*
PFOA	20	171	18	10.53 %	4617	89	1.93 %
PFNA	20	171	4	2.34 %	4617	10	0.22 %
PFHpA	10	171	5	2.92 %	4617	77	1.67 %
PFOS	40	171	5	2.92 %	4617	88	1.91 %
PFHxS	30	171	2	1.17 %	4617	52	1.13 %
PFBS	90	171	0	0 %	4617	6	0.13 %

*PFHpA – perfluoroheptanoic acid (C7); PFBS – perfluorobutane sulfonate; PFHxS – perfluorohexane sulfonate.

The occurrence of PFCs in NJ PWS in the 2009-10 NJDEP study was also compared to similar occurrence studies in other nations by Post et al. (2013). PFOA was detected more frequently and at a higher maximum concentration in the 2009-10 New Jersey PWS study than in comparable drinking water studies in France, Spain, and China which had RLs similar to the RL in the NJ study.

Occurrence in NJ private wells

A statewide study of PFOA or other PFCs in New Jersey private wells has not been conducted. PFOA was detected at >40 ng/L, with a maximum above 400 ng/L, in 59 of 104 private wells within a radius of slightly more than 2 miles of a New Jersey industrial source (DuPont, 2009);

contamination of the distant wells was likely due to air deposition. More recently, PFOA has been detected in private wells near another facility which used and discharged a mixture of PFCs that consisted primarily of PFNA and also contained PFOA (DWQI, 2015c).

HUMAN BIOMONITORING

Human biomonitoring studies show that exposure to PFOA and/or its precursors is ubiquitous in the U.S. and throughout the world. PFOA has a human half-life of several years and remains in the body for a long period of time after exposure occurs. Data on blood serum concentrations from the general population, communities with contaminated drinking water, and workers with occupational exposure are summarized below. Consumption of contaminated drinking water results in increased blood serum concentrations, while the highest blood serum concentrations have been found in occupationally exposed workers. PFOA is detected in human breast milk, amniotic fluid, and umbilical cord blood, demonstrating that exposure occurs during prenatal and postnatal development, and it has also been detected in human seminal fluid.

Blood Serum

General population

PFOA and other PFCs are present in the serum of the general population in the United States and in countries worldwide. This topic was recently comprehensively reviewed by Kato et al. (2015).

Data from archived serum samples from the United States and Norway indicate that human exposure to PFOA has been ongoing for decades, and that exposure increased greatly in the 1980s in these two locations. Analysis of serum samples collected up to 56 years ago found that the median level in serum from pregnant California women sampled in 1960-63 (n=40) was 0.27 ng/ml, approximately 10-fold lower than the median in serum from California women sampled in 1981-86 (n=30) and 2009 (n=35), which were 2.71 and 2.08 ng/ml, respectively (Wang et al., 2011a). In pooled serum samples from Norwegian men (age 40-50) collected over a 29 year period (1977-2006), PFOA levels gradually increased from 0.58 ng/ml in 1976 to 4.9 ng/ml in 2001, an 8-fold increase, followed by a yearly decline to 2.7 ng/ml in 2006. A similar temporal pattern was seen in serum samples collected from Norwegian children and male and female adults of other age groups between 1976 and 2007 (Haug et al., 2009).

The largest studies of the U.S. general population are from the National Health and Nutrition Examination Survey (NHANES) conducted by the U.S. Centers for Disease Control and Prevention (CDC) (Kato et al., 2011; CDC, 2015) and American Red Cross blood donors (Olsen et al., 2012). PFOA is one of four PFCs (PFOA, PFOS, PFNA, and perfluorohexane sulfonate [PFHxS]) that have been detected in the serum of greater than 99% of a representative sample of the U.S. population, age 12 or older, in NHANES (Kato et al., 2011). PFOA and these other PFCs are biologically persistent, with human half-lives of several years, as discussed in the

Toxicokinetics section below.

Data from six cycles of NHANES monitoring between 1999-2000 and 2011-12 show that serum PFOA levels have decreased in the U.S. general population during this time period (Table 3). In the first NHANES (1999-2000), the geometric mean serum concentration was 5.21 ng/ml and the 95th percentile was 11.9 ng/ml, while the most recent NHANES (2011-12) found a geometric mean and 95th percentile of 2.08 and 5.68 ng/ml, respectively. In the NHANES surveys, PFOA concentrations were lower in those 12-19 years of age than in older individuals, and were somewhat higher in males than females. In data from the three ethnic groups that were analyzed over time, levels were consistently lowest in Mexican Americans, intermediate in non-Hispanic blacks, and highest in non-Hispanic whites (CDC, 2015).

Table 3. Serum PFOA concentration from NHANES (ng/ml)

Year	*Geometric Mean*	*Percentile*				*n*
		50th	*75th*	*90th*	*95th*	
2011-12	2.08	2.08	3.03	4.35	5.68	*1904*
2009-10	3.07	3.20	4.60	6.00	7.50	*2233*
2007-08	4.12	4.30	5.90	7.90	9.60	*2100*
2005-06	3.92	4.20	6.20	9.00	11.3	*2120*
2003-04	3.95	4.10	5.80	7.80	9.80	*2094*
1999-2000	5.21	5.20	6.90	9.40	11.9	*1562*

CDC, 2015

A similar pattern of decreasing serum PFOA concentrations over time was seen in three studies of American Red Cross blood donors in 2000-2001, 2006, and 2010 (Olsen et al., 2012). Each study included samples from 600-645 subjects from six locations throughout the U.S., with an approximately equal number in each of five 10-year age categories (20-29 through 60-69 years of age) from each location. Geometric means and 95th percentile concentrations, respectively, were 4.7 and 12.0 ng/ml in 200-01, 3.44 and 7.9 ng/ml in 2006, and 2.44 and 6.6 ng/ml in 2010. As in the NHANES studies, serum concentrations were generally higher in males than females.

Serum PFOA levels are generally comparable to those found in the U.S in developed countries throughout the world, including Europe, Asia, and Australia (Post et al., 2012; Kato et al., 2015). In contrast to industrialized nations where serum PFOA is almost universally detected, PFOA was detected at > 0.5 ng/ml in only 12 of 55 serum samples from Afghan children and adults, with a maximum of 1.5 ng/ml; relatively low serum levels have also been reported in other developing countries where exposure to PFOA and other PFCs may be lower than in industrialized nations (Hemat et al., 2010).

PFOA concentrations in pooled serum samples from children (age 3-11) in 2001-2002 NHANES ranged from about 6-8 ng/ml, significantly higher than in pooled serum samples from adults in this study (Kato et al., 2009). Median and maximum serum PFOA levels in 300 Texas children, age <1 to 12 years, were 2.85 ng/ml and 13.50 ng/ml; adults were not included in this study. In the Texas study, the median level did not differ between genders, and was lower in those less than 3 years of age than in the older age groups (Schecter et al., 2011). Exposures to infants and young children are discussed in detail in the section on developmental exposures below.

Communities with drinking water exposures

Continued exposure to even relatively low concentrations of PFOA in drinking water concentrations results in substantial increases in serum levels. The quantitative relationship between drinking water exposure and human serum PFOA levels is discussed below.

A recent study (Hurley et al., 2016) found substantially increased serum PFOA levels in individuals served by PWSs reporting detection of PFOA in UCMR3 monitoring. PFOA detections were relatively low, ranging from 20 ng/L (the UCMR3 RL) to 53 ng/L, with a mean of 28 ng/L. The study group consisted of middle aged and older California women (n=1,333; 70% between 60 and 79 years of age). Of this group, 4.5% resided in a zipcode where a PWS reporting detection of PFOA in UCMR3 monitoring is located. The distribution of serum concentrations differed significantly ($p<0.0001$) in those served by a PWS where PFOA was detected ("exposed") as compared to those served by a PWS without a detection ("unexposed"). The median serum PFOA concentrations in the "exposed" group was 38% higher (3.46 ng/ml) than in the "unexposed" group (2.51 ng/ml). The authors note that the contribution of drinking water to serum PFOA is likely actually greater than observed in the study since some of those classified as "exposed" may have received their drinking water from another point of entry (e.g. treatment plant) within the PWS that detect PFOA. Additionally, the serum PFOA levels of some participants classified as "not exposed" may have been increased by PFOA in drinking water at concentrations below the UCMR3 RL of 20 ng/L.

Public water supply wells and private wells in several Ohio and West Virginia communities were contaminated by PFOA emissions from an industrial facility. In Little Hocking, Ohio, the concentration in drinking water was 3550 ng/L in 2002-2005, and the median serum PFOA concentration in 282 individuals tested in 2004-2006, with occupationally exposed individuals excluded, was 371 μg/L (Emmett et al., 2006a). The C8 Health Study (described in more detail below) is a much larger study of Little Hocking and several other communities in this vicinity with drinking water PFOA concentrations ranging from >50 ng/L to over 3000 ng/L (Post et al., 2009a). In approximately 69,000 C8 Health Study participants, including some with occupational exposures, the median serum PFOA concentration in 2005-2006 was 28.2 ng/ml, as compared to the median of 4 ng/ml in the 2003-2004 NHANES study (Steenland et al., 2009a). The upper 25% of C8 Health Study participants had serum PFOA levels greater than 71 ng/ml, only slightly

below the highest concentration found in 2003-2004 NHANES, 77.1 ng/ml. As in the NHANES and Red Cross blood donor studies of the general population discussed above, the median serum concentrations in males (33.7 ng/ml) were higher than females (23.7 ng/ml) in this large study of an exposed population.

Emmett et al. (2006a) observed higher serum levels in children ages 2-5 than in older children and adults in their study of Little Hocking, Ohio residents with exposure to PFOA in drinking water. Adults over 60 years of age also had higher levels than other age groups. In the larger C8 Health Study population, serum concentrations were also higher in children and older adults, with the lowest levels in those age 20-29 (Steenland et al., 2009a). More recently, Shin et al. (2011) estimated a median one-year old child:maternal serum ratio of 1.27:1 from data on 40 child-mother pairs in the C8 Health Study. In a much larger study of almost 5000 mother-child pairs from the C8 Health Study, serum levels in children up to age 12 were higher than in their mothers in this population; in the youngest age category ($\leq$ 5 years), mean levels were 44% higher than maternal levels (Mondal et al., 2012).

Serum PFOA levels were also higher than in the general population in several other communities with exposure from drinking water. These include communities in Germany whose surface water source of drinking water was contaminated by runoff from industrial waste used as a soil amendment (Hölzer et al., 2008); in Alabama where a river used as a public water supply source was contaminated by industrial discharge, and PFOA from contaminated biosolids applied to agricultural land reached drinking water wells (ATSDR, 2013); in Minnesota communities where private wells and public water supply wells were contaminated by disposal of industrial waste (Landsteiner et al., 2014); in Pease, NH where public water supply wells were contaminated by military use of aqueous firefighting foam (NHDHHS, 2015); and in Hoosick Falls, NY, where drinking water was contaminated by releases from an industrial facility (NYS DOH, 2016)

Occupationally exposed workers

Serum PFOA levels in workers at facilities where PFOA is made or used in fluoropolymer production are much higher than in the general population. Biomonitoring data from workers at such facilities were reviewed by Olsen (2015). Mean or median serum concentrations of several 1000 ng/ml (several ppm) were reported for some job categories at some facilities, with maximum serum concentrations of over 100,000 ng/ml (100 ppm), although levels in most workers were lower. In the C8 Health Study participants, the median serum level among those currently working at the Washington Works plant where PFOA was used in fluoropolymer production (n=1,171) was 148 ng/ml, as compared to 24 ng/ml in those who did not work there at or prior to the time of sampling (Steenland et al., 2009a).

Serum concentrations of PFOA and other PFCs are also elevated in professional ski waxing technicians due to exposures to fluorinated ski waxes that contain both the compounds themselves and their precursors (reviewed by Olsen, 2015).

Other human biological matrices

Seminal plasma

PFOA and other PFCs were found in human seminal plasma in a study of Sri Lankans. The mean and median concentrations were 6.38 and 4.02 ng/ml, respectively, and PFOA concentrations were significantly correlated with serum PFOA concentrations (Guruge et al., 2005).

Amniotic fluid

PFOA was detected in amniotic fluid in a study in the United States (Stein et al., 2012). The median blood serum:amniotic fluid concentration ratio was about 13:1.

Umbilical cord blood serum

PFOA and other PFCs were detected in numerous studies of umbilical cord blood from the general population worldwide in studies reviewed by Post et al. (2012). Locations of these studies included Baltimore, Maryland (Abelberg et al, 2007), Ontario, Canada (Monroy et al, 2008), Denmark (Fei et al., 2007), Germany (Midasch et al., 2007), Norway (Gützkow et al., 2011), the Faroe Islands (Needham et al., 2011), Australia (Toms et al., 2009), South Africa (Hannsen et al., 2010), Korea (Kim et al., 2011), and Taiwan (Lien et al., 2011). Mean serum (or plasma) levels in these studies ranged from 1.1 ng/ml in Korea (Kim et al., 2011) to 4.4 ng/ml in Taiwan (Lien et al., 2011). No geographic pattern was apparent from this dataset, as the levels reported from Africa, Australia, Europe, and North America fell in between the levels in the two Asian studies. More recent additional data are reviewed by Kato et al. (2015).

Breast milk

PFOA was detected in human breast milk in studies from locations worldwide (reviewed by Liu et al., 2010; White et al., 2011a; Post et al., 2012) including Massachusetts (Tao et al, 2008a), Japan (Tao et al., 2008b), China (So et al., 2006; Liu et al., 2010), Korea (Kim et al., 2011), Belgium (Roosens et al., 2010), Spain (Llorca et al., 2010), Norway (Haug et al., 2011; Thomsen et al., 2010), and Sweden (Sunstrom et al., 2011). Concentrations in breast milk were generally similar in studies from different parts of the world. In studies using sensitive analytical methods enabling detection of lower concentrations, median PFOA levels were 36 ng/L (Massachusetts; Tao et al., 2008a), 67 ng/ml (Japan; Tao et al., 2008b), and 46 ng/L (China; Liu et al., 2010), while PFOA was not detected or was infrequently found in breast milk in some other studies with higher detection limits (Fromme et al., 2010; von Ehrenstein et al., 2009). In the studies cited above, PFOA was frequently found in breast milk at concentrations higher than 40 ng/L, with some detections exceeding 100 ng/L (for example, in Belgium; Roosens et al., 2010).

Notably, breast milk concentrations were much higher in both rural and urban samples from Shanghai province (urban mean, 616 ng/L; rural mean, 814 ng/L) than in 12 other Chinese provinces (mean, 46 ng/L). Maternal exposures were likely higher in Shanghai than in the other areas sampled because PFOA levels are higher in Shanghai drinking water and surface water, likely because many fluorochemical manufacturing plants are located there (Liu et al., 2010).

SOURCES OF HUMAN EXPOSURE

The human body burden of PFOA results from exposure to both PFOA itself and to precursor compounds that can be metablized to PFOA, such as FTOH and diPAPs (D'eon and Mabury, 2011a; Lee and Mabury, 2011). Sources of exposure to PFOA and/or its precursors include drinking water, food, migration from food packaging into food, treated fabrics (carpets, upholstery, and clothing), protective sprays, ski waxes, cosmetics and personal care products, house dust, and inhalation of indoor and outdoor air (Trudel et al., 2008; Guo et al., 2009; Gewurtz et al., 2009; Freberg et al, 2010; Nilsson et al., 2010; Fraser et al., 2012; Knobeloch et al., 2012; Fujii et al., 2013; Fraser et al., 2013; Kotthoff et al., 2015). Migration into food from non-stick (PTFE-coated) cookware is not considered to be a significant exposure source (Trudel et al., 2008).

The relative contributions from direct exposure to PFOA and exposure to its precursors, used in products including food contact paper and stain resistant carpet and textile coatings, is uncertain and varies among individuals (D'eon and Mabury, 2011b; Gebbink et al., 2015a). Other classes of precursor molecules, some of which are known or suspected to be metabolically converted to PFOA in humans, have also been detected in human serum (Lee and Mabury, 2011). Some of these precursor compounds are known to be present in consumer products at much higher levels than PFOA itself (Lee and Mabury, 2011).

Efforts have been made to model the relative contributions of consumer products, indoor and outdoor air, house dust, diet, and/or other sources to exposures of PFOA and other PFCs in the general population. Some of these studies estimated the contributions of precursors (Fromme et al., 2008; Vestergren and Cousins, 2009: Gebbink et al., 2015b) while others did not (Washburn et al., 2005; Tittlemeier et al., 2007; Trudel et al., 2008; Cornelius et al., 2012); a high level of uncertainty is associated with the precursor estimates.

Most of these studies predict that diet is the predominant exposure source. Typical adult total exposures of about 2-3 ng/kg/day in Europe or North American were estimated in several studies (Fromme et al., 2009; Trudel et al., 2008; Vestergren and Cousins, 2009), while some more recent studies give higher dietary estimates (6.1 ng/kg/day in Flanders, Belgium; Cornelis et al., 2012) or lower dietary estimates (0.6 ng/kg/day in Norway, Haug et al., 2010a; 0.2 ng/kg/day in

The Netherlands, Noorlander et al., 2011; 0.16 ng/kg/day (location not specified), Gebbink et al., 2015b). Such dietary exposure estimates, in general, are highly uncertain because there are relatively few data on PFOA levels in food, analytical methods for food lack sufficient sensitivity, detection limits vary greatly among food types, and PFOA levels differ greatly in samples of the same foods obtained from different sources and/or locations.

PFOA has been detected in at least some samples of several types of foods including milk, butter, meats, fish, vegetables (including potatoes), bread, and microwave popcorn, but was not detected in most food samples tested (reviewed by D'Hollander et al., 2010; Domingo et al., 2012).

Commercially available infant formula products do not appear to be a major source of exposure to PFOA or other PFCs in the U.S. Tao et al. (2008a) evaluated PFCs in 21 samples of five brands of infant formula representing >99% of the U.S. market. Products tested included milk-, organic- and soy-based formula, packed in cans, glass, or plastic, in liquid, powdered, and concentrated liquid forms. PFOA was not detected (<0.048 ng/L) in any sample. Other PFCs (for which detection levels varied) were also not detected or were infrequently found (PFOS – one detection at 11.3 ng/L; perfluorohexane sulfonate (PFHxS)-two detections at up to 3.59 ng/L). In this study, PFCs were also analyzed in 12 samples of 11 brands of dairy milk purchased in Albany, NY in 2008, an there was only one detection of PFHxS at 3.83 ng/L. However, it should be noted that exposure to infants occurs when powdered or concentrated formula is prepared with drinking water contaminated with PFOA.

Llorca et al. (2010) analyzed two brands of dry infant cereal and three brands of powdered milk-based infant formula purchased in Spain. PFOA concentrations in the cereals were 166 and 438 ng/kg, and in the formulas, 374, 488, and 723 ng/kg. The concentrations in these products when prepared for consumption were not given.

PFOA and other PFCs can be taken up into plants grown on contaminated soil (e.g. from application of PFOA-contaminated biosolids) or irrigated with contaminated water, including into the parts of some vegetables and grains that are consumed by humans and by grazing livestock (Stahl et al., 2009; Lechner and Knapp, 2011; Yoo et al., 2011). The potential for human exposure to PFOA through this route generally depends on the part of the plant that is consumed. In general, shorter chain PFCs are preferentially taken up into the fruit of the plant, while longer chain PFCs such as PFOA are preferentially taken up into the root and shoot parts of the plant (Blaine et al., 2013a, b, 2014; Felizeter et al., 2012, 2014). In the C8 Health Study population, consumption of locally grown or home grown vegetables was associated with higher serum PFOA levels (Emmett et al., 2006a; Steenland et al., 2009a; Hoffman et al., 2011).

PFOA is much less bioaccumulative in fish than other PFCs that have a longer fluorinated carbon chain, including PFOS (Conder et al., 2008). Thus, consumption of fish from waterways contaminated with PFOA does not result in the high exposures typical of other persistent organic contaminants, including PFOS, which are bioaccumulative in fish (Hölzer et al., 2011). However, PFOA has been detected in edible fish and other seafood, and consumption of aquatic organisms may represent a significant portion of total dietary exposure in some populations (Haug et al., 2010 b; Zhang et al., 2011).

Several studies suggest that PFOA and its precursors in indoor air and/or house dust may be a major exposure source for some individuals (Haug et al., 2011; Shoeib et al., 2011; Schlummer et al., 2013; Gebbink et al., 2015a). Fraser et al. (2013) reported that the concentration of the PFOA precursor 8:2 FTOH in indoor air in offices is a predictor of serum PFOA concentration. Levels of this compound were greatly elevated in offices with new carpets compared to other offices.

Greater exposures to PFOA may occur in young children than in older individuals because of age-specific behaviors such as greater drinking water and food consumption on a body weight basis, hand-to-mouth behavior resulting in greater ingestion of house dust, and more time spent on floors where treated carpets are found (Section 5.1; Trudel et al., 2008; Shoeib et al., 2011).

Occupational exposure to PFOA is believed to occur primarily through inhalation (Vestergren and Cousins, 2009).

Exposures from drinking water

It is well established that serum PFOA concentrations are greatly elevated in communities with highly contaminated drinking water resulting from environmental discharges (discussed in Biomonitoring, above). As discussed in Biomonitoring (above) and Toxicokinetics (below), continued exposure to even relatively lower drinking water concentrations which are more widespread (Section 3 above) can also substantially increase total human exposure, as indicated by serum PFOA levels.

The total exposure studies discussed above provide varying conclusions about the relative importance of drinking water to total exposure; these conclusions are highly dependent on the concentration of PFOA in drinking water assumed in the analyses. For example, Fromme et al. (2008) and Cornelis et al. (2012) concluded that drinking water contributed <1% to total exposure, assuming drinking water levels of 1 ng/L and 2 ng/L, respectively, while Noorlander et al. (2011) estimated that 55% of exposure comes from drinking water, assuming 9 ng/L. Vestergren and Cousins (2009) and Thompson et al. (2011) demonstrated that the contribution of drinking water to total exposure depends on the concentration of PFOA, and Thompson et al. (2011) predicted that a drinking water level of 9.66 ng/L contributed 24% to total exposure.

PFOA exists in drinking water in its non-volatile anionic form, and the formation of inhalable water droplets during showering or bathing is minimal. Therefore, inhalation exposure is not expected to be significant from non-ingestion uses of drinking water such as showering, bathing, laundry, and dishwashing (Trudel et al., 2008; USEPA, 2016e). In contrast, these are important exposure routes for volatile drinking water contaminants. Similarly, dermal absorption of PFOA during showering, bathing, or swimming is not expected to be significant compared to exposure through ingestion, based on analysis by NJDOH (2014) using skin permeability data from Franko et al. (2012).

TOXICOKINETICS

Absorption, Distribution, Metabolism, and Excretion

Summary

PFOA is well absorbed orally, and can also be absorbed dermally and by inhalation. The ammonium (APFO) or sodium (NaPFO) salts dissociate to PFOA (the anionic form) in the body. PFOA is water soluble and distributes primarily to the liver and serum, and, to a lesser degree, to the kidney. Unlike most other bioaccumulative organic compounds, it does not distribute to fat. In the serum, PFOA is almost totally bound to albumin and other proteins. Since it is chemically non-reactive, it is not metabolized. The rate of excretion of PFOA varies widely among species, and in some cases between males and females of the same species. The excretion rate is largely dependent on the extent of secretion and reabsorption by organic anion transporters in the kidney. In humans, the half-life is several years, and half-lives in male and female mice and male rats are days to weeks, so that PFOA reaches steady-state in these species/genders after continued dosing. However, PFOA is rapidly excreted in female rats (half-life of 2-4 hours) and does not reach steady-state after continued once-daily dosing. For this reason, the rat is not an ideal model for studying developmental effects of PFOA. Because of the large variations in half-lives, the internal dose resulting from a given administered dose varies widely among species and, in some cases, genders of the same species. For this reason, interspecies (e.g. animal-to-human) comparisons are made on the basis of internal dose, as indicated by serum level, rather than administered dose.

Absorption

PFOA is well absorbed by the oral route (Lau et al., 2007). More than 95% of a single dose of 0.1 to 25 mg/kg APFO (the ammonium salt of PFOA) was absorbed in male and female rats (Kemper, 2003). It was also well absorbed in mice, rats, hamsters, and rabbits in studies by Hundley et al. (2006). About 98.7% of an oral dose given to pregnant rats on gestation day (GD) 8 or 9 was excreted in the urine within 24 hours (Gibson and Johnson, 1983). Additionally, a recent study in mice (Fujii et al., 2015) estimated the oral absorption of PFOA as 98.7% in males and 99.8% in females. The extent of oral absorption was determined by comparing fecal excretion after intravenous dosing (representing biliary excretion of PFOA into the

gastrointestinal tract) and oral dosing (representing both unabsorbed PFOA and biliary excretion). PFOA is well absorbed by humans exposed orally, as demonstrated by elevated serum concentrations in residents of communities with contaminated drinking water (discussed above).

PFOA penetrated rat and human skin in an *in vitro* system (Fasano et al., 2005), and dermal exposure caused liver toxicity in rats (Kennedy, 1985) and immune effects in mice (Fairley et al., 2007). The dermal permeability coefficient of PFOA (14,000 ng/L [14 µg/L] in water, pH 5.01) was estimated as 8.8×10^{-5} cm/hr (Fasano et al., 2005). As above, dermal absorption is not expected to be a significant source of exposure from contaminated drinking water (NJDOH, 2014).

Inhalation exposure to APFO in rats caused hepatic effects (Kennedy et al., 1986). Elevated serum levels of PFOA in workers in facilities making or using PFOA are likely to result primarily from inhalation exposure (Olsen, 2015). As above, PFOA does not volatilize from water, and inhalation is not expected to be a significant source of exposure from contaminated drinking water (Trudel et al., 2008; USEPA, 2016e).

Distribution

After oral administration, the highest concentrations of PFOA are found in the liver and serum, followed by the kidney, with lower concentrations in other organs (Vanden Heuvel et al., 1991; Kemper, 2003; Hundley et al., 2006; Fujii et al., 2015). After *in utero* exposure to mice, PFOA persisted in bone until adulthood (Koskela et al., 2016). In the serum, PFOA is almost totally bound to albumin and other proteins (SRI, 2003: Han, 2003). Unlike many other persistent bioaccumulative compounds, PFOA does not distribute to fat, and the concentrations of PFOA in the fat after dosing are very low (Vanden Heuvel et al., 1991; Hundley et al., 2006; Fujii et al., 2015).

The fraction of the dose found in the liver is dose-dependent and varies between male and female animals. For example, in male rats two hours after a single intravenous dose, 52% of a low dose (0.041 mg/kg) and 27% of a higher dose (16.56 mg/kg) were found in liver (Kudo et al., 2007). Serum concentrations were similar in male and female CD-1 mice after a single oral gavage dose of 1 or 10 mg/kg PFOA (Lou et al., 2009). However, the concentration in the liver was higher in males than in females.

In another study in which rats were given a single dose of 25 mg/kg, the absolute concentrations in the livers of males were higher than in females, as expected based on the slower excretion by male rats (see below), and the percentage of PFOA in the cytosolic fraction of the liver was higher in females (49%) than in males (26%) (Han et al., 2005).

The subcellular distribution within the liver of male rats was also found to be dose-dependent (Kudo et al., 2007). Over 40% of a low dose (0.041 mg/kg) distributed to the 8000xg pellet (nuclei, mitochondria, and cellular debris), followed by lysosomes and peroxisomes, microsomes, with the least amount found in cytosol (less than 5%), while over 43% of a higher dose (16.56 mg/kg) distributed to cytosol, followed by the 8000xg pellet, lysosomes and peroxisomes, and microsomes. In another part of this study using a range of doses, there was a dose-dependent increase in the percentage found in cytosol.

Metabolism

PFOA is chemically unreactive due to its carbon-fluorine bonds, one of the strongest found in organic chemistry (Vaalgamaa et al., 2011. Therefore, it is not metabolized by biological systems.

Excretion

PFOA is excreted in the urine and the feces, and the proportion in the urine versus the feces varies among species (Hundley et al., 2006; Cui et al., 2010). It is believed that PFOA undergoes enterohepatic circulation (Kudo and Kawashima, 2003) since oral administration of cholestyramine (an anion exchange resin which is not absorbed from the gastrointestinal tract) increased the fecal elimination of PFOA in male rats by 10-fold (Johnson et al., 1984).

Data from several studies indicate that blood loss (e.g. through menstruation, blood donation, or venesection) is an additional excretion route for PFCs (Harada and Koizumi, 2009; MDH, 2013; Taylor et al., 2014; Lorber et al., 2015)

The half-life of PFOA varies among species, and it also differs between males and females in some species, most notably rats and hamsters (Table 4; Hundley et al., 2006, Lau et al., 2007). PFOA is excreted much more quickly in female rats ($t_{1/2}$ = 2-4 hours) than in male rats ($t_{1/2}$ = 4-6 days), while the excretion rate in hamsters is much more rapid in males than in females. The half-life in both sexes of mice is similar (17 days in females and 19 days in males), while excretion is rapid in both sexes of rabbits with half-lives of 5.5 hours in males and 7 hours in females.

The differences in excretion rates between species and between male and female rats are thought to be due to variations in renal clearance rates. These rates are controlled by specific organic anion transporters that are responsible for the active transport (secretion or reabsorption) of many organic anions, including endogenous substances and xenobiotics, across membranes in several organs including the kidney (Weaver et al., 2010; Han et al., 2012). The specific transporters believed to be responsible for renal reabsorption of PFOA have been identified in male rats as Oatp1a1 and in humans as OAT4 and URAT1 (Han et al., 2012).

Table 4: Serum/plasma elimination half-lives of PFOA			
Species	*Females*	*Males*	*References*
Rat	2–4 hours	4–6 days	Johnson et al. (1979); Kemper and Jepson (2003)
Mouse	17 days	19 days	Lau et al. (2005)
Rabbit	7 hours	5.5 hours	Hundley et al. (2006)
Dog	8–13 days	20–30 days	Hanhijarvi et al. (1988)
Cynomolgus Monkey	30 days	21 days	Butenhoff et al. (2004a)
Human (males and females combined)	3.8 years (retired workers)		Olsen et al. (2007)
	2.3 years (adults after cessation of exposure from contaminated drinking water)		Bartell et al. (2010a)
	3.3 years (average of adults and children after cessation of exposure from contaminated drinking water)		Brede et al. (2010)
	Adults and children after cessation of exposure to contaminated drinking water. Highly exposed group: 2.9 years (initial 4 years post-exposure); 10.1 years (>4 years post-exposure). Less exposed group: 8.5 years (initial 9 years post-exposure); no apparent decline (>9 years post-exposure).		Seals et al. (2011)

Adapted from Lau, 2012

Renal excretion of PFOA appears to be under hormonal control (Ylinen et al., 1989; Kudo et al., 2002). Kudo et al. (2002) found that the clearance of PFOA in female rats (15 ml/min/kg) is greater than the glomerular filtration rate (10 ml/min/kg), suggesting that PFOA is actively excreted through renal tubular secretion, while the renal clearance was much lower in male rats, 0.6 ml/min/kg (Kudo et al., 2002). Castration of male rats increased the clearance to a rate similar to that in females, and this increase was reversed by administration of testosterone to the castrated rats (Ylinen et al., 1989; Kudo et al., 2002). Administration of estradiol to male rats increased the renal excretion of PFOA, and administration of testosterone to female rats reduced the clearance to a rate similar to that of male controls (Ylinen et al., 1989; Kudo et al., 2002). Probenecid, an inhibitor of renal tubular secretion of organic anions, greatly reduced the clearance of PFOA in female and castrated male rats, but had little effect on the excretion rate in control male rats (Hanhijarvi et al., 1982; Kudo et al., 2002).

The gender-dependent differences in excretion rate in rats appear to develop between 3 and 5 weeks of age (Hinderliter et al., 2006). At 4 weeks of age, serum concentrations 24 hours after a single oral dose of 10 mg/kg PFOA were similar (within 3-fold) in male and female rats, while at 5 weeks and older, serum levels were at least 30-fold higher in males than in females receiving the same dose. This greater difference in older rats resulted from age-dependent changes in both males and female. At 5 weeks of age or older, serum levels in males at this dose were about 5-fold higher than serum levels at 4 weeks, while serum levels in females 5 weeks or older are 2-3 fold lower than at 4 weeks of age.

Human half-life

The half-life of PFOA in humans is several years and does not appear to differ significantly between males and females. Inter-individual differences in half-life may be due to differences in renal transport by OATs. A mean half-life of 3.8 years was estimated from data from 26 retired workers with occupational exposure, with no difference found between men and women (Olsen et al., 2007). Bartell et al (2010a) estimated a half-life of 2.3 years in a more heterogeneous study population consisting of 200 adults exposed to PFOA in drinking water. This estimate was based on average decreases in serum level of 26% and 24% for a one-year period after treatment to remove PFOA was initiated in two water districts contaminated by PFOA emissions from a West Virginia manufacturing facility. There was no evidence of age- or gender-dependence in elimination rates in this study. During the second year of follow-up of the same individuals, serum PFOA levels decreased more slowly (9% and 15% in the two water districts), suggesting either ongoing exposures from sources other than residential drinking water or that kinetics do not follow first-order elimination (Bartell et al., 2010b).

Seals et al. (2011) studied the rate of decline of serum PFOA levels in former residents of Little Hocking (n = 602) and Lubeck (n = 971), the two water districts with the highest drinking water PFOA levels of the six districts included in the C8 Health Study. Median serum levels in current and former residents of Little Hocking (current residents, 241.0 ng/ml; former residents regardless of years elapsed, 60.6 ng/ml) were much higher than in Lubeck (current residents, 69.4 ng/ml; former residents regardless of years elapsed, 31.0 ng/ml), due to the much higher drinking water PFOA concentrations in Little Hocking than Lubeck. The number of years elapsed since the former residents moved and thus stopped consuming contaminated water ranged from less than one year to almost 25 years. The data on the relationship between years elapsed since moving and serum PFOA levels suggest that PFOA elimination is biphasic and dependent on serum concentration. In former residents of Little Hocking, the half-life was 2.9 years for the first 4 elapsed years, and about 8.5 years after the first 4 elapsed years. In former residents of Lubeck, the half-life was about 8.5 years for the first 9 elapsed years, with no apparent decline in serum levels after 9 elapsed years.

PFOA levels in serum of exposed individuals were also studied in Arnsberg, Germany, where the Moehne River which is used a drinking water source was contaminated by runoff from PFOA-contaminated industrial waste applied to agricultural land (Brede et al., 2010). In a two-year study of 138 individuals before and after drinking water treatment removal was initiated, the geometric mean PFOA plasma levels declined by 39% in children and mothers, and by 26% in men; the geometric mean half-life was estimated as 3.26 years.

Isomer-specific kinetics

PFOA exists as a mixture of linear and branched isomers, and the isomer profile varies depending on the manufacturing process used. PFOA made by the telomerization process is primarily linear, while PFOA produced by electrochemical fluorination consists of a mixture of linear and branched isomers. Differences in the rates of elimination of the isomers have been investigated (Loveless et al., 2006; DeSilva et al., 2009). Loveless et al. (2006) reported that, after equivalent doses, serum levels of branched PFOA were lower than for linear PFOA in rats and mice. Similarly, after subchronic administration to rats, most branched isomers were eliminated more quickly than linear PFOA, with the exception of two minor unidentified branched isomers which had half-lives about twice that of linear PFOA (DeSilva et al., 2009). In humans, branched isomers were also more rapidly eliminated than linear isomers (Zhang et al., 2013; Gao et al., 2015). PFOA isomer profiles differed in maternal and cord serum within human infant-mother pairs, indicating that most branched isomers cross the placenta more efficiently than the linear forms (Beesoon et al., 2011). It has recently been reported that linear PFOA has a higher binding affinity than branched PFOA for human serum albumin and serum proteins in general, providing a potential explanation for the more rapid excretion of branched isomers of PFOA (Beesoon and Martin, 2015).

Toxicokinetics Relevant to Developmental Exposures

Summary

It is important to consider toxicokinetics relevant to developmental exposures of PFOA in detail. Developmental effects are the most sensitive known endpoints for PFOA toxicity in experimental animals, and prenatal exposure is associated with decreased fetal growth in humans (see Toxicology and Epidemiology sections, below).

The toxicokinetics of PFOA during gestation and lactation have been studied in rats and mice but have not been evaluated in non-human primates. In rodents, PFOA is present in fetuses of dosed dams, as well as in the placenta and amniotic fluid. PFOA is also present in the breast milk of gestationally exposed dams.

Because it is excreted very quickly in female rats but very slowly in humans, the rat is not an ideal model for study of developmental effects of PFOA. In contrast, the mouse is a preferable

model for evaluation of developmental effects because PFOA is excreted slowly in female mice. For this reason, many recent developmental studies have been conducted in mice.

In humans, PFOA has been measured in amniotic fluid, maternal serum, umbilical cord blood, and breast milk. PFOA concentrations are similar in maternal serum and umbilical cord blood serum, which is reflective of serum levels in the newborn. PFOA exposure in breast-fed infants is greatest during the first few months of life because both PFOA concentrations in breast milk and the rate of fluid consumption are highest during this time period. As a result, serum PFOA concentrations in breast-fed infants increase several fold from levels at birth within the first few months of life. Exposures to infants who consume formula prepared with contaminated water are also highest during this time period. These greatly elevated exposures during the first months of life are of special concern because the neonatal period is a sensitive time period for the toxicological effects of PFOA.

Rats

As discussed above, PFOA is excreted very rapidly by female rats (half-life of 2-4 hours). Because of its rapid excretion, PFOA is not continuously present in female rats dosed once daily, and the fetuses are thus not exposed continuously to PFOA from such a dosing regimen. Because PFOA is highly persistent in humans, the rat is not an ideal model for evaluation of developmental effects of PFOA.

In female rats given a single oral dose of PFOA, the maximum plasma concentration occurred about 1.25 hours after dosing (Kemper and Jepson, 2003). About 98.7% of an oral dose given to pregnant rats on gestation day (GD) 8 or 9 was excreted in the urine within 24 hours (Gibson and Johnson, 1983).

Kinetics of PFOA were studied in pregnant rats (strain not stated) dosed once daily by gavage with 3, 10, or 30 mg/kg/day on GD 4 to postnatal day (PND) 21, and their pups (Hinderliter et al., 2005). Because the gender difference in kinetics in rats develops at about 5 weeks of age (Hinderliter et al., 2006), plasma levels were similar in male and female pups until PND 21, the time period evaluated in this study. Plasma was taken from the dams 2 hours after dosing to allow maximum detection of PFOA, since virtually all PFOA is excreted within 24 hours after dosing of female rats. Mean PFOA concentrations in maternal plasma approximately 2 hours after doses of 3, 10, or 30 mg/kg were 11,000, 27,000, and 67,000 ng/ml, respectively. On GD 21, levels in fetal plasma were about half of those in the dams. Concentrations in milk were fairly constant on PND 3 through PND 21, and were about 10-fold lower than maternal serum levels. Pup plasma levels were about 4-fold lower than maternal plasma levels on PND 3, and about 10-fold lower at later time points. PFOA was also detected in the placenta and amniotic fluid. Interpretation of these data is complicated by the fact that maternal plasma levels varied

widely during the course of the day between the daily doses, and milk and fetal/pup levels are compared to maternal plasma levels in samples taken near their daily peaks.

Mice

In contrast to female rats, PFOA is slowly excreted in female mice with a half-life of several weeks (discussed above). Therefore, the fetus is continuously exposed when pregnant mice are dosed once daily. Because PFOA is persistent in humans, the mouse is a preferable model for evaluation of PFOA's developmental effects than the rat, and many recent developmental studies have been conducted in mice.

Fenton et al. (2009) studied the disposition of PFOA in pregnant CD-1 mice and their pups after a single oral gavage dose of 0.1, 1, or 5 mg/kg on GD 17. On GD 18 prior to delivery, the PFOA concentration in the amniotic fluid was about half of the concentration in maternal serum. PFOA concentrations in the whole pup on GD 18 were similar to those in maternal serum. On PND 1 (the earliest time at which serum was measured in pups), pup serum PFOA concentrations were about 1.5 times the concentrations in maternal serum. The exposure of pups at this stage was thought by the authors to result primarily from *in utero* exposure rather than through lactation.

PFOA concentrations in maternal serum and in aspirated milk followed a U-shaped curve over time between PND 1 (serum) or PND 2 (milk) and PND 18, with decreases between the earliest time point (PND 1 or 2) and mid-lactation (PND 8, pups, and PND 11, milk), and increases from mid-lactation to PND 18. This increase between PND 8 and 18 was thought to result from decreased dilution of maternal serum and milk at PND 18. PFOA concentrations in pup serum and in whole pups decreased over time from postnatal day 1 to 18 on a ng/ml or ng/g basis, while the total PFOA pup body burden increased from GD 18 to PND 8, and decreased between PND 8 and 18, presumably because the intake of milk has decreased during this period. Milk concentrations were lower than maternal serum concentrations at all doses and time points, ranging from 11% to 56% of the serum concentration, with the higher percentages at early and late lactation time points.

Serum PFOA concentrations in lactating CD-1 mouse dams and their pups were also measured in a cross-fostering study of mammary gland developmental effects (White at al., 2009) in which dams were treated by gavage with 5 mg/kg/day from GD 8-17. After birth, litters of similar ages and exposures were mixed and fostered, resulting in four treatment groups: untreated dam with unexposed pup, treated dam with unexposed pup, untreated dam with pup exposed during gestation, and treated dam with pups exposed during gestation. Consistent with the single dose study (Fenton et al., 2009), serum PFOA levels on PND 1 in pups exposed *in utero* were about 50% higher than in treated dams. On PND 1, PFOA in the pups is primarily attributable to *in utero* exposure, rather than lactational exposure during the first postnatal day, since serum PFOA concentrations in pups not exposed *in utero* but nursing on treated dams were only about 3% of

the concentrations in pups exposed *in utero*. Serum levels in treated dams and pups decreased between PND 1 and PND 10, with a greater decrease in pups exposed *in utero* that nursed from untreated dams than in pups exposed in *utero* that nursed from treated dams. Serum levels from pups not exposed *in utero* that nursed from treated dams rose over time, and by PND 10 serum levels in these pups were similar to levels in the treated dams they nursed from. Interestingly, serum levels in untreated dams that nursed pups exposed *in utero* increased between PND 1 and PND 10, presumably due to maternal behavior (grooming, and ingestion of urine and feces) resulting in ingestion of PFOA from the pups.

In a more recent study of effects on mammary gland development from lower doses of PFOA (Macon et al., 2011), pregnant CD-1 mice were dosed by gavage on GD 10 to 17 with 0, 0.01, 0.1, or 1 mg/kg/day. At PND 1, serum levels in the pups were 24, 285, 2304, and 16,306 ng/ml in the untreated, 0.01, 0.1, and 1 mg/kg groups, respectively. The pup serum levels decreased over time to 3, 17, 132, and 2683 ng/ml at PND 21. Maternal serum levels were not measured by Macon et al. (2011). However, in other studies in which serum data are available for dams and pups within the same study (e.g. Fenton et al., 2009), serum levels on PND 1 were higher in pups than dams.

White et al. (2011) provide data on serum PFOA concentrations in CD-1 dams and female pups exposed to 5000 ng/L (5 μg/L) PFOA in drinking water over multiple generations. Results of this study are discussed in the section on Developmental Effects. Exposure began in P0 dams on GD 7 and continued throughout the F1 and F2 generations (except during F1 breeding and early gestation, to avoid exposing control males). Serum concentrations were 74.8 ng/ml in the P0 dams and 86.09 ng/ml in the F1 dams at weaning on PND 22, as compared to 4 and 2 ng/ml, respectively, in the corresponding control groups of dams. At this time point, the P0 dams had been exposed for about 32 days and the F1 dams had been exposed throughout their lifetimes beginning *in utero*, except during breeding and early gestation. As discussed below, serum concentrations in humans with ongoing exposure PFOA in drinking water are, on average, more than 100-fold higher than the concentration in drinking water. Thus, the serum PFOA concentrations in the mice exposed to 5000 ng/ml (5 μg/L) in drinking water are much lower than the average serum concentrations of more than 500 ng/ml expected in humans chronically exposed to this drinking water concentration.

Serum PFOA concentrations were similar in F1 and F2 pups, and concentrations in the pups were lower than in the dams at weaning. The serum concentrations in the F1 and F2 pups at PND 22 were 21.3 and 26.6 ng/ml, respectively; at PND 42, they were 48.9 and 57.4 ng/ml; and at PND 63, they were 66.2 and 68.4 ng.ml.

Humans

Relationship between maternal and fetal/neonatal exposures

PFOA has been detected in umbilical cord blood serum in studies of the general population worldwide. In seven studies reviewed by Post et al. (2012) in which both maternal and cord blood were analyzed, the mean cord blood serum:maternal serum (or plasma) ratio ranged from 0.68:1 to 1.26:1, with a mean ratio of less than 1:1 in all but one study. However, cord:maternal serum (or plasma) ratios for some individual neonate-maternal pairs within these studies were greater than 1:1. Since umbilical cord serum (or plasma) is reflective of neonatal serum (or plasma), these data indicate that serum (or plasma) levels are generally similar in the neonate and the mother. A more recent review by Kato et al. (2015) that evaluated 12 studies in total also concluded that the maternal:cord serum ratios for PFOA is approximately 1:1.

Exposure to infants through breast milk and infant formula

PFOA is detected in human breast milk worldwide (reviewed by Liu et al., 2010; White et al., 2011a; Post et al., 2012). Factors which may potentially affect the concentration of PFOA in breast milk include whether the mother has previously nursed other infants and how soon after birth the sample is taken (Tao et al., 2008a; Haug et al., 2011; Thomsen et al., 2010). Thomsen et al. (2010) found that average breast milk concentrations were highest initially and decreased by about 7.7% per month, or about 94% during the first year of breast feeding, presumably due to decreased maternal body burden resulting from excretion into breast milk.

Breast milk PFOA concentrations were reported to be about 1% of mean general population serum levels by Tao et al. (2008a), and to be 2.5% and 9% of median maternal serum levels by Kim et al. (2011) and Liu et al. (2011), respectively. These data suggest a breast milk:maternal serum ratio of about 1:100 to 1:11. Based on a breast milk:maternal serum ratio of greater than or equal to 1:100 (Tao et al., 2008a; Kim et al., 2011; Liu et al, 2011) and a serum:drinking water ratio of greater than or equal to 100:1 (discussed below), the initial PFOA concentration in breast milk is expected to be greater than or equal to the concentration in the maternal drinking water source (Post et al., 2012).

Exposures to infants to PFOA from breast milk or formula are higher than in older individuals exposed to the same concentration of PFOA in drinking water. Mean breast milk consumption is 150 ml/kg/day during the first post-partum month when PFOA levels in breast milk are highest (Thomsen et al., 2010), and it is 83 ml/kg/day from 6-12 months of age (USEPA, 2008a). Similarly, the mean drinking water intakes in infants who consume drinking water (e.g. in formula prepared with water) are 137 ml/kg/day from birth to 1 month of age, and 53 ml/kg/day at 6-12 months of age (USEPA, 2011b). These fluid intakes are much higher than the mean drinking water consumption rates in lactating women, 26 ml/kg/day (USEPA, 2011b), and the general population (11 years of age or older), 13 ml/kg/day (USEPA, 2008a). Although breast milk or

formula consumption on a body weight basis decreases as the infant gets older, it remains much higher than adult water consumption throughout infancy.

As noted above, serum PFOA levels are similar in newborns and in their mothers. Several studies, summarized below, have consistently demonstrated that serum PFOA concentrations in breast-fed infants increase by several fold during the first few months of life, presumably because both breast milk PFOA concentrations and intake of breast milk on a body weight basis are highest during this time period. Infants fed with formula prepared with contaminated drinking water also receive the greatest exposures during the first few months of life because the rate of fluid intake is highest then.

Serum PFOA levels were measured in umbilical cord blood at delivery and at 6 month and 19 months of age in infants from the German general population (Fromme et al., 2010). Average body burdens, as indicated by serum levels, were increased, typically by several-fold, from birth to 6 months by exposure through breast milk. Levels declined between 6 months and 19 months, a time point at which breast feeding had stopped or was decreased, but remained higher at 19 months than at birth (Figure 5).

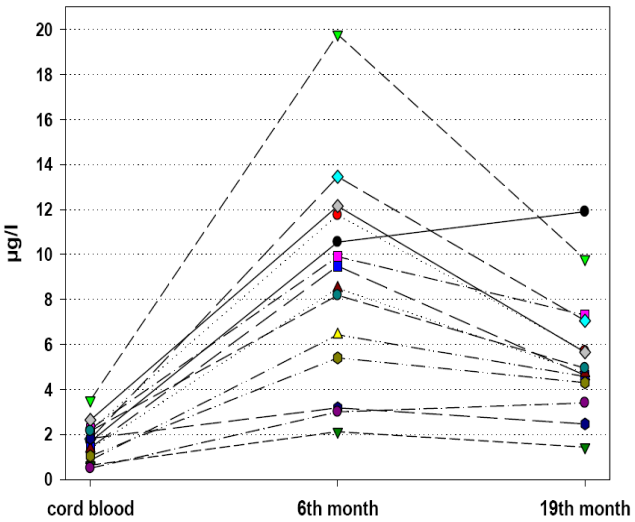


Figure 5. PFOA concentration in cord blood and blood collected in infants around six and nineteen months after birth (Fromme et al., 2010)

Duration of breastfeeding was also associated with higher serum PFOA concentrations in infants (n=49) from a community with PFOA-contaminated drinking water (the C8 Health Study). The increases were estimated as 6% per month of breastfeeding and 96% for one year of breastfeeding (Mondal et al., 2014). The authors noted that these values may underestimate the actual increases from exposure through breastfeeding, because they are based on comparisons to non-breastfed infants from the same communities who may also have had increased serum PFOA concentrations from exposures via formula prepared with contaminated drinking water.

Similarly, a study of Faroese infants (n= 80) with serum PFOA data at birth and 11, 18, and 60 months estimated an increase in serum PFOA concentrations of about 28% per month during the period of exclusive breast feeding (median of 4.5 months in the study group) and about 4% per month during the period of partial breast feeding (median of 4 additional months) (Mogensen et al., 2015). Serum PFOA concentration did not increase in non-breastfed (e.g. formula-fed) infants; presumably, the drinking water in this location was not contaminated with PFOA. Data for 12 infants from the study are shown in Figure 6.

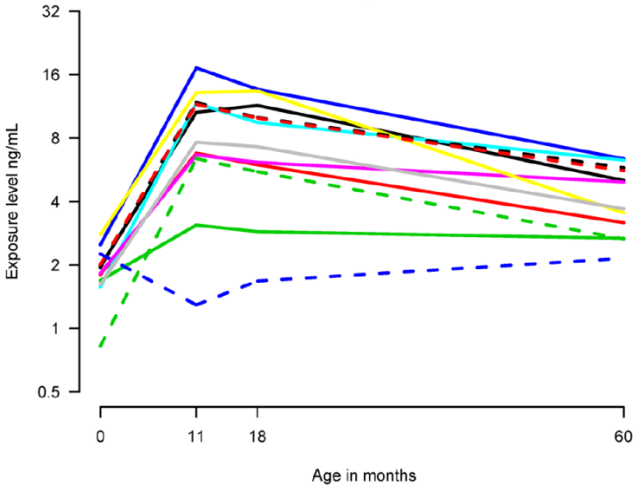


Figure 6. Serum PFOA concentrations over time in 12 infants from Mogensen et al. (2015).

Finally, Verner et al. (2016a,b) developed a pharmacokinetic model that predicts PFOA doses and plasma levels in breastfed infants and children, and their mothers. Monte Carlo simulations were used to predict the distribution of child:mother ratios for doses and plasma levels starting at birth (Figure 7). Doses (ng/kg/day) to infants were much higher than in their mothers during the first year of life. The infant:mother dose ratio peaked right after birth, with a median ratio of about 75:1 and a maximum of 231:1, and declined thereafter (Figure 7, right side). The infant:mother plasma level ratio peaked during the first year of life, with predicted ratios of 4.5-fold (median), 7.8-fold (95th percentile), and 15.3-fold (maximum) higher plasma PFOA concentrations in infants than in their mothers during the period of greatest infant exposure (Figure 7, left side).

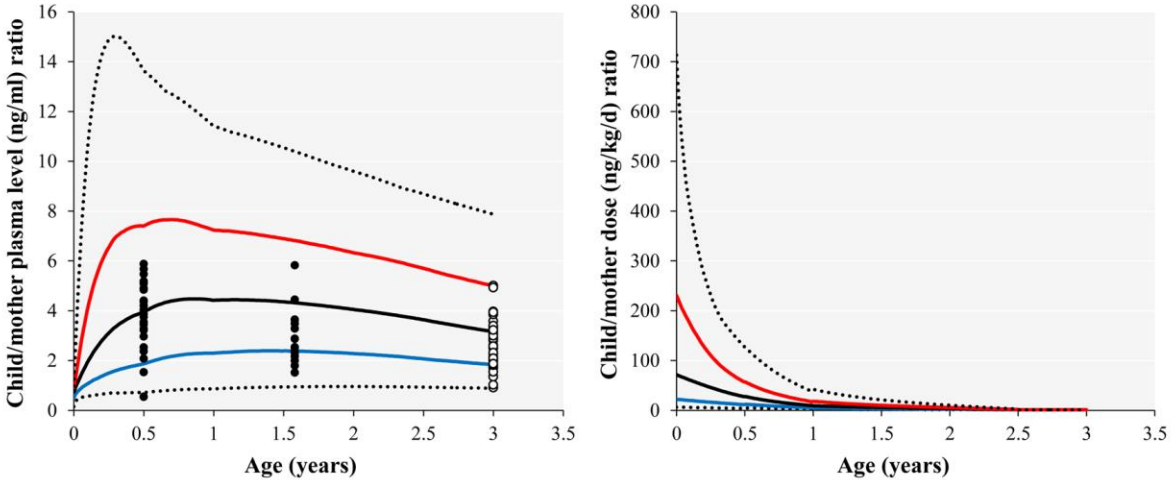


Figure 7. Monte Carlo simulations (n = 10 000) of child/mother ratios of plasma PFOA levels (ng/ml; right side of figure) and doses (ng/kg/day; left side of figure) for a breastfeeding period of 30 months. The black line represents the 50th percentile, the blue line represents the 5th percentile, the red line represents the 95th percentile, and the dotted lines represent minimum and maximum values (Verner et al., 2016a,b).

While peak serum PFOA concentrations occur during the first year of life, levels remain elevated for at least several additional years. Serum PFOA levels in children up to age 5 (or older) were higher than in adults in communities exposed through contaminated drinking water (Emmett et al., 2006a; Steenland et al., 2009a; Mondal et al., 2012, discussed above). In the study of Faroese children (Mogensen et al., 2015), serum PFOA levels declined after their peak in infancy but remained elevated above initial levels at birth until at least age 5 years, the last time point assessed. Similarly, the model developed by Verner et al. (2016a) predicts that plasma PFOA concentrations will remain several fold higher than at birth until at least age 3 years, the last time point modeled.

In summary, both breast-fed and formula-fed infants receive much greater exposures to PFOA from contaminated drinking water (directly or indirectly) than older individuals. Serum PFOA levels peak during the first year of life and remain elevated for several years. These elevated exposures during early life are of special concern because effects from neonatal exposure are sensitive endpoints for the toxicity of PFOA.

Relationship between administered dose and internal dose

Repeated Dose Animal Studies

Information on kinetics in repeated dose animal studies was reviewed by Post et al. (2012). At higher doses, the kinetics of PFOA in rodents and primates (Griffith and Long, 1980; Ylinen et al., 1990; Mylchreest, 2003; Butenhoff et al., 2004a; Perkins et al., 2004; Loveless et al., 2006; Lau et al., 2006; Das et al., 2010) are not consistent with one-compartment or simple first-order models (Anderson et al., 2006; Clewell, 2009). Serum levels did not increase proportionally with increasing dose, except at lower doses in some studies. Additionally, steady-state was reached more rapidly at high doses than predicted by classical kinetics (4 to 5 half-lives).

However, at lower doses closer to those relevant to human environmental exposures, kinetics are consistent with first order processes, and serum levels are proportional to administered dose (Clewell, 2009; Lou et al., 2009; Loveless et al., 2006; Das et al., 2010). Available data indicate that serum levels in mice from doses below the administered range can be estimated by linear extrapolation from data on doses of 1 mg/kg/day or lower. The kinetics are consistent with the saturation of OATs responsible for renal reabsorption at high doses, resulting in a higher excretion rate at high doses than at low doses (Anderson et al., 2006; Clewell, 2009).

Human studies

Relationship between drinking water and serum concentrations in exposed communities

In communities with drinking water contaminated by PFOA, mean and median serum PFOA levels higher than in the general population. Variations in serum PFOA concentrations among individuals using the same source of drinking water arise from inter-individual differences in daily water consumption rates (L/kg/day) and/or toxicokinetic factors.

The relationship between drinking water concentration and serum concentration has been extensively evaluated for PFOA. It is well established that ongoing human exposure to PFOA in drinking water increases serum levels, on average, by at least 100 times the drinking water concentration. This conclusion is based on data from several studies of populations whose public water supplies or private wells were contaminated with a wide range of PFOA concentrations (60 ng/L to 13,300 ng/L).

In 282 residents of Little Hocking, Ohio at least six years of age exposed for two years or more, with occupationally exposed individuals excluded, the median ratio between the PFOA concentration in serum (371 ng/ml) and drinking water (3,550 ng/L) was 105:1 (25th-75th percentile range, 62:1-162:1), with a higher median ratio in young children (Emmett et al., 2006a).

This approximate 100:1 central tendency value for the serum:drinking water ratio was confirmed in communities with lower drinking water PFOA concentrations (Post et al., 2009a) based on data from approximately 70,000 residents of Little Hocking and five other Ohio and West Virginia water districts, when background serum levels found in the general population from non-water sources of exposure were taken into account. Drinking water levels in four of these districts were in the range of about 60 ng/L to 400 ng/L, while levels in a fifth district and Little Hocking were higher (Anderson-Mahoney et al., 2008).

Additionally, Hoffman et al. (2011) studied the relationship between drinking water concentrations and serum levels in the same region of Ohio and West Virginia in a study of 108 individuals using 62 private wells, with 1 to 4 participants using each well. Since the PFOA concentrations differed in each private well, this study included a greater range of drinking water

concentrations than studies of the six affected public water districts in this vicinity. The median and mean PFOA levels in the wells were 200 ng/L and 800 ng/L, respectively, and the maximum concentration was 13,300 ng/L. An adjusted robust regression model of the serum and drinking water data provided an estimated serum:drinking water ratio of 141:1 (95% CI: 135:1 – 148:1), while a one-compartment pharmacokinetic model based on assumed water intake of 1.41 L/day and half-life of 2.3 years provided an estimated ratio of 114:1.

An approximate ratio of 100:1 or greater between serum and drinking water concentrations is also consistent with observations in 98 Minnesota residents tested 34 months after exposure to contaminated drinking water ended (MDH, 2009), when the expected post-exposure decline in serum levels is considered.

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

Pharmacokinetic modeling of the relationship between dose and serum concentration

Biologically based pharmacokinetic modeling predicts a linear relationship in humans between external dose and internal dose, as measured by serum PFOA level, at doses relevant to environmental exposures such as from contaminated drinking water (Clewell, 2009). Increased serum levels that are linearly proportional to exposure levels have been observed in communities using contaminated drinking water (discussed above). However, non-linear kinetics such as observed in animals at higher doses, may occur at higher (occupational) human exposures (Clewell, 2009).

Lorber and Egeghy (2011) developed a simple single-compartment pharmacokinetic model that predicts the relationship in humans between PFOA dose (ng/kg/day) and serum concentration (ng/ml) based on volume of distribution and elimination rate, as follows:

$$\text{Serum concentration (ng/ml)} = \frac{\text{Dose (ng/kg/day)}}{\text{Volume of distribution (ml/kg)} \times \text{Elimination rate (day}^{-1}\text{)}}$$

This model uses a volume of distribution of 170 ml/kg (0.17 L/kg), based on a model calibrated with data from an Australian population exposed to PFOA through drinking water (Thompson et al., 2010), and an elimination rate of 0.0008 day^{-1}, based on the human half-life of 2.3 years observed in the C8 Health Study population (Bartell et al., 2010a). The product of these two

values provides a clearance factor that relates serum concentration (ng/ml) to dose (ng/kg/day) of 0.14 ml/kg/day (or 0.00014 L/kg/day).

The USEPA Office of Water (2016a) used the same values for volume of distribution and human half-life (elimination rate) selected by Lorber and Egeghy (2011) to derive the same clearance factor, 0.00014 L/kg/day. This value is very close to the clearance factor of 0.127 ml/kg/day (0.000127 L/kg/day) from the earlier unpublished model developed by Clewell (2006) that is discussed in Post et al. (2012).

USEPA (2016f) presents the following equation, which is equivalent to the equation presented by Lorber and Egeghy (2011) above:

$$\text{Serum Concentration } (\mu g/L) \times \text{Clearance } (1.4 \times 10^{-4} \text{ L/kg/day}) = \text{Human Dose } (\mu g/kg/day)$$

The relationship between the concentration of PFOA in drinking water and serum predicted by the clearance factor of 0.00014 L/kg/day (Lorber and Egeghy, 2011; USEPA, 2016a) was compared with the empirically observed average ratio of > 100:1 in communities with drinking water exposure to PFOA (discussed above) as follows:

The daily dose from a given concentration of PFOA in drinking water is:

$$\text{Human Dose } (\mu g/kg/day) = \text{Drinking Water Concentration } (\mu g/L) \times 0.016 \text{ L/kg/day}$$

Where: 0.016 L/kg/day is the mean daily water ingestion rate in the U.S. (USEPA, 2011b).

Therefore:

$$\text{Drinking Water Conc. } (\mu g/L) \times 0.016 \text{ L/kg/day} = \text{Serum Conc. } (\mu g/L) \times \text{Clearance } (1.4 \times 10^{-4} \text{ L/kg/day})$$

And:

$$\frac{\text{Serum Concentration } (\mu g/L)}{\text{Drinking Water Concentration } (\mu g/L)} = \frac{0.016 \text{ L/kg/day}}{1.4 \times 10^{-4} \text{ L/kg/day}} = 114:1$$

The serum:drinking water ratio of 114:1 based on the clearance factor and average daily water consumption is consistent with the observed ratios in communities exposed to contaminated drinking water. This calculation verifies that the clearance factor accurately predicts the relationship between human dose and human serum level. The clearance factor can therefore be used in the development of a Reference Dose (RfD) for PFOA from the Target Human Serum Level (RfD in terms of serum level).

Increases in serum levels associated with PFOA in drinking water

The increase in serum PFOA level, on average, expected from ongoing consumption of a given concentration of PFOA in drinking water can be predicted using the clearance factor, 0.00014 L/kg/day, and an assumed drinking water ingestion rate (L/kg/day).

The mean daily water ingestion rate in the U.S. is 0.016 L/kg/day (from above), and the daily water ingestion rate based on the upper percentile factors (2 L/day water consumption; 70 kg body weight) used to derive Health-based MCLs is 0.029 L/kg/day. For each 10 ng/L in drinking water, ongoing exposure at the mean ingestion and upper percentile ingestion rates are predicted to increase serum PFOA by 1.2 ng/ml and 2.0 ng/ml, respectively. Increases in serum levels from various concentrations of PFOA in drinking water, and the percent increases from the most recent median serum level, 2.1 ng/ml, from NHANES (2011-12; CDC, 2015) are shown in Table 5 and Figure 8.

Table 5. Increase in serum PFOA concentrations predicted from various concentrations of PFOA in drinking water

Drinking Water Conc. (ng/L)	Mean Water Ingestion Rate (0.016 L/kg/day)			Upper Percentile Water Ingestion Rate (0.029 L/kg/day)		
	Increase in serum (ng/ml)	Total serum* (ng/ml)	% increase from drinking water*	Increase in serum (ng/ml)	Total serum* (ng/ml)	% increase from drinking water*
1	0.1	2.2	5%	0.2	2.3	10%
10	1.1	3.2	52%	2.0	4.1	95%
20	2.3	4.4	110%	4.0	6.1	190%
40	4.6	6.7	219%	8.0	10.1	381%
100	11.4	13.5	543%	20.0	22.1	952%
400	45.6	47.7	2171%	80.0	82.1	3810%

*Total serum concentrations and % increases from drinking water are based on assumption of 2.1 ng/ml in serum (U.S. median value from NHANES, 2011-12; CDC, 2015) from non-drinking water exposures.

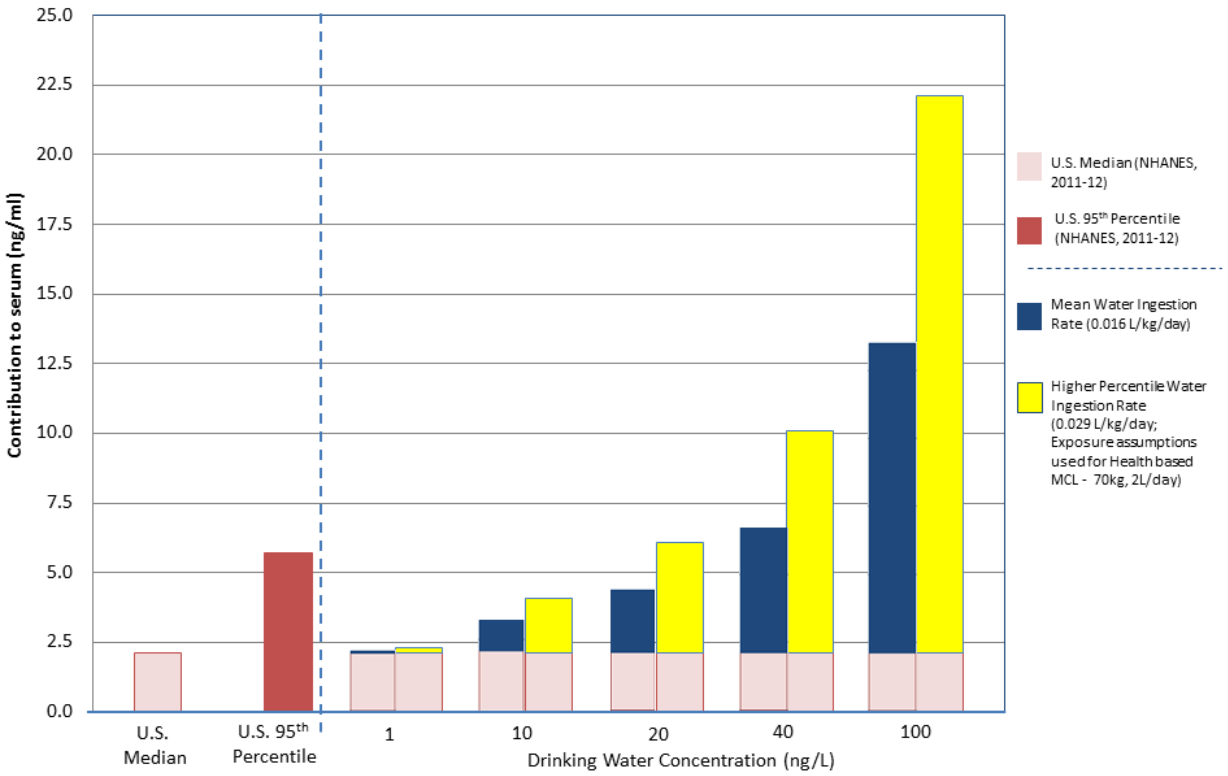


Figure 8. Increases in serum PFOA concentrations predicted from mean and upper percentile consumption of drinking water with various concentrations of PFOA, as compared to U.S median and 95th percentile serum PFOA levels (NHANES, 2011-12).

It is evident from Table 5 and Figure 8 that relatively low concentrations of PFOA in drinking water are associated with substantial increases in serum PFOA concentrations; this has recently been observed in a study of serum PFOA levels in individuals served by PWS with PFOA detections in UCMR3 (median UCMR3 detection – 28 ng/L; Hurley et al., 2016). For example, ongoing exposure to 20 ng/L at the upper percentile ingestion rate is predicted to result in a serum concentration of 6.1 ng/ml, which is above the 95th percentile in the U.S population of 5.7 ng/ml (NHANES, 2011-12; CDC, 2015). With an average (mean) water ingestion rate, exposure to 40 ng/L is expected to result in an elevation in serum level to 6.7 ng/ml, also above the 95th percentile from NHANES. Additionally, it should be kept in mind that (as discussed above), the increases in serum levels in infants are expected to be several fold higher than those shown in Table 5 and Figure 8.

HEALTH EFFECTS - HUMAN STUDIES

Overview

The epidemiological database for PFOA is much larger than for most other drinking water contaminants, including those previously evaluated by the DWQI. Considering the large body of epidemiologic studies assessing associations with PFOA, the Health Effects Subcommittee chose

to narrow and focus the human health effects section of this report. Studies of selected health endpoints were comprehensively reviewed, while information on other endpoints is summarized in the text. Conclusions of reviews of selected additional key health endpoints performed by other groups were also evaluated by the Subcommittee and are cited. This method allowed the Subcommittee to focus its resources while maintaining a high level of scientific review.

The basis for selection of endpoints for comprehensive review was largely supported by a previous detailed evaluation of the scientific literature on PFOA by the Health Effects Subcommittee in 2009-2010, and a subsequent comprehensive review of PFOA as an emerging drinking water contaminant (Post et al., 2012). These efforts represent a large amount of work that had already been completed in reviewing information relevant to the development of a Health-based MCL recommendation for PFOA and served as a starting point for the evaluation presented in this document.

Health endpoints evaluated comprehensively include: serum cholesterol/lipids, liver enzymes/bilirubin and liver disease, uric acid, thyroid function and thyroid disease, and antibody concentrations following vaccination. Some of the factors considered in selection of these endpoints were the extent and consistency of the data, whether the effect has been observed at exposures relevant to potential drinking water exposures, and evidence for reverse causality. Comprehensive evaluation involved the review of peer-reviewed studies identified through an *a priori* literature search and screening criteria. An individual study table summarizing the study design, location, study population characteristics, outcome and exposure assessment, study population exposure, statistical methods, results, major limitations which addresses risk of bias, and funding source for each reviewed study can be found in Appendix 4, and tables summarizing all studies of each endpoint are found below. Two other critical endpoints, fetal growth following developmental exposure and cancer, were recently comprehensively reviewed by other authoritative scientific groups. Review reports by these groups are evaluated and summarized in this document.

In total, 54 epidemiological studies assessing associations with serum cholesterol/lipids, liver enzymes/bilirubin, uric acid, thyroid function and thyroid disease, and/or antibody concentrations following vaccination were evaluated in depth. The studies were conducted on populations in the U.S., Canada, and several European and Asian countries. The studies evaluated the general population, communities with drinking water contaminated with PFOA, and occupationally-exposed workers, thereby assessing health effects over a wide range of PFOA exposures and serum concentrations.

In human environmental health effect studies in general, confounding by co-exposure to contaminants other than the one being evaluated may be particularly important since it may bias results. In some instances, PFOA has been shown to be strongly correlated with other co-

occurring PFCs which may not have been controlled for, and the same may be true for other environmental contaminants. This confounding bias could impact studies in any type of population, but may play a more important role in occupational populations which may be more likely than the general population to be exposed to co-occurring contaminants at meaningful levels. In general, co-exposure to other chemicals could also be more likely in communities where there are high levels of environmental contamination. However, this is not likely the case in the C8 Health Project, a large community study of populations with drinking water exposure to PFOA (discussed in more detailed below), since PFOA is the only contaminant that was reported to be present at elevated levels in drinking water or other environmental media.

As is the case for epidemiologic studies of environmental contaminants in general, the nature of these observational epidemiology studies, in contrast to experimental studies, limits our ability to definitively conclude that PFOA causes health effects. However, the findings from observational epidemiology studies are useful in assessing consistency, strength of association, exposure-response, temporality, specificity, and biologic plausibility - criteria which are useful in assessing causation.

Studies of Exposure Levels Found in the General Population

For the endpoints that were comprehensively reviewed, the majority of studies evaluated the general population and/or study populations with general population-level exposures to PFOA. Twenty nine (29) studies with general population, low-level exposures were identified. The serum PFOA concentrations (based on a measure of central tendency, which was presented as median, mean, or geometric mean) in these studies range from 0.9 to 7.1 ng/ml. A strength of the general population studies is their use of serum PFOA levels as the basis for exposure assessment. Because of the long human half-life of PFOA, serum levels do not rapidly fluctuate with short term variations in exposure, and serum levels taken at a single time therefore reflect long-term exposures. Serum levels thus provide an accurate measure of internal exposure for each study participant, an advantage over studies based on external exposure metrics such as drinking water concentrations. Among these studies, the large majority are cross-sectional (23 studies, plus one which includes a cross-sectional component).

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

Studies in Exposed Communities

For the endpoints selected for comprehensive evaluation, 15 studies evaluated highly-exposed individuals residing in communities with known PFOA drinking water contamination or in close proximity to a factory utilizing or producing PFOA. A large majority of these studies (14) occurred among communities in the Mid-Ohio Valley near the DuPont Washington Works plant in Parkersburg, WV. This industrial facility used large amounts of PFOA in the manufacturing of

a fluoropolymer, polytetrafluoroethylene (PTFE), and discharged PFOA to the environment resulting in widespread drinking water contamination. Many of the studies in this population are the result of the settlement of a class-action lawsuit by residents exposed to PFOA-contaminated drinking water which mandated that DuPont fund a health study called the C8 Health Project. Additional epidemiologic studies of associations with PFOA and health endpoints in this population have also been published by other researchers.

The C8 Health Project is a community health study of approximately 70,000 Ohio and West Virginia residents of all ages (infants to very elderly) with at least one year of exposure to drinking water contaminated with PFOA at >50 ng/L to over 3000 ng/L (Frisbee et al, 2009; C8 Science Panel, 2014). The C8 Health Project was conducted by the C8 Science Panel, which consisted of three epidemiologists chosen jointly by the parties involved in the legal settlement. This study is notable because of its large size, the wide range of exposure levels, and the large number of parameters evaluated. Data collected included serum levels of PFOA and other PFCs, clinical laboratory values, and health histories. The median serum PFOA concentration in this population was 28 ng/ml (ppb), and serum concentrations in the lowest two deciles were within the U.S. general population range at the time (<10 ng/ml).

The C8 Science Panel was charged with determining if "probable links" exist between diseases and PFOA exposure in the C8 study population, based on the results of their studies and other information from the scientific literature. Probable links were defined as "…. given the scientific evidence available, it is more likely than not that a connection exists between C8 exposure and a particular human disease among class members…". Probable links were established with PFOA exposure and six health endpoints (clinically defined high cholesterol, kidney and testicular cancer, ulcerative colitis, thyroid disease, and pregnancy-induced hypertension). For a number of other endpoints, no probable link with PFOA exposure was reported. Associations were also found with additional health endpoints for which no probable link evaluation was conducted because they were not considered to be clinically defined diseases. These endpoints include increased serum levels of liver enzymes, uric acid, C-reactive protein, and others. C8 Science Panel reports and citations for peer-reviewed publications presenting the results of these studies are found at the C8 Science Panel website (C8 Science Panel, undated, b).

Occupational Studies

There are 14 peer-reviewed occupational studies of the endpoints chosen for detailed evaluation, three of which also studied exposed community populations (Wang et al., 2012, Winquist and Steenland, 2014a, and Winquist and Steenland, 2014b). Eight of the 14 occupational studies are cross-sectional. Locations include industrial facilities in the U.S., Italy, Belgium, and China. Occupational studies are often considered useful for evaluating effects of environmental contaminants because exposure levels are generally higher than in general population or in communities exposed through site-specific environmental contamination. Mean or median serum

PFOA levels in occupational studies reviewed in this report were generally over 1,000 ng/ml (ppb), several orders of magnitude higher than the median concentrations in the general population or in communities with drinking water exposure.

Associations of PFOA with some clinical parameters, including cholesterol, liver enzymes, and uric acid, exhibit a steep dose-response curve in the lower exposure range found in the general population, with a much flatter slope (approaching a plateau) at higher exposure such as those found occupationally (discussed in more detail below). For dose-response curves of this type, the associations found in populations with lower exposures may not be observed in workers because even the least exposed workers used as the comparison/reference group in occupational studies may have exposure levels that are high enough to fall on the much flatter upper portion of the dose-response curve.

Occupational studies may also have a selection bias from a "healthy worker effect" whereby workers usually have lower overall mortality and morbidity than individuals of the same age as a whole, since severely ill and disabled persons are typically not included in the workforce, especially in industrial settings (Shah, 2009). Longer duration of employment may also increase the effects of this bias, since sick people will be more likely to leave or change to safer work. Therefore, data based on duration of employment may not accurately reflect higher prevalence or larger magnitude of effects that are associated with longer exposures to the contaminant being evaluated.

Another issue with occupational studies of PFOA is the small number of exposed female employees which limits the ability of the occupational epidemiology to adequately address specific effects among women.

An additional issue is the possibility of effect modification due to exposure to other chemicals. Exposure to other PFCs, including PFOS at the 3M Decatur plant, may have played a role in the observed associations. Differences in exposures to other chemicals among manufacturing facilities may result in differences in degree of association with various effects.

Comprehensively Reviewed Endpoints

Serum lipids

Associations of serum lipids and PFOA were evaluated in 24 studies, each of which included one or more of the following endpoints: total cholesterol, high density lipid cholesterol (HDL), non-HDL, ratio of total cholesterol to HDL, low-density lipid cholesterol (LDL), very low-density lipid cholesterol (VLDL), ratio of HDL to LDL, and triglycerides. There is also one additional study which only evaluated expression of genes related to cholesterol transport in humans (Fletcher et al., 2013). Study details are provided in the tables for individual studies (Appendix 4) and the summary table for serum lipids (Table 6A).

In total, 20 studies evaluated serum total cholesterol and two evaluated self-reported clinically defined high cholesterol (Steenland et al., 2015 and Winquist and Steenland, 2014). Among the 20 serum total cholesterol studies, 15 were cross-sectional (Emmett et al., 2006b; Eriksen et al., 2013; Fisher et al., 2013; Frisbee et al., 2010; Fu et al., 2014; Geiger et al., 2014; Gilliland et al., 1996; Nelson et al., 2010; Olsen et al., 2000; Olsen and Zobel 2007; Sakr et al., 2007a; Starling et al., 2014; Steenland et al., 2009; Wang et al., 2012; and Zeng et al., 2015) and two studies included cross-sectional and other analyses (Costa et al., 2009; and Olsen et al., 2003). The cross-sectional studies include seven studies of the general population or individuals with low-level exposures (Eriksen et al., 2013; Fisher et al., 2013; Fu et al., 2014; Geiger et al., 2014; Nelson et al., 2010; Starling et al., 2014; and Zeng et al., 2015); four studies of residents of highly exposed communities (Emmett et al., 2006b; Frisbee et al., 2010; Steenland et al., 2009; Wang et al., 2012); and five studies of occupationally exposed individuals (Gilliland et al., 1996; Olsen et al., 2000; Olsen and Zobel 2007; Sakr et al., 2007a; and Wang et al., 2012). Five remaining studies evaluating serum total cholesterol and PFOA include an occupational case-control study (Costa et al., 2009), and four cohort studies including one study of residents of a highly-exposed community (Fitz-Simon et al., 2013) and three studies of occupationally exposed individuals (Olsen et al., 2003; Olsen et al., 2012; and Sakr et al., 2007b).

Six of seven cross-sectional studies of the general population or populations with low-level exposures found evidence of statistically significant positive associations with serum cholesterol and PFOA. These studies of general population level exposures include a study nested in a larger cohort in Denmark of adults, aged 50 to 65 years, with mean serum PFOA concentration of 7.1 ng/ml (Eriksen et al., 2012); a general population study in Canada with a PFOA geometric mean of 2.5 ng/ml (Fisher et al., 2013); a small study of individuals randomly selected from attendees at a health check-up clinic with a median serum PFOA concentration of 1.4 ng/ml (Fu et al., 2014); a study of children in the U.S. general population with a serum PFOA mean concentration of 4.3 ng/ml (Geiger et al., 2014); a study of the general U.S. population aged 12 years older with a median PFOA concentration of 3.8 ng/ml (Nelson et al., 2010); and a study of subjects recruited from the control group of another study in Taiwan with median PFOA exposures of 1.1 ng/ml in boys and 0.9 ng/ml in girls (Zeng et al., 2015). A study of pregnant women recruited from a larger cohort in Norway, with a median serum PFOA concentration of 2.3 ng/ml, did not find a statistically significant positive association with PFOA and serum cholesterol; however, results showed a positive and increasing association of cholesterol with increasing quartiles of PFOA (Starling et al., 2014).

Two large cross-sectional studies evaluated individuals residing in communities located in the mid-Ohio Valley with drinking water contaminated with PFOA. One study included 12,476 children aged 1 to 17.9 years with a mean serum PFOA concentration of 69.2 ng/ml (Frisbee et al., 2010) and the other included 46,294 individuals aged 18 years or older with a median serum

PFOA concentration of 27 ng/ml (Steenland et al., 2009). Both studies found a positive, statistically significant association of serum PFOA and cholesterol. A third smaller (n=371) cross-sectional study from the water district in the mid-Ohio Valley with the highest PFOA levels in its drinking water, with a much higher median serum PFOA concentration, 354 ng/ml, did not find a statistically significant association (Emmett et al., 2006)b. A fourth study from China, which in addition to a study of 132 residents located near a plant utilizing PFOA with a median PFOA concentration of 284 ng/ml also included a worker study, did not find an association with serum cholesterol in either group (Wang et al., 2012).

Of the five occupational cross-sectional studies, only one U. S. occupational study (n=840) with a median serum PFOA concentration of 189 ng/ml found a positive statistically significant association with serum cholesterol (Sakr et al., 2007a). The remaining four occupational cross-sectional studies which did not find evidence of an association include two U.S. male only worker studies, one with a mean serum PFOA concentration of 3,300 ng/ml and a sample size of 115 (Gilliland et al., 1996), and one with a mean serum PFOA concentration of 1,190 ng/ml with a sample size of 265 (Olsen et al., 2000). The third study took place in both the U.S. and Belgium with a median PFOA concentration of 2210 ng/ml and a sample size of 506 (Olsen and Zobel 2007) and the fourth cross-sectional study included 55 workers in China with a median PFOA concentration of 1,636 ng/ml (Wang et al., 2012).

Five of the 20 studies had study designs other than cross-sectional. A longitudinal analysis of workers from Belgium and U.S. with a range of PFOA means of 1,220 to 1,900 ng/ml (Olsen et al., 2003), and another longitudinal worker cohort analysis from the U.S. with a range of PFOA exposure from 1,010 to 1,160 ng/ml (Sakr et al., 2007b), both found evidence of an association with PFOA and serum cholesterol. A third occupational cohort study utilizing matched-pair analysis of 98 to 179 workers (highly exposed of 881 ng/ml PFOA mean v. lower exposed of 28.9 ng/ml PFOA mean) did not find a statistically significant association (Olsen et al., 2012). None of these studies found evidence of a statistically significant inverse association with serum cholesterol and PFOA. An Italian male occupational case-control study with PFOA median concentration 4,400 among formerly exposed workers and a median of 5,700 ng/ml among currently exposed workers, with cross-sectional analysis, found evidence of a positive association (Costa et al., 2009). Among the cohort studies, a longitudinal study of individuals in highly-exposed mid-Ohio Valley communities, with geometric mean PFOA concentrations of 74.8 ng/ml at baseline and 30.8 ng/ml at follow-up, found evidence of a positive association (Fitz-Simon et al., 2013).

Although 15 of the 20 studies evaluating associations of PFOA and serum cholesterol were cross-sectional, thereby limiting the interpretation of temporality since exposures and outcomes are measured at the same point in time, four studies included longitudinal analyses (Fitz-Simon et al., 2013; Sakr et al., 2007b; Costa et al., 2009; Olsen et al., 2003). Each of these studies had

multiple measurement data, and all four found a significant correlation over time between cholesterol and PFOA levels (Fitz-Simon et al., 2013; Steenland et al., 2010b). In summary, the epidemiologic data provide evidence of consistency, strength and dose-response, including some evidence of temporality, of PFOA and serum cholesterol.

Several of the studies mentioned above showed statistically significant trends for increased serum cholesterol with increasing serum PFOA. A decile analysis of PFOA with total cholesterol among a large study of residents of a highly exposed community showed an increasing effect of PFOA on cholesterol and additionally the odds of clinically defined hypercholesterolemia (≥240 mg/dL) increased 40-50% from the lowest to the highest quartile of PFOA (Steenland et al., 2009). A statistically significant trend of increasing serum cholesterol with increasing PFOA was also reported in at least five other studies (Frisbee et al., 2010, Fu et al., 2014, Geiger et al., 2014; and Zeng et al., 2015).

In summary, general population level exposure studies (seven), found consistent evidence of a positive association between PFOA and serum cholesterol. Additionally, three very large studies (two cross-sectional and a cohort study) of highly exposed community populations found evidence of a positive association between PFOA and serum cholesterol. Two longitudinal occupational studies also found a positive association, along with one case-control occupational study. In contrast, results from two much smaller cross-sectional studies of highly exposed community populations (with higher median population exposures than the three larger studies) and a matched-pairs occupational study did not find an association. Although findings from the occupational cross-sectional studies in general (four out of five) found no evidence of an association, they may be biased toward the null by a healthy worker effect. This is suggested by a similar pattern of inconsistency among these study's findings as compared to the findings from the corresponding database were also noted for other serum lipid endpoints (HDL and LDL – discussed below).

In general, studies of the general population, as well as large, mid-exposure range community studies and occupational studies with longitudinal designs, found consistent evidence of an association, while a few smaller, higher exposure range community and occupational studies found no evidence. None of the 20 studies evaluated found evidence of an inverse association.

A review by Steenland et al. (2010a) summarized and evaluated the epidemiologic literature on PFOA and cholesterol available at that time. The authors noted that the lower the range of PFOA that was studied, the greater the change in cholesterol per unit change in PFOA. They suggest that, as discussed in Occupational Studies (above), an exposure-response relationship that is steep at low PFOA concentrations and then flattens out (i.e. approaches a plateau) at higher serum PFOA concentrations is a possible explanation for the observed differences in effect magnitudes. Therefore, studies of populations with high serum PFOA concentrations may not detect an association of PFOA with serum cholesterol if there is a steep dose-response curve for

the association in the lower exposure ranges. For dose-response curves of this type, associations may not be evident in populations with higher exposures since even the least exposed individuals in the comparison group may have exposures that fall on the much flatter (approaching a plateau) portion of the exposure/response curve.

Associations of PFOA and high density lipid cholesterol (HDL), non-HDL, ratio of total cholesterol to HDL, low-density lipid cholesterol (LDL), very low-density lipid cholesterol (VLDL), ratio of HDL to LDL, and/or triglycerides were evaluated in 20 studies. All but two of these 20 studies also evaluated serum cholesterol.

HDL and PFOA were evaluated in 19 studies. It should be noted that an increase in HDL is considered to be beneficial, as compared to increases in total cholesterol, LDL, and non-HDL, which are considered to be undesirable. None of these studies found an association with increased HDL, while four of the 19 studies found evidence of statistically significant decreased association with HDL (Gilliland et al., 1996; Olsen et al., 2000; Olsen and Zobel 2007; and Wang et al., 2012). Interestingly, these four studies are all occupational cross-sectional studies which also did not find evidence of an association with PFOA and increased serum cholesterol (described above), whereas the only other additional occupational cross-sectional study found no evidence of an association with HDL but did find a statistically significant positive association between PFOA and cholesterol (Sakr et al., 2007a). These differences in findings suggest that these occupational cross-sectional studies may be biased from a healthy worker effect. There was no evidence of statistically significant associations with HDL in any of the other 15 studies (Costa et al., 2009; Fisher et al., 2013; Fitz-Simon et al., 2013; Frisbee et al., 2010; Fu et al., 2014; Geiger et al., 2014; Lin et al., 2011; Nelson et al., 2010; Olsen et al., 2003; Olsen et al., 2012; Sakr et al., 2007a; Sakr et al., 2007b; Starling et al., 2014; Steenland et al., 2009; Wang et al., 2012 [resident study]; and Zeng et al., 2015).

Non-HDL was evaluated in four studies: two general population cross-sectional studies (Fisher et al., 2013; and Nelson et al., 2010), a U.S. occupational longitudinal study (Olsen et al., 2012), and a large cross-sectional study of residents in highly exposed communities (Steenland et al., 2009). Three of the studies found statistically significant positive associations with non-HDL and PFOA (Fisher et al., 2013; Nelson et al., 2010; and Olsen et al., 2012), while the occupational longitudinal study had a negative association with non-HDL which was not statistically significant (Olsen et al., 2012).

The ratio of total cholesterol to HDL was evaluated in three studies with inconsistent findings. A general population study in Canada did not find evidence of a statistically significant association (Fisher et al., 2013), U.S. occupational longitudinal study found a statistically significant negative association (Olsen et al., 2012), and a large study of residents from a highly exposed community found a statistically significant positive association (Steenland et al., 2009).

Associations of LDL and PFOA were evaluated in 16 studies. Fourteen of the studies are cross-sectional, which includes seven low level exposure populations (Fisher et al., 2013; Fu et al., 2014; Geiger et al., 2014; Lin et al., 2013; Nelson et al., 2010; Starling et al., 2014; and Zeng et al., 2015), three studies of residents from a highly exposed community (Frisbee et al., 2010; Steenland et al., 2009; Wang et al., 2012), and five studies of occupationally exposed individuals (Gilliland et al., 1996; Olsen et al., 2000; Olsen and Zobel 2007; Sakr et al.; 2007a ; and Wang et al., 2012). The other two studies of LDL and PFOA include an occupational longitudinal study (Sakr et al., 2007b) and a cohort study of residents from the highly exposed community, mid-Ohio Valley (Fitz-Simon et al., 2013).

Among the cross-sectional studies of populations with low level exposure, three found evidence of statistically significant positive associations with LDL (Fu et al., 2014; Geiger et al., 2014; and Zeng et al., 2015) and four found no statistically significant evidence of an association (Fisher et al., 2013; Lin et al., 2013; Nelson et al., 2013; and Starling et al., 2014). Of the three cross-sectional studies of residents from a highly exposed community; the two large studies in the mid-Ohio Valley, one which included children and the other of adults, found evidence of statistically significant positive association (Frisbee et al., 2010, and Steenland et al., 2009); while the third smaller study of 132 residents in China found no evidence of an association (Wang et al., 2012). Four of the five occupational cross-sectional studies found no association (Gilliland et al., 2996; Olsen et al., 2000; Olsen and Zobel, 2007; and Wang et al., 2012) while only one of the studies found evidence of a statistically significant association with both LDL and VLDL (Sakr et al., 2007a). Additionally, an occupational longitudinal study found a positive, non-statistically significant association with LDL (Sakr et al., 2007b) while a cohort study of residents from a highly exposed community found a statistically significant positive association (Fitz-Simon et al., 2013). Finally, the ratio of HDL to LDL was evaluated in a cross-sectional study which assessed both occupational and highly exposed residential populations and found a negative association with the worker population and no evidence of a statistically significant association with the residential population (Wang et al., 2012).

In summary, positive associations with PFOA and LDL were inconsistent among low level exposure populations, and largely unassociated in occupational studies, but there is consistent evidence of an association with PFOA and LDL among larger studies of the highly exposed mid-Ohio Valley communities: two cross-sectional studies one among children and another among adults, and a longitudinal study.

Sixteen studies evaluated triglycerides with inconsistent findings. Four of the studies found evidence of positive statistically significant association (Frisbee et al., 2010; Olsen et al., 2003; Olsen and Zobel, 2007; and Zeng et al., 2015), one found evidence of a negative statistically significant association (Lin et al., 2013), and 11 studies found no evidence of a statistically

significant association (Costa et al., 2009; Fisher et al., 2013; Fitz-Simon et al., 2013; Fu et al., 2014; Geiger et al., 2014; Lin et al., 2011; Olsen et al., 2000; Sakr et al., 2007a; Sakr et al., 2007b; Starling et al., 2014; and Wang et al., 2012).

Selection bias may be an issue in Fu et al. (2014) since the study included only individuals attending a health clinic check-up such that individuals concerned with existing health issues may be more likely to be included. Selection bias may also be an issue in Lin et al. (2013), which included individuals with an abnormal urinalysis from a population-based screening program in which the final study population was made up of 246 (37%) individuals with elevated blood pressure. Information bias is unlikely to have an impact in the general population studies which relied on serum concentrations and clinical biomarkers. In contrast, some occupational studies relied on medical record abstraction of clinical parameters. Other limitations of occupational studies include small sample size that may limit power to detect associations, possibility of healthy worker effect, inclusion of few or no women, and the possibility that exposure in the least exposed groups may be well above the population exposure range in occupationally exposed individuals.

The biological plausibility of the association of PFOA and serum cholesterol was investigated in a study of associations of serum PFOA and changes in expression of genes involved in cholesterol metabolism. In this cross-sectional study, expression of 13 genes involved in cholesterol metabolism (cholesterol biogenesis, peroxisome proliferation, cholesterol transport, downstream transcriptional activation of PPAR-alpha, and mobilization of cholesterol) was evaluated in whole blood from 290 subjects from a highly exposed community (geometric mean serum PFOA, 32.2 ng/ml). Statistically significant associations between genes involved in cholesterol transport and mobilization and PFOA were found, and the affected genes differed in men and women. The authors state that these change in gene expression "appear consistent with PFOA promoting a hypercholesterolemic environment" (Fletcher et al., 2013).

Effects of PFOA on serum lipids in laboratory animals are considered in evaluating the biological plausibility of the associations of PFOA and cholesterol found in humans. Serum cholesterol was not affected by PFOA in a 6-month study of cynomolgus monkeys (Butenhoff et al., 2002), while triglycerides were significantly increased compared to controls at several time points in the monkeys in the mid- and high dose groups (10 and 20/30 mg/kg/day). As discussed above, the dose-response curve for increased cholesterol in humans appears steepest at serum PFOA levels below about 40 ng/ml, with a much flatter dose-response at higher serum levels. In the monkey study, the mean serum PFOA level in the control group was 134 ng/ml. If the dose-response curve is similar in monkeys such that effects on serum cholesterol are steepest at lower serum PFOA levels, effects may not be observable because the control exposure levels could be high enough to fall on the much flatter portion of the dose-response curve.

Although PFOA is consistently associated with increased serum cholesterol in humans, serum cholesterol and triglycerides are generally decreased by PFOA in rodents. This effect in rodents is attributed to PPAR-alpha activation (Lau, 2013). As discussed in detail in the Mode of Action section below, PPAR-alpha activators typically reduce serum lipids in both rodents and humans, and this is the basis for the use of fibrates as hypolipidemic drugs in humans. It is well established that the effects of PFOA in rodents differ from those of other PPAR-alpha activators, and that PFOA affects rodents through both PPAR-alpha independent and PPAR-alpha dependent pathways. It is possible that the dissimilar effects of PFOA on serum lipids in humans and rodents could arise from a different balance of PPAR-alpha dependent and independent processes with opposite effects on this endpoint.

Two recent studies, discussed in detail in the Mode of Action section, suggest the important possibility that the contrasting effects on serum cholesterol observed in humans and animals may result from differences in dietary fat content, rather than intrinsic interspecies biochemical or physiological differences (Tan et al., 2013; Rebholz et al., 2016). In these studies, mice were fed a diet with a high fat content, similar to that of a typical U.S. diet, instead of standard lower fat laboratory chow. Serum cholesterol was either increased or unaffected in mice fed the high fat diets. In contrast, serum cholesterol was decreased, as is typically seen in mice fed the regular diet in Tan et al. (2013), while Rebholz et al. (2016) did not include a regular diet group. These results suggest that effects of PFOA on cholesterol may be similar in rodents and humans consuming the same dietary fat content, and they provide evidence for biological plausibility for PFOA's effects on serum cholesterol in humans.
In summary, the epidemiologic database for serum cholesterol and PFOA, which included twenty studies, provides evidence of consistency, strength and dose-response, including some evidence of temporality. Associations with clinically defined hypercholesterolemia were reported in some studies. These findings provide evidence supporting a causal relationship between PFOA and serum cholesterol. Overall, the epidemiologic evidence suggests no evidence of an association with HDL and PFOA. There were a limited number of epidemiologic studies evaluating an association with non-HDL or the ratio of total cholesterol to HDL and PFOA. The epidemiologic database for PFOA and LDL appears inconsistent. Although there is some evidence of an association with LDL, it remains limited due to the interpretation of other studies which found no evidence of an association. There is limited epidemiologic evidence evaluating associations of VLDL, the ratio of HDL to LDL, and triglycerides with PFOA.

Liver enzymes/bilirubin

A total of 16 studies evaluated associations between PFOA and clinical biomarkers used in the diagnosis and/or evaluation of treatment of liver function or metabolic disease. These biomarkers include the following liver enzymes: alanine aminotransferase (ALT /SGPT), gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST /SGOT), and alkaline phosphatase (ALP), as well as total bilirubin or unspecified bilirubin (TB) and direct bilirubin (DB). Additionally,

two studies assessed PFOA and liver disease (LD). Study details are provided in the tables for individual studies (Appendix 4) and the summary table for liver enzymes/bilirubin (Table 6B).

Two larger cross-sectional general population studies utilizing different survey cycles of the U.S. National Health and Nutrition Examination Survey (NHANES) both found evidence of statistically significant positive associations with PFOA and the liver enzyme ALT (Gleason et al., 2015 and Lin et al., 2010). Two other low cross-sectional studies of a population with low-level exposure cross-sectional studies have also evaluated this association. A study that was based on a population recruited from a larger cohort in Taiwan (n=608) found a positive statistically significant correlation (Yamaguchi et al., 2013), while a small study (n=141) of pregnant women in China did not find a significant correlation between PFOA and ALT (Jiang et al., 2014). Of the three cross-sectional studies of mid-Ohio Valley residents, the smaller study (n=371) with a higher median and narrower range of PFOA exposure found no evidence of an association (Emmett et al., 2006b), while the two larger studies (n=47,092) with a wider range of exposures found a consistent positive statistically significant association with ALT and PFOA (Gallo et al., 2012; and Gallo et al., 2016). Nine additional occupational studies investigated associations of ALT and PFOA with inconsistent findings. Among these studies only one cross-sectional study found evidence of a positive association (Olsen et al., 2007), one found evidence of a negative association (Gilliland et al., 1996) and four cross-sectional studies found no consistent evidence of an association (Olsen et al., 2000; Olsen et al., 2003; Sakr et al., 2007a; and Wang et al., 2012). An occupational case-control study, with cross-sectional components, found some evidence of a positive association (Costa et al., 2009); one longitudinal occupational study found evidence of a negative association (Olsen et al., 2012), and a second longitudinal occupational study found no evidence of an association (Sakr et al., 2007b).

Although results of occupational studies were inconsistent, both cross-sectional general population studies found evidence of an increasing trend (Gleason et al., 2015 and Lin et al., 2010). The much larger studies of a highly-exposed community also found increasing levels of ALT with increasing serum concentrations of PFOA (Darrow et al., 2016; Gallo et al., 2012). Further, the associations noted by Gallo et al. (2012) were consistent both between water districts and among individuals within the same district, which also increased the strength of evidence. Additionally, the modeled serum PFOA exposure assessment used by Darrow et al. (2016) complements evidence from previous studies because these estimates are not affected by reverse causation.

Thirteen studies evaluated associations of PFOA and GGT: six studies found evidence of a positive statistically significant association (Costa et al., 2009; Gallo et al., 2012; Gleason et al., 2015; Lin et al., 2010; Olsen and Zobel, 2007; and Sakr et al., 2007a) and the remaining seven studies found no statistically significant evidence of an association (Darrow et al., 2016; Emmett et al., 2006b; Gilliland et al., 1996; Olsen et al., 2000; Olsen et al., 2003; Sakr et al., 2007b; and

Yamaguchi et al., 2013). Twelve studies also evaluated the association of PFOA and AST; three found evidence of a positive statistically significant negative association (Gleason et al., 2015; Sakr et al., 2007b; Yamaguchi et al., 2013); two studies found some evidence of a negative association (Gilliland et al., 1996 and Wang et al., 2012) and seven other studies found no evidence (Emmett et al., 2006; Jiang et al., 2014; Olsen et al., 2000; Olsen et al., 2003; Olsen and Zobel 2007; Olsen et al., 2012; and Sakr et al., 2007a).

Eight studies evaluated the association of PFOA and the liver enzyme ALP. Only one found some limited evidence of a positive statistically significant association (Costa et al., 2009), while the other seven studies found no evidence of an association (Emmett et al., 2006b; Gleason et al., 2015; Olsen et al., 2000; Olsen et al., 2003; Olsen and Zobel, 2007; Olsen et al., 2012; and Sakr et al., 2007b).

Thirteen studies evaluated the association of PFOA and either total or direct bilirubin. A component of total bilirubin is direct bilirubin, a product of hemoglobin metabolism for which increased serum concentrations reflect increases in liver and bile duct disease. Therefore, total bilirubin serves only as an inferential measure of liver function. Among studies of total bilirubin, three studies found evidence of a statistically significant association (Costa et al., 2009; Olsen and Zobel, 2007; and Sakr et al., 2007b); one study found a positive statistically significant association (Gleason et al., 2015); and seven found no association with total bilirubin (Emmett et al., 2006b; Jiang et al., 2014; Lin et al., 2010; Olsen et al., 2000; Olsen et al., 2003; Olsen et al., 2012; and Sakr et al., 2007a). Two additional studies found no association with direct bilirubin (Gallo et al., 2012; and Darrow et al., 2016), and Olsen et al. (2000) also found no association with total or direct bilirubin.

Three studies investigated association with PFOA and clinical liver disease. Melzer et al., 2011 found no statistically significant association of PFOA and current liver disease in a cross-sectional study of the U.S. general population (NHANES). Also Darrow et al. (2016) found no evidence of an association with modeled serum PFOA and medically-validated liver disease when categorized as either any liver disease or restricted to enlarged liver, fatty liver, or cirrhosis among the highly exposed C8 Health Study community. A retrospective cohort of a U.S. occupational population also found no association with self-reported liver disease and estimated serum PFOA concentration (Steenland and Zhao et al., 2015).

As previously described, cross-sectional studies limit interpretation of temporality. Information bias is unlikely to have an impact in the general population studies which relied on serum concentrations and clinical biomarkers. Small sample sizes in some studies may have limited their power to detect associations (Emmett et al., 2006b; Jiang et al., 2014; Wang et al., 2012; and Yamaguchi et al., 2013). In addition to small sample size, some occupational studies relied on abstraction of clinical parameters from medical records. Other limitations of occupational

studies include the possibility of healthy worker effect, inclusion of few or no women, and the possibility that exposure in the least exposed groups may be well above the population exposure range in occupationally exposed individuals.

Toxicological and mode of action data support the biological plausibility of hepatic effects of PFOA in humans. As discussed in detail in the Toxicology and Mode of Action sections below, it is well established that hepatic toxicity is a sensitive effect of PFOA in experimental animals. Based on studies in non-human primates, standard strains of rodents, and rodents lacking a functional PPAR-alpha receptor, hepatic effects of PFOA are considered relevant to humans.

In summary, the evaluation of epidemiologic studies provides evidence of some inconsistencies among the group of studies evaluated. However, there was consistency among the larger non-occupational studies, as well as evidence of specificity, exposure-response, strength, and biologic plausibility for PFOA and ALT. These findings provide evidence supporting a causal relationship between PFOA and ALT. The epidemiologic evidence of an association with PFOA and GGT, AST, and bilirubin is inconsistent, while there was no evidence of an association with PFOA and ALP. There is also limited epidemiologic evidence of a causative relationship with PFOA and liver disease, and the available studies did not find an association.

Thyroid hormones, TSH, and thyroid disease

Twenty studies were identified as evaluating thyroid hormones, TSH, hypo-and hyperthyroidism, thyroid disease in general, and/or other thyroid conditions. Study details are provided in the tables for individual studies (Appendix 4) and the summary table for thyroid effects (Table 6C).

Thyroid stimulating hormone (TSH) was the most commonly evaluated thyroid endpoint, and there was limited evidence of a positive statistically significant relationship with PFOA. Three general population studies which include a cross-sectional U.S. population study (Jain, 2013), a South Korean prospective birth cohort (Kim et al., 2011), and a prospective cohort study in Canada (Webster et al., 2014) found some evidence of a positive statistically significant association of elevated TSH and PFOA. The remaining 12 studies found limited or no evidence of a positive association. These 12 studies are all cross-sectional study design, which include six general population studies (Bloom et al., 2010; Ji et al., 2012; Lin et al., 2013; Shrestha et al., 2015; Wang et al., 2014; and Wen et al., 2013), three studies of residents in a highly exposed community (Emmett et al., 2006b; Knox et al., 2011; Lopez-Espinosa et al., 2012), and three occupational studies (Olsen et al., 1998; Olsen et al., 2003; Olsen and Zobel 2007). Three of the 12 studies also included components of other study designs in addition to the cross-sectional

design: birth cohort (Lopez-Espinosa et al., 2012), longitudinal (Olsen et al., 1998), and prospective birth cohort (Wang et al., 2014)

Additionally, total thyroxine (TT4) has been extensively evaluated with little evidence of a positive statistically significant association. Only two studies found some evidence of statistically significant positive association (de Cock et al., 2014, and Knox et al., 2011), while 11 others found no evidence of a statistically significant association (Jain 2013; Ji et al., 2012; Kim et al., 2011; Lin et al., 2013; Lopez-Espinosa et al., 2012; Olsen et al., 2003; Olsen and Zobel 2007; Shrestha et al., 2015; Wang et al., 2014; Webster et al., 2014; and Wen et al., 2013). A case-control study of hypothyroxemic pregnancy matched with non-hypothyroxemic pregnant women in Canada evaluated the association of PFOA and maternal hypothyroxemia, a common condition in pregnant women characterized by low maternal free thyroid hormone (fT4) and normal TSH levels, and found no evidence of a statistically significant association (Chan et al., 2011).

Eight studies evaluated PFOA and associations with total triiodothyronine (TT3). Four of these studies found some evidence of a statistically significant positive association, including two larger (n=1,540 and 1,180) cross-sectional studies of the U.S. general population (Jain 2013 and Wen et al., 2013, respectively) as well as both of the occupational studies (Olsen et al., 2003; Olsen and Zobel 2007).Three studies did not find any statistically significant evidence of an association (Kim et al., 2011; Shrestha et al., 2015; and Wang et al., 2014), while a large (n=50,113) cross-sectional study of the mid-Ohio Valley which found some evidence of an inverse association (Knox et al., 2011). Two of these studies also evaluated free triiodothyronine (FT3) and neither found evidence of a statistically significant association (Jain, 2013; and Wen et al., 2013). These same two studies also evaluated associations of PFOA and thyroglobulin and found no evidence of a statistically significant association (Jain, 2013 and Wen et al., 2013).

Three studies evaluated the association of PFOA and hypo- and hyperthyroidism, with mixed results. Hypothyroidism is a condition in which the thyroid gland is under-active and is characterized by elevated TSH serum levels combined with low serum FT4. Hyperthyroidism is a condition involving an over-active thyroid gland and is characterized by very low TSH hormone and raised FT4. Lopez-Espinosa et al. (2012) found a borderline statistically significant positive association with measured PFOA concentrations and self-reported subclinical hypothyroidism, but found non-statistically significant results for modeled PFOA, including modeled *in utero* exposure to PFOA, and subclinical measures of hypothyroidism. Odds ratio for PFOA and hyperthyroidism were mixed and not statistically significant. A study by Wen et al. (2013) of the U.S. adult general population found a statistically significant positive association of hypothyroidism among women but not men, and a statistically significant negative association of hyperthyroidism among men and not women. Winquist and Steenland (2014b) found increasing hazards with increasing PFOA exposure for hypothyroidism, although the trend was not

statistically significant, while retrospective and prospective analyses were statistically significantly positively associated among men. A statistically significant trend of hyperthyroidism and increasing PFOA exposure was found overall and for women.

Five studies evaluated thyroid disease in general, which may also include hypo- and hyperthyroidism. Three studies found some evidence of a statistically significant positive association with PFOA and thyroid disease. A large study of highly exposed children in the mid-Ohio Valley found a positive statistically significant association among measured PFOA concentrations, median of 29 ng/ml, and parent-reported thyroid disease, but this association was not statistically significant with modeled PFOA (Lopez-Espinosa et al., 2012). A cross-sectional study of the U.S. general population found increasing odds ratio of self-reported thyroid disease, both ever and current, with increasing quartiles of PFOA among women but not men (Melzer et al., 2011). A large retrospective cohort study with prospective analyses found evidence of a positive association with thyroid disease and increasing quintiles of PFOA which was strongest among women for retrospective analyses, but prospective analyses found no clear associations with PFOA and thyroid disease (Winquist and Steenland, 2014b). The remaining two studies, a small study in a highly exposed community wiht median serum PFOA concentration of 354 ng/ml and a relatively narrow range of exposures (Emmett et al., 2006b), and a retrospective occupational cohort with a median PFOA exposure of 113 ng/ml (Steenland et al., 2015), found no evidence of a statistically significant association with thyroid disease and PFOA.

As discussed above, the C8 Science Panel concluded there was a probable link with PFOA and thyroid disease. The C8 Science Panel summarized the epidemiologic evidence of an association with PFOA and thyroid function or disease and, although the evidence was deemed inconsistent, concluded that "…the presence of some independent pieces of evidence indicative of an association was not easily dismissed, despite a lack of coherence among them." The C8 Science Panel determined the strongest evidence for an association was increased occurrence of medically validated thyroid disease (hyperthyroidism in women, hypothyroidism in men) with increasing measured PFOA exposure (2005-2006) in the prospective analyses (2005-2010). Therefore, despite inconsistencies in the evidence, the Panel concluded that there was evidence of a probable link between C8 and thyroid disease (C8 Science Panel, 2012).

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

associations (Bloom et al., 2010; Kim et al., 2011b; Mundt et al., 2007; and Webster et al., 2014).

Although thyroid endpoints were not evaluated in most toxicology studies of PFOA, data from a limited number of studies, reviewed in the Toxicology section below, support the biological plausibility of effects of PFOA on human thyroid function. Although no effects on thyroid hormones were seen in a very small 29-day study of cynomolgus monkeys, changes in TSH, T3, and T4 occurred at multiple time points in a 6-month study in this species. Thyroid hormones were significantly decreased in rats in a short duration exposure study using a high dose of PFOA. Thyroid endpoints have not been evaluated in longer duration, lower dose, or rodent studies.

Overall, studies evaluating thyroid hormones, TSH, and thyroid disease provide inconsistent evidence of any associations with PFOA. The C8 Science Panel concluded that, despite the inconsistencies of findings among studies, compelling evidence for associations with thyroid disease could not be dismissed, and a probable link with PFOA and thyroid disease was determined.

Uric acid

Uric acid is a product of purine metabolism with both oxidant and antioxidant properties, and elevated levels are a marker of kidney disease. Additionally, some studies have shown that elevated uric acid is associated with cardiovascular disease and may trigger hypertension (Klein et al., 1973; Fang et al., 2000; and Freedman et al., 1995). Seven studies evaluated the association of uric acid and serum PFOA concentrations; three of these also assessed clinically defined hyperuricemia. Study details are provided in the tables for individual studies (Appendix 4) and the summary table for uric acid (Table 6D).

These studies include six cross-sectional studies and one occupational study which was a case-control study with cross-sectional components. All of these studies found strong, positively statistically significant associations of uric acid and PFOA (Costa et al. 2009; Geiger et al., 2013; Gleason et al., 2015; Sakr et al., 2007a; Shankar et al., 2011; and Steenland et al., 2010) with the exception of Lin et al., 2013 which did not find a statistically significant association. Additionally, all three studies which evaluated clinically defined hyperuricemia found strong evidence of a positive statistically significant association (Geiger et al., 2013; Shankar et al., 2011; and Steenland et al., 2010b).

Although the six studies with evidence of statistically significant association are mainly cross-sectional, they represent the general population, residents from a highly exposed community, and an occupationally exposed population. These studies therefore evaluated a wide range of serum PFOA concentrations - about 4 ng/ml in the general population studies (Geiger et al., 2013;

Gleason et al., 2015; Shankar et al., 2011), a median of about 28 ng/ml in a highly exposed community population (Steenland et al., 2010b), and a median serum PFOA concentration range from 428 ng/ml (Sakr et al., 2007a) to 4,400 -5,700 ng/ml (Costa et al., 2009) among occupationally exposure populations. Also, importantly, these studies evaluated a wide range of age groups as well, including children less than 19 years of age (Geiger et al., 2013), adolescents and adults greater than 11 years of age (Gleason et al., 2015), and adult populations 20 years or older (Costa et al., 2009, Shankar et al., 2011; and Steenland et al., 2010b).

The general population studies which evaluated associations of uric acid with increasing serum concentrations of PFOA found strong exposure-response relationships (Geiger et al., 2013; Gleason et al., 2015; and Shankar et al., 2011). Additionally, Steenland et al. (2010b) found a significant trend and some evidence of increased changes in uric acid with high level of serum PFOA concentrations among residents from a highly exposed community. Lin et al. (2013) did not find evidence of a statistically significant trend in mean uric acid across categories of PFOA exposure but there does appear to be a small, non–significant increase in uric acid level as serum PFOA concentrations increase.

Information bias is unlikely to have an impact in the general population studies which relied on serum concentrations and clinical biomarkers. Selection bias may have impacted findings in Lin et al. (2013), which included individuals with an abnormal urinalysis from a population-based screening program in which the final study population was made up of 246 (37%) individuals with elevated blood pressure.

Reverse causality is a potential explanation for increased uric acid with increasing PFOA. It has been proposed that PFOA could be higher in individuals with reduced excretion due to reduced kidney function, and that this would also result in increased uric acid (Kataria et al., 2015). Also, Kataria et al., (2015) reviewed toxicology evidence and suggests that PFOA and other PFCs can adversely impact renal function. Unfortunately, a hypothesis of reverse causality cannot be assessed because the six studies which evaluated uric acid and PFOA are limited by their cross-sectional design in which exposures and outcomes are measured at the same point in time.

Epidemiologic evidence provides evidence of consistency among findings, strength of findings with clinically defined outcomes, and exposure-response with PFOA and uric acid. These findings provide evidence supporting a causal relationship between PFOA and uric acid. However, there are limitations in use of the epidemiologic evidence to draw conclusions regarding temporality and there remain some questions of biologic plausibility due to possible reverse causality explanation.

Antibody concentrations following vaccination

Five studies evaluated associations of serum PFOA concentrations and antibody concentrations following six types of vaccines. Study details are provided in the tables for individual studies (Appendix 4) and the summary table for this effect (Table 6E). Only one type of vaccine (tetanus) was evaluated in three of the five studies, each of four vaccine types were evaluated in two studies each, and one (mumps) was evaluated in only one study. These five studies include a prospective cohort following 656 singleton births recruited from birth and followed to seven years of age (n=587) in the Faroe Islands (Grandjean et al., 2012). In this group of children with a median serum PFOA concentration at 5 years of age of 4.1 ng/ml, researchers found strong statistically significant evidence of a decrease in antibody concentrations following a tetanus or diphtheria vaccination at age 5 and 7 years. Another prospective birth cohort study in Norway collected blood samples at delivery from 99 pregnant women with a subsequent follow-up sample of 56 children at 3 years of age (Granum et al. 2013). The median PFOA maternal serum concentration was 1.1 ng/ml. Investigators found strong evidence of decreased rubella-induced antibodies with increasing PFOA maternal serum concentrations, but associations with PFOA and responses to tetanus, measles, and influenza vaccines were not statistically significant. A prospective cohort of 12 adults recruited from hospital staff in Denmark with median serum PFOA concentration of 1.7 ng/ml found no statistically significant associations with antibody response to tetanus or diphtheria vaccines (Kielsen et al., 2015). A larger prospective cohort of 411 adults from the mid-Ohio valley with a high median serum PFOA concentration of 31.5 ng/ml found evidence of decreasing antibody concentrations following Influenza A H3N2 vaccination; however no statistically significant associations were found with responses to vaccines for Influenza Type B or Influenza Type A H1N1 (Looker et al., 2014). The largest of the studies includes a sample size of 1,191 adolescents aged 12 to 19 years of age from the U.S. general population with a geometric mean serum PFOA concentration of 4.1 ng/ml. This cross-sectional study found some evidence of a statistically significant decrease of rubella- and mumps vaccine induced antibody concentration, but a decreased antibody response to measles vaccine was not statistically significant (Stein et al., 2016).

Associations between decreased antibody concentration and increasing PFOA concentration may be related to a threshold such that limited evidence of associations was found among the two studies with median serum PFOA concentrations below 2 ng/ml (Granum et al, 2013 and Kielsen et al., 2015). Both of these studies also had small sample sizes which may have restricted the power of the study to detect a statistically significant decrease.

Specificity of the observed association may also be difficult to interpret since responses to many different vaccines were evaluated, with each type of vaccine included only in a few (and often in only one or two) studies. Unlike many of the other outcomes evaluated in studies of the human health effects of PFOA, four of the studies that assessed associations with antibody concentrations following vaccination had a prospective study design, allowing temporality

assessment. Since the exposures and outcomes were followed over time, it can be concluded that exposures preceded the outcome. There was limited evidence or exploration of exposure-response relationships.

Data from other human studies and toxicology studies provide support for biological plausibility of decreased immune system response to vaccines in humans. As discussed in the Toxicology and Mode of Action sections, PFOA suppressed the immune system in studies of both non-human primates and rodents. Fletcher et al. (2009) reported several statistically significant associations between several markers of immune function (decreased IgA; decreased IgE in females only; increased anti-nuclear antibody; decreased C-reactive protein) and serum PFOA levels in communities with drinking water exposure to PFOA in a C8 Science Panel status report (Fletcher et al., 2009). As yet, only the information on C-reactive protein has been published (Genser et al., 2015). Genser et al. (2015) found consistent and significant associations of serum PFOA with this effect, both within each of the six water districts included in the study and on an aggregated basis. They concluded that these within- and between-district associations strengthen the evidence of causality for this effect.

Review of epidemiologic studies provides evidence of consistent findings among studies of decreased antibody concentrations following vaccination and PFOA. However, there are a limited number of comparisons across the same vaccination types, making consistency/specificity difficult to evaluate. While there is epidemiologic evidence of temporality, evidence of an exposure-response is limited.

Additionally, the National Toxicology Program (NTP) recently completed a draft systematic review of immunotoxicity of PFOA, based on consideration of human and animal studies, along with mechanistic data (NTP, 2016). The draft NTP assessment concluded that PFOA is presumed to be an immune hazard to humans based on (1) a high level of evidence from animal studies and a moderate level of evidence from human studies that PFOA suppresses antibody response, and (2) a high level of evidence from animal studies and a low level of evidence from human studies that PFOA increases hypersensitivity-related outcomes. NTP also considered additional, although weaker, evidence primarily from epidemiological studies that PFOA reduced infectious disease resistance and increased autoimmune disease. NTP states that the evidence for effects on multiple aspects of the immune system supports the overall conclusion that PFOA alters immune function in humans.

Endpoints Evaluated by Other Researchers:

Fetal growth

A collaborative team of scientists developed a methodology for the systematic review of environmental health data titled the “Navigation Guide.” The Navigation Guide is intended as a “systematic and rigorous approach to research synthesis” that will “reduce bias and maximize

transparency in the evaluation of environmental health information" (Woodruff and Sutton, 2014). The Navigation Guide methodology utilizes a three-step process in which a study question is specified, evidence is selected, and the quality and strength of the evidence is evaluated. Developmental exposure to PFOA and fetal growth was selected by the research group as a proof-of-concept of the methodology. This effort included evaluation of human epidemiologic data (Johnson et al., 2014), animal toxicology data (Koustas et al., 2014), and a synthesis of both types of data to develop overall conclusions (Lam et al., 2014).

The application of the Navigation Guide methodology to human studies required that each identified study was evaluated for risk of bias, and that the quality and strength of the evidence across all studies was rated. The authors reviewed 19 datasets available from 18 studies which met the inclusion criteria. Additionally, a meta-analysis of 10 studies with combinable study attributes was performed. Investigators concluded there was a low risk of bias across the studies. The meta-analyses of birth weight resulted in an overall continuous regression estimate per ng/ml serum PFOA of -18.9 g (95% CI -29.8, -7.9), an estimate of -0.06 cm (95% CI -0.1, -0.02) in birth length, a -0.01 (95% CI 0.03, 0.01) reduction in ponderal index (birth weight/length^3 x 100), and a -0.03 cm (95% CI -0.08, 0.01) reduction in head circumference. The quality of the human evidence was rated as "moderate"'. By evaluating the quality of studies, direction of effect estimate, confidence of effect, and other possible compelling attributes, it was concluded that there is "sufficient" human evidence, the strongest descriptor for strength of evidence, that developmental exposure to PFOA reduces fetal growth in humans (Johnson et al., 2014).

The conclusions of another recent meta-analysis of human data (Verner et al., 2015) were generally consistent with Johnson et al. (2014). Verner et al. (2015) estimated a decrease in birth weight per ng/ml PFOA in maternal or cord blood serum of -14.7 g (95% CI -21.7, -7.8). They note that their analysis was less formal than the one conducted by Johnson et al. (2014) and that it did not include two studies included in the analysis of Johnson et al. (2014).

The animal evidence was also reviewed by the Navigation Guide research group, and it was concluded that there is "sufficient" evidence that exposure to PFOA adversely affects fetal growth in animals (Koustas et al., 2014). The human and non-human evidence was integrated to develop an overall conclusion on whether developmental exposure to PFOA affects fetal growth in humans. The human and non-human mammalian evidence were both rated as being of "moderate" quality and "sufficient" strength. The authors concluded that there is "sufficient" evidence that developmental exposure to PFOA adversely affects human health based on decreased fetal growth in both human and non-humans (Lam et al., 2014).

Although previous research in the C8 Health Study population did not find an association with clinically defined birth weight and PFOA (Stein et al., 2009, C8 Science Panel, 2011), a subsequent study in this population found associations with low birth weight evaluated as a

continuous variable (Savitz et al., 2012). Thus, findings from the C8 Health Study do not contradict conclusions reached by the Navigation Guide.

Evidence supporting biological plausibility for decreased fetal growth from PFOA is provided by studies (discussed in the Toxicology section) showing that PFOA adversely effects prenatal and postnatal growth and development. As discussed in the Mode of Action section, cellular receptors that play a role in the developmental toxicity of PFOA are found in human tissues, and developmental effects observed in laboratory animals are believed to be relevant to humans.

Potential impact of glomerular filtration rate on association of PFOA and fetal growth

Glomerular filtration rate (GFR) is the flow rate of fluids being filtered through the kidneys. GRF increases during the first half of pregnancy and declines slightly during the second half (Gibson 1973). Decreased GFR has been associated with lower infant birth weight in some studies (reviewed by Lam et al., 2014; Morken et al., 2014). Because decreased GFR might also result in higher serum PFOA levels due to slower excretion, it has been hypothesized that lower GFR could be a confounding factor for the association of PFOA and decreased fetal growth.

Lam et al. (2014) considered the evidence for reverse causality related to decreased GFR for the association of PFOA with decreased fetal growth. They concluded that the available data did not justify revision of their conclusion of sufficient human evidence for association of PFOA and decreased fetal growth. Vesterinen et al. (2015) subsequently adapted and applied the Navigation Guide methodology, described above, to assess the evidence of an association between fetal growth and GFR. The authors identified 35 relevant studies (31 human observational, two non-human observational, and two non-human experimental), all of which were rated as either “low” quality or “very low” quality. All three of these evidence streams were classified as “inadequate,” indicating that the association between GFR and fetal growth was “not classifiable.” Based on their review, Vesterinen et al. (2015) concluded that the current evidence is insufficient to support the plausibility of a reverse causality hypothesis for the associations between environmental chemicals during pregnancy and fetal growth. In an additional recent study not included in the review of Vesterinen et al. (2015), Morken et al. (2014) analyzed data from 953 pregnant Norwegian women (470 with pre-eclampsia and 483 without pre-eclampsia) and estimated GFR in the second trimester based on plasma creatinine. The association of estimated GFR and infant birth weight was not significant at $p<0.05$ when women with pre-eclampsia, which is associated with decreased kidney function, were excluded from the analysis. When these women with pre-eclampsia were included, the association was significant.

Verner et al. (2015) used data from three studies that provide data on maternal GFR and birth weight on an individual basis to modify a physiologically based pharmacokinetic (PBPK) model for PFOA during pregnancy (previously developed by Loccisano et al., 2013) to include the relationship between GFR and birth weight. A Monte Carlo simulation using the modified model

was used to estimate the population distribution of PFOA levels in maternal and cord blood plasma during pregnancy and at delivery. Based on an assumed PFOA half-life of 3.8 years, the model simulation predicted a decrease in birth weight per ng/ml PFOA of -7.9 g (CI -9.4, -6.4) based on maternal plasma at term and -7.1 g (95% CI -8.5, -5.8) based on cord plasma at delivery. When a shorter half-life of 2.3 years (which is likely more appropriate, discussed in Toxicokinetics section) was assumed, greater effects on birth weight per ng/ml PFOA were predicted: -9.6 g (95% CI -11.0, -8.2) based on maternal plasma at delivery and -8.1 g (95% CI -9.4, -6.8) based on cord plasma at delivery. The data from the simulation were compared to the results of a meta-analysis of the effect of maternal or cord plasma PFOA on birth weight (-14.7 g per ng/ml, 95% CI -21.7, -7.8) to estimate the effect of GFR on this association. Results of this study suggests that GFR may confound a portion (less than 50%) of the association between PFOA and decreased birth weight, and that it would be desirable to control for GFR in studies of PFOA and birth weight to account for potential confounding by GFR.

Based on review of the relevant information, the Health Effects Subcommittee concludes that confounding by GFR does not account for the major portion of the decrease in fetal growth that is associated with PFOA.

Cancer

The USEPA Science Advisory Board (USEPA, 2006) described PFOA as "likely to be carcinogenic to humans" according to the criteria provided in the USEPA Guidelines for Carcinogen Risk Assessment (2005b). This determination was made prior to the publication of the epidemiology studies summarized below and was based on the toxicology and mode of action data available at that time.

The International Agency for Research on Cancer (IARC) is the specialized cancer agency of the World Health Organization (WHO). One of IARC's major activities is the evaluation of the evidence of human carcinogenicity of specific exposures, including environmental contaminants, by international expert working groups which it convenes. IARC considers human, animal, and mechanistic data in making its determinations of evidence for cancer risk to humans. It has classified PFOA as "possibly carcinogenic to humans" (Group 2B) based on "limited evidence that PFOA causes testicular and renal cancer, and limited evidence in experimental animals" (Benbrahim-Tallaa et al., 2015). A brief summary of the basis for this classification states that human data considered by IARC in making this determination included increases in cancer among workers in the DuPont Washington Work plant in Parkersburg, WV (Steenland and Woskie, 2012) and among highly exposed members of the C8 Health Project study population (Barry et al., 2013 and Vieira et al., 2013). The complete report supporting IARC's conclusion will be published in Volume 110 of the IARC Monographs; the release of this volume is still pending. The epidemiological studies cited by Benbrahim-Tallaa et al. (2015) as the basis for IARC's conclusion are presented in more detail below.

More recently, the USEPA Office of Water (2016a) concluded that PFOA has suggestive evidence of carcinogenic potential for PFOA based on the human studies mentioned above that found an association of serum PFOA with kidney and testicular tumors in communities with drinking water exposure and increased incidence of tumors in one more organs in two chronic rat bioassays.

Steenland and Woskie (2012) studied the mortality of 5,791 workers at the DuPont chemical plant in Parkersburg, West Virginia from 1952-2008. Exposure to PFOA in workers was estimated using a job exposure matrix developed from serum data available for a smaller set of workers. Standardized Mortality Ratios (SMRs) were calculated using other DuPont workers in the region and the U.S. population as referent groups. PFOA exposure was categorized into quartiles. The SMR for kidney disease increased with increasing quartiles of exposure (Quartile 1: SMR=1.07, 95% CI 0.02, 3.62 (based on 1 death); Quartile 2: SMR=1.37, 95% CI 0.28, 3.99 (based on 3 deaths); Quartile 3: SMR=0.00, 95% CI 0.00, 1.42 (based on 0 deaths); Quartile 4: SMR=2.66, 95% CI 1.15, 5.24 (based on 8 deaths). Mesothelioma and chronic renal disease (malignant and non-malignant) also both had exposure-response relationships with PFOA. When investigators lagged models by 10-years and 20-years, kidney cancer and chronic renal disease remained most elevated in the highest quartile of exposure compared to the lower quartiles. An important potential confounder in this occupational cohort could be tetrafluoroethylene (TFE) which was also used in the manufacture fluoropolymers at the Parkersburg, WV facility and has been identified as a rodent kidney carcinogen (NTP, 1997). However, the authors believe that appreciable exposures would have been unlikely, since TFE exposure would have been well controlled due to its explosive and volatile nature.

Vieira et al. (2013) performed an ecological study in 13 counties which encompass six PFOA-contaminated water districts in Ohio and West Virginia located near the DuPont chemical plant in Parkersburg, West Virginia. Cases of 18 types of cancer were obtained from the Ohio and West Virginia cancer registries, in which the final data set included 7,869 Ohio cases and 17,328 West Virginia cases. To calculate adjusted odds ratios (OR) with logistic regression, controls were selected from the state cancer registries as all other cases of cancers (excluding cases of kidney, pancreatic, testicular, and liver cancers, the types which have been associated with PFOA in animal or human studies). Exposure estimates were based on estimated 1995 median serum PFOA concentrations in the six water districts. The analysis was adusted for age, sex, diagnosis year, smoking status, and insurance provider. The first of two analyses, using water district of residence as the exposure of interest and including both Ohio and West Virginia cases, found the odds of testicular cancer was statistically significantly increased (OR=5.1, 95% CI 1.6-15.6) and the odds of kidney, non-Hodgkin lymphoma, and prostate cancer were also increased, in the highest exposed water district of Little Hocking, although not statistically significant. Kidney cancer was also elevated in the third highest exposed water district of Tuppers Plain

(OR=2.0, 95% CI 1.3-3.1). Lung cancer was statistically significantly associated with PFOA in the total exposed group and in two of the water districts. For the second analysis, serum PFOA levels were estimated at a finer geographic scale and restricted to Ohio-only cases. Kidney cancer was elevated in both the very highly exposed category (serum concentration of 110-665 ng/ml; OR=2.0, 95% CI 1.0, 3.9) and the highly exposed category (serum concentration of 30.8-109 ng/ml; OR=2.0, 95% CI 1.3, 3.2) groups.

Barry et al. (2013) evaluated cancer incidence among individuals exposed to PFOA through contaminated drinking water in the mid-Ohio Valley, as well as subjects who worked at the local chemical plant where PFOA was used (n=32,254). Associations between self-reported cancers and cumulative (based on retrospective yearly estimates for each individual) serum PFOA concentrations were evaluated using a proportional hazards model. The model was adjusted for time-varying smoking, time-varying alcohol consumption, sex, education, and 5-year birth year period. Analyses were restricted to the 21 types of reported primary cancers validated through medical records and state cancer registries (n=2,507, 70% validated). Among the cancer types, only testicular cancer was statistically significantly associated with cumulative serum PFOA concentration (OR=1.34, 95% CI 1.00, 1.79), and the effect was stronger when restricted to community members only (OR=1.73 95% CI 1.24,2.40). Thyroid cancer was statistically significantly associated with cumulative serum PFOA concentrations among occupational workers (OR=1.93, 95% CI 1.00, 3.71) but not among the community study population. Results for cumulative serum PFOA concentrations by quartiles show that estimated risk ratios for kidney cancer (p-value=0.10) and testicular cancer (p-value=0.05) generally increased across quartiles. This pattern was less consistent for thyroid cancer (p-value=0.20), except when restricted to the occupational study population (p-value=0.04). Since this is largely a survivor cohort, differences in cancer survival rates could cause cancer types with shorter than 5-year survival to be likely to be captured by the study. Additionally, if individuals with cancers caused by high exposure were more likely to die before being captured in the cohort, results could be biased towards the null (less likely to find an association). Therefore, results about these cancer types should be interpreted with caution.

A comprehensive review of PFOA as an emerging drinking water contaminant (Post et al., 2012) found evidence that occupational studies showed some consistency of increased cancer mortality and/or incidence for bladder, kidney, and prostate cancer. Post et al. (2012) also reviewed studies showing that white blood cell neoplasms (Leonard, 2003; Leonard et al., 2008), thyroid cancer (Leonard et al., 2008), and carcinoid tumors (Morel-Symons et al., 2007) were also increased at one industrial facility.

Information on human breast cancer and PFOA is of interest because toxicological findings provide some evidence suggesting biological plausibility, although the available toxicological data do not support firm conclusions on this topic. Developmental exposures to PFOA cause

delayed mammary gland development in mice. Several researchers have stated that, in general, such disruptions of mammary gland development may result in adverse effects later in life resulting in increased cancer risk, although no information specific to PFOA is available on this question (Fenton 2006; Rudel and Fenton, 2009; IOM, 2011; Rudel et al., 2011; Fenton et al., 2012). Developmental exposures to PFOA in mice also caused increased numbers of darkly stained foci (described in detail in the Toxicology section) that persisted into adulthood and are considered permanent (White et al., 2009). Gore et al. (2015) notes that abnormalities of this type can be associated with increased breast cancer risk. However, chronic carcinogenicity studies have not been conducted in mice.

Epidemiological studies of associations of PFOA with breast cancer did not evaluate early life exposures. Studies of workers with occupational exposures to PFOA include very few women and are thus not informative about disease incidence in females. The incidence of breast cancer was not increased in a study of many types of cancer in the C8 Health Study population in Ohio and West Virginia communities with drinking water exposure to PFOA that included about 17,000 women (Barry et al., 2013). A small study (31 cases and 98 controls) in the Inuit population in Greenland found significantly increased risk of breast cancer associated with PFOA, perfluorooctane sulfonic acid (PFOS), total perfluorinated carboxylates, and total perfluorinated sulfonates (Bonefeld-Jorgensen et al., 2011). A subsequent study of this small group of cases and controls found a greatly elevated odds ratio for breast cancer in women with both high PFC levels and specific polymorphisms of xenobiotic metabolizing enzymes that affect levels of hormones such as estrogens, suggesting that inter-individual variations in these polymorphisms may affect sensitivity to the effects of PFCs on breast cancer risk (Ghisari et al., 2014). Because this was the only such study and it is of small size, these data are considered preliminary and suggest the need for further research on this topic.
In regard to biological plausibility for human carcinogenicity, PFOA caused hepatic, testicular Leydig cell, and pancreatic acinar cell tumors in two chronic studies in male rats (discussed in Toxicology section). Potential human relevance of these tumors is discussed in the Mode of Action section. Although liver tumors in rodents caused by compounds that activate PPAR-alpha are generally not considered relevant to humans, activation of this receptor may not be the sole mode of action for liver tumors caused by PFOA. Additionally, a PPAR-alpha mode of action may be involved in hepatic effects in the fetus, infants, and children. Therefore, the potential for human relevance of the tumors observed in rodents cannot be dismissed. The mode of action for the testicular and pancreatic tumors has not been established.

Additional endpoints

In addition to the epidemiologic endpoints reviewed above, there remain a large number of peer-reviewed studies that have evaluated other endpoints. Associations with PFOA have been found in some of these studies and not in others. Although these endpoints are not evaluated in this document, other organizations and publications may provide a more comprehensive review

Additional endpoints evaluated in studies but not reviewed in this report include biomarkers of kidney function or damage such as blood urea nitrogen, serum creatinine, renal glomerular filtration rate (eGFR) and chronic kidney disease; heart disease and hypertension; cerebrovascular disease including stroke, diabetes, immune function (with the exception of immune response following vaccination which was reviewed above) including asthma and other allergies, autoimmune disease including osteoarthritis, lupus, juvenile diabetes, rheumatoid arthritis, multiple sclerosis, and Crohn's disease; osteoporosis and bone mineral density; neurological and neurodegenerative disorders including self-reported memory impairment and Parkinson's disease; cognitive and behavioral developmental milestones; performance testing, and attention deficit hyperactivity disorder (ADHD) in children; reproductive and developmental outcomes (with the exception of lower birth weight and birth size of neonates), including decreased sperm count, longer time to pregnancy, birth defects, miscarriage and stillbirth, and overweight and obesity measured by BMI and waist circumference in offspring. It is noted that the C8 Science Panel concluded that there are "probable links" with PFOA exposure in the C8 Health Study population for two adverse health conditions, pregnancy-induced hypertension and ulcerative colitis, that were not reviewed above.

Health effects that occur later in life from prenatal and early life exposures to environmental contaminants are a current focus of research in toxicology and epidemiology (Barouki et al., 2012; Heindel and Vandenberg, 2015). Exposures of developing tissues to toxic substances during sensitive time periods can result in increased risk or severity of later disease or dysfunction (Heindel and Vandenberg, 2015). Recent studies have found that developmental exposures to PFOA cause toxicological effects that persist into adulthood and/or become evident later in life, including hepatic toxicity, delayed mammary gland development, and bone morphology and mineral denisty (Macon et al., 2011; White et al., 2009; Filgo et al., 2015; Quist et al., 2015; Koskela et al., 2016). However, the effects of developmental exposures to PFOA on these endpoints have not been evaluated in humans.

Summary of conclusions for epidemiologic information

Of the endpoints that were evaluated comprehensively by the Health Effects Subcommittee, the evidence for association with PFOA is strongest for serum cholesterol which demonstrates consistency, strength, dose-response and some evidence of temporality. PFOA was associated with clinically defined hypercholesterolemia in a community exposed through drinking water. There was also consistency among the larger non-occupational studies, as well as evidence of specificity, exposure-response and strength for associations between PFOA and both serum cholesterol and the liver enzyme ALT. These findings provide evidence supporting a causal relationship between PFOA and both cholesterol and ALT. There is evidence of consistency, strength, and exposure-response with PFOA and uric acid as well, but interpretation for temporality is limited.

In general, the human data for PFOA are notable because of the consistency between results among human epidemiologic studies in different populations for some endpoints, the concordance with toxicological findings from experimental animals, the use of serum concentrations as a measure of internal exposure, the potential clinical importance of the endpoints for which associations are observed, and the observations of associations within the exposure range of the general population.

As mentioned above, the epidemiologic databases for cholesterol, the liver enzyme ALT, and other endpoints are sufficient to support one or more of the criteria for causality. The sufficiency of the database to support these criteria is particularly evident when compared to the human epidemiologic databases for numerous other environmental contaminants for which quantitative risk assessment is based on animal toxicological studies in the absence of substantial support from human epidemiologic data (for example, 1,2,3-trichloropropane and methyl tertiary butyl ether [MTBE]).

For some endpoints, studies of populations from varied locations including the U.S., Canada, and several European and Asian countries, with study populations including children, young adults, adults, the elderly, and pregnant women, demonstrate a congruence of consistent findings. Although the human epidemiologic database is largely driven by cross-sectional studies, consistency is supported from studies with stronger study designs such as cohort and/or case-control, especially in studies of the general populations and populations exposed to drinking water contaminated with PFOA.

A particular strength of epidemiologic studies of PFOA and other PFCs is the availability of serum concentrations, a direct and accurate measure of internal dose in individuals. In contrast, human studies of most other environmental contaminants usually rely on estimates of external exposure. Exposure assessment tends to be susceptible to misclassification that may lead to biased findings. However, information bias is unlikely to have an impact in most general population studies of PFOA because of their reliance on serum concentrations and clinical biomarkers

Application of the Navigation Guide systematic review methodology to epidemiologic data resulted in the conclusion that there is sufficient evidence that developmental exposure to PFOA adversely affects fetal growth. The methodology developed and utilized to assess the relationship of PFOA and fetal growth was substantive and robust such that conclusions reached through application of the Navigation Guide are reasonable and supportable.

Reviews by several authoritative groups concluded that there is evidence of carcinogenic potential in humans for PFOA. The USEPA SAB (2006) described PFOA as “likely to be

carcinogenic to humans" based on the criteria provided in USEPA (2005b) cancer risk assessment guidance. More recently, IARC (2015) concluded that, based on its evaluation criteria, PFOA is possibly carcinogenic to humans. Finally, the USEPA Office of Water (2016a) concluded that there is suggestive evidence of human carcinogenicity for PFOA.

For some other endpoints, limited evidence of an association with PFOA was found. There is consistent evidence of decreased antibody concentrations following vaccination, with evidence of temporality. However, most of the vaccine types were evaluated in only one or two studies, limiting the ability to determine specificity, and there is limited evidence of exposure-response. The epidemiologic database for PFOA and LDL appears inconsistent, although there is some evidence of an association. Epidemiologic evidence for other serum lipid endpoints is limited. The epidemiologic evidence of a casual association with PFOA and GGT, AST, and bilirubin lacks consistency, and there is a limited evidence of associations with PFOA and liver disease. Evidence of thyroid disease remains limited.

There was no evidence of an association with PFOA and the liver enzyme ALP. Overall the epidemiologic evidence suggests no evidence of an association with HDL and PFOA. Overall studies evaluating thyroid hormones and TSH provide limited or no evidence of any associations with PFOA.

Although the magnitude of change for the parameters associated with PFOA is generally relatively small, they are of public health concern because population-level changes of this magnitude in parameters such as serum cholesterol or liver enzymes will result in a shift in the overall distribution of values such that the numbers of individuals with clinically abnormal values is increased. Additionally, small changes in a clinical biomarker may be an indicator of other effects which were not assessed. For example, birth weight represents a gross measure of development, and relatively small decreases in birth weight may be an indication of changes in other subtler developmental parameters which were not assessed.

The steepest dose-response for associations of some health endpoints with PFOA has been observed within the lower range of serum PFOA concentrations. Epidemiologic studies of smaller numbers of highly exposed participants may not be inclusive of these low level exposure ranges and thus may not detect associations. Therefore, an important limitation of studies of participants with extremely high serum PFOA concentrations may be that there are an insufficient number of study participants with low(er) level exposures who can serve as a comparison.

To illustrate this point, Figures 9A and 9B show changes in total serum cholesterol across deciles of serum PFOA concentrations in adults (Steenland et al., 2009) and children (Frisbee et al., 2010) from the mid-Ohio Valley. In both children and adults, associations increased steeply at

low ranges of serum PFOA concentrations, with a much flatter dose-response at higher levels. In the absence of data from the lower range of serum PFOA concentrations, modeling techniques might not be able to detect statistically significant changes of this effect with increasing serum PFOA concentrations.

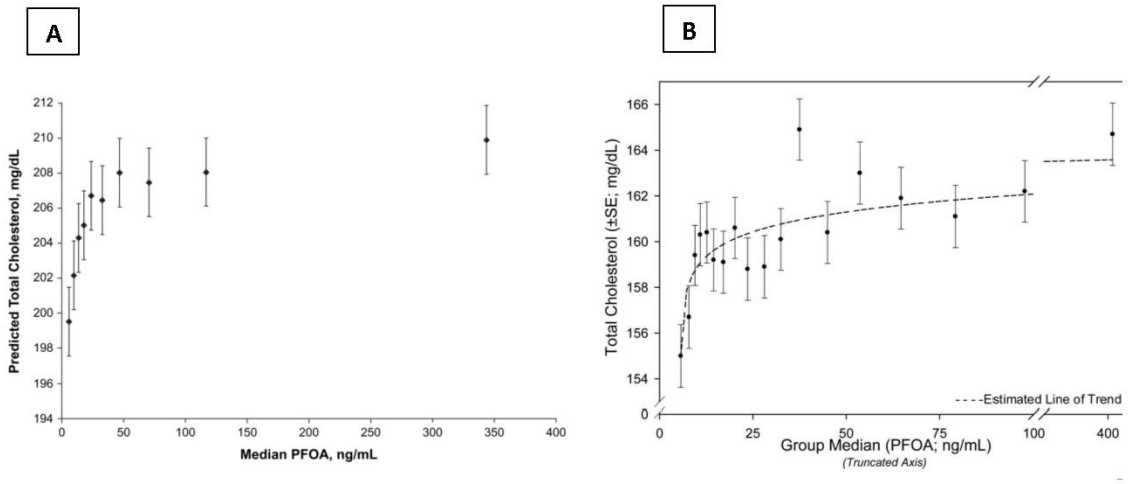


Figure 9. Adjusted-predicted total cholesterol change with increasing group median deciles. (A). Adults, Steenland et al., 2009 (B). Adolescents, Frisbee et al., 2009

For instance, in Emmett et al. (2006b), almost all participants had highly elevated serum PFOA levels (25th percentile value was 184 ng/ml), and comparisons were made only within the study population. Most or all of the lowest exposed individuals in this study may have had serum levels that fall on the flatter portion of the dose-response curve for serum cholesterol, limiting the ability to detect an association. Additionally, Olsen and Zobel (2007) provide data on serum cholesterol and other lipids in workers with PFOA exposure which indicate that the median serum PFOA level in the lowest exposed group, 60 ng/ml, lies on the flatter portion of the dose-response curve in Figure 9A and 9B above.

In summary, the consistency between results in different populations, the concordance with toxicological findings from experimental animals, the potential clinical importance of the endpoints for which associations are observed, and the observation of associations within the exposure range of the general population justify concerns about exposures to PFOA through drinking water. Although there is evidence to support causality for some epidemiological endpoints, the epidemiological data have limitations and therefore are not used as the quantitative basis for the Health-based MCL. Instead, the human data are considered as part of the weight of evidence for the health effects of PFOA and are used to support a public health-protective approach in development of a recommended Health-based MCL based on animal toxicology data.

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

Citation	Study Population	Study Details	TC	HDL	Non-HDL	TC/ HDL	LDL	VLDL	HDL/ LDL	TG	Genes
1. Costa et al., 2009	Italy., Occupational: Cases – Male workers engaged in the PFOA production department; Controls – Male workers never exposed to PFOA	**Study Design:* Exposure case-control & Cross-sectional **Study Size*: n=160 **Study Population Age*: 20-63 years **Exposure Range (Median)*: 4400 – 5700 ng/ml	↑	—						—	
2. Emmett et al., 2006b	U.S., Highly exposed community	**Study Design:* Cross-sectional **Study Size*: n=371 **Study Population Age*: 2.5-89 years **Exposure Range (Median)*: 354 ng/ml	—								
3. Eriksen et al., 2013	Demark, nested in a larger cohort, General population	**Study Design:* Cross-sectional **Study Size*: n=753 **Study Population Age*: 50-65 years **Exposure Range (Mean)*: 7.1 ng/ml	↑								
4. Fisher et al., 2013	Canada, General population	**Study Design:* Cross-sectional **Study Size*: n=5,604 **Study Population Age*: 6-79 years **Exposure Range (Geo Mean)*: 2.46 ng/ml	↑-	—	↑-	—	—			—	
5. Fitz-Simon et al., 2013	U.S., Highly exposed community	**Study Design:* Cohort **Study Size*: n=560 **Study Population Age*: 20-60 years **Exposure Range (Geo Mean)*: 30.8 – 74.8 ng/ml	↑-	—			↑			—	
6. Fletcher et al., 2013	U.S., Highly exposed community	**Study Design:* Cross-sectional **Study Size*: n=290 **Study Population Age*: 20-60 years **Exposure Range (Median)*: 30.1 ng/ml									[1]↑-
7. Frisbee et al., 2010	U.S., Highly exposed community	**Study Design:* Cross-sectional **Study Size*: n=12,476 **Study Population Age*: 1-17.9 years **Exposure Range (Mean)*: 69.2 ng/ml	↑	—			↑			↑-	
8. Fu et al., 2014	China, random selection of attendees to health check-up clinic (General population)	**Study Design:* Cross-sectional **Study Size*: n=133 **Study Population Age*: 0-88 years **Exposure Range (Median)*: 1.43 ng/ml	↑	—			↑			—	

Citation	Study Population	Study Details	TC	HDL	Non-HDL	TC/ HDL	LDL	VLDL	HDL/ LDL	TG	Genes
9. Geiger et al., 2014	U.S., General population	**Study Design:* Cross-sectional **Study Size*: n=815 **Study Population Age*: ≤ 18 years **Exposure Range (Mean)*: 4.3 ng/ml	↑-	—			↑			—	
10. Gilliland et al., 1996	U.S., Occupational - men	**Study Design:* Cross-sectional **Study Size*: n=115 **Study Population Age*: Mean 39.2 years **Exposure Range (Mean)*: 3300 ng/ml	—	↓-			—				
11. Lin et al., 2011	Taiwan, Recruited from hypertension cohort (General population)	**Study Design:* Cross-sectional **Study Size*: n=287 **Study Population Age*: 12-30 years **Exposure Range (Median)*: 2.39 ng/ml		—						—	
12. Lin et al., 2013	Taiwan, Individuals with abnormal urinalysis results from population-based screening program in Taiwan	**Study Design:* Cross-sectional **Study Size*: 664 (246 w/ elevated blood pressure and 398 w/ normal blood pressure) **Study Population Age*: 12-30 years **Exposure (Median)*: 3.49 ng/ml					—			↓	
13. Nelson et al., 2010	U.S., General population	**Study Design:* Cross-sectional **Study Size*: n=416 to n=860 **Study Population Age*: 12-80 years **Exposure Range (median)*: 3.8 ng/ml	↑-	—	↑		—				
14. Olsen et al., 2000	U.S., Occupational – male workers	**Study Design:* Cross-sectional **Study Size*: n=265 **Study Population Age*: Adults **Exposure Range (Median)*: 1190 ng/ml	—	↓-			—			—	
15. Olsen et al., 2003	Belgium & U.S., Occupational	**Study Design:* Cross-sectional & Longitudinal **Study Size*: n=421 & 174 **Study Population Age*: Adults **Exposure Range (Mean)*: 1220-1990 ng/ml	↑	—						↑	
16. Olsen and Zobel 2007	Belgium & U.S., Occupational - male	**Study Design:* Cross-sectional **Study Size*: n=506 **Study Population Age (Mean)*: 37-41 **Exposure Range (Median)*: 2210 ng/ml	—	↓-			—			↑-	

Citation	Study Population	Study Details	TC	HDL	Non-HDL	TC/ HDL	LDL	VLDL	HDL/ LDL	TG	Genes
17. Olsen et al., 2012	U.S., Occupational, direct employed and contract workers	**Study Design:* Longitudinal **Study Size*: n=179 **Study Population Age*: Adults **Exposure Range (Mean)*: Baseline/mean change - direct employed (881/-218.3 ng/ml), contract (28.9/32.1 ng/ml)	—	—	—	↓					
18. Sakr et al., 2007a	U.S., Occupational	**Study Design:* Cross-sectional **Study Size*: n=840 **Study Population Age*: Adults **Exposure Range (Median)*:189 ng/ml	↑	—			↑	↑		—	
19. Sakr et al., 2007b	U.S., Occupational	**Study Design:* Longitudinal **Study Size*: n=454 **Study Population Age*: Adults **Exposure Range (Median)*: 1040/1160 ng/ml	↑	—			—			—	
20. Starling et al., 2014	Norway, Pregnant women recruited from larger cohort	**Study Design:* Cross-sectional **Study Size*: n=891 **Study Population Age*: not stated - Adults **Exposure Range (Median)*: 2.25 ng/ml	—	—			—			—	
21. Steenland et al., 2009	U.S., Highly exposed community	**Study Design:* Cross-sectional **Study Size*: n=46,294 **Study Population Age*: ≥ 18 years **Exposure Range (Median)*: 27 ng/ml	↑	—	↑	↑	↑				
22. Steenland et al., 2015	U.S., Occupational	**Study Design:* Retrospective cohort **Study Size*: n=3,713 **Study Population Age*: Adult (mean year of birth 1951) **Exposure Range (Median)*: 113 ng/ml	[2]—								
23. Wang et al., 2012	China, Occupational (male) and Highly exposed community	**Study Design:* Cross-sectional **Study Size*: Workers: n=55 / Residents: n=132 **Study Population Age*: Adult **Exposure Range (Median)*: 1636 / 284 ng/ml	—/ —	↓/ —			—/ —		↓/ —	—/ —	
24. Winquist and Steenland, 2014a	U.S., Occupational (male) and Highly exposed community	**Study Design:* Retrospective cohort (prospective analyses) **Study Size*: Workers: n=3,713/ Residents: n=28,541 **Study Population Age*: Adult **Exposure Range (Median)*: 112/ 24 ng/ml	[2]↑								

Citation	Study Population	Study Details	TC	HDL	Non-HDL	TC/ HDL	LDL	VLDL	HDL/ LDL	TG	Genes
25. Zeng et al., 2015	Taiwan, recruited from control group of another study	**Study Design:* Cross-sectional **Study Size*: n=255 **Study Population Age*: 12-15 years **Exposure Range (Median)*: Boys=1.1, Girls=0.9 ng/ml	↑	—			↑			↑	

↑ = statistically significant increased association, ↓ = statistically significant decreased association, ↑- = inconsistent positively associated finding (findings from different models resulted in both statistically and non-statistically significant associations), ↓- = inconsistent negatively associated finding, — = not statistically significant, [statistical significant determined at α=0.05]
TC= total cholesterol, HDL= high density lipoprotein cholesterol, LDL=low density lipoprotein cholesterol, TG=triglycerides
1. Changes in the expression of genes 2. Self-reported high cholesterol

Table 6B. Summary of findings from epidemiologic studies of PFOA and liver enzymes/bilirubin									
Citation	**Study Population**	**Study Details**	**ALT**	**GGT**	**AST**	**ALP**	**TB**	**DB**	**LD**
1. Costa et al., 2009	Italy, Occupational (male)	**Study Design:* Exposure case-control & Cross-sectional **Study Size*: n=160 **Study Population Age*: 20-63 years **Exposure Range (Median)*: 4400 – 5700 ng/ml	↑-	↑-		↑-	↓-		
2. Darrow et al., 2016	U.S., Highly exposed community	**Study Design: Cross-sectional* **Study Size*: up to n=32,254 including 3,713 workers **Study Population Age*: >20 years of age **Exposure Range (Median)*: 16.5 ng/ml	↑	—				↓	—[1]
3. Emmett et al., 2006b	U.S., Highly exposed community	**Study Design:* Cross-sectional **Study Size*: n=371 **Study Population Age*: 2.5-89 years **Exposure Range (Median)*: 354 ng/ml	—	—	—	—	—		
4. Gallo et al., 2012	U.S., Highly exposed community	**Study Design:* Cross-sectional **Study Size*: n=47,092 **Study Population Age*: ≥ 18 years **Exposure Range (Median)*: 23.1 ng/ml	↑	↑-				—	
5. Gilliland et al., 1996	U.S., Occupational (male)	**Study Design:* Cross-sectional **Study Size*: n=115 **Study Population Age*: Mean 39.2 years **Exposure Range (Mean)*: 3300 ng/ml	↓-	—	↓-				
6. Gleason et al., 2015	U.S., General population	**Study Design:* Cross-sectional **Study Size*: n=4,333 **Study Population Age*: ≥ 12 years **Exposure Range (Median)*: 3.7 ng/ml	↑	↑	↑-	—	↑		
7. Jiang et al., 2014	China, pregnancy women	**Study Design:* Cross-sectional **Study Size*: 141 **Study Population Age*: Not stated - adults **Exposure Range (Median)*: 4.2 ng/ml	—		—		—		
8. Lin et al., 2010	U.S., General population	**Study Design:* Cross-sectional **Study Size*: n=2,216 **Study Population Age*: ≥ 18 years **Exposure Range (Mean)*: 4.5 ng/ml	↑	↑			—		
9. Melzer et al., 2011	U.S., General population	**Study Design:* Cross-sectional **Study Size*: n=3,974 **Study Population Age*: ≥ 20 years **Exposure Range (Geo Mean)*: Men=4.9, Women=3.8 ng/ml							—

Citation	Study Population	Study Details	ALT	GGT	AST	ALP	TB	DB	LD
10. Olsen et al., 2000	U.S., Occupational – (male)	**Study Design:* Cross-sectional **Study Size*: n=265 **Study Population Age*: Adults **Exposure Range (Median)*: 1190 ng/ml	—-	—	—	—	—	—	
11. Olsen et al., 2003	Belgium & U.S., Occupational	**Study Design:* Cross-sectional & Longitudinal **Study Size*: n=421 & 174 **Study Population Age*: Adults **Exposure Range (Mean)*: 1220-1990 ng/ml	—	—	—	—	—		
12. Olsen and Zobel 2007	Belgium & U.S., Occupational (male)	**Study Design:* Cross-sectional **Study Size*: n=506 **Study Population Age (Mean)*: 37-41 **Exposure Range (Median)*: 2210 ng/ml	↑-	↑-	—	—	↓-		
13. Olsen et al., 2012	U.S., Occupational	**Study Design:* Longitudinal **Study Size*: n=98-179 **Study Population Age*: Adults **Exposure Range (Mean)*: Baseline/mean change -direct employed (881/-218.3ng/ml), contract (28.9/32.1ng/ml)	↓-		—	—	—		
14. Sakr et al., 2007a	U.S., Occupational	**Study Design:* Cross-sectional **Study Size*: n=840 **Study Population Age*: Adults **Exposure Range (Median)*:189 ng/ml	—	↑	—		—		
15. Sakr et al., 2007b	U.S., Occupational	**Study Design:* Longitudinal **Study Size*: n=454 **Study Population Age*: Adults **Exposure Range (Median)*: 1040/1160 ng/ml	—	—	↑	—	↓		
16. Steenland et al., 2015	U.S., Occupational	**Study Design:* Retrospective cohort **Study Size*: n=3,713 **Study Population Age*: Adult (mean year of birth 1951) **Exposure Range (Median)*: 113 ng/ml							—
17. Wang, et al., 2012	China, Occupational (male) and Highly exposed community	**Study Design:* Cross-sectional **Study Size*: Workers: n=55 / Residents: n=132 **Study Population Age*: Adults **Exposure Range (Median)*: 1636 / 284 ng/ml	—/ —		—/ ↓				
18. Yamaguchi et al., 2013	Taiwan, recruited from larger cohort	**Study Design:* Cross-sectional **Study Size*: n=608 **Study Population Age*: 16-76 years **Exposure Range (Median)*: 2.1 ng/ml	↑	—	↑				

↑ = statistically significant increased association, ↓ = statistically significant decreased association, ↑- = inconsistent positively associated finding (findings from different models resulted in both statistically and non-statistically significant associations), ↓- = inconsistent negatively associated finding, — = not statistically significant, [statistical significant determined at α=0.05], —- findings from some analyses are negative, positive, and non-significant
ALT (SGPT)=alanine aminotransferase, GGT=gamma-glutamyl transferase, AST (SGOT)=aspartate aminotransferase, ALP=alkaline phosphatase, TB=total bilirubin or unspecified bilirubin, DB=direct bilirubin, LD=liver disease
[1]Includes findings from both any liver disease and second category of liver disease which includes enlarged liver, fatty liver, and cirrhosis

Table 6C. Summary of findings from epidemiologic studies of PFOA and thyroid hormones and diseases												
Citation	**Study Population**	**Study Details**	**TSH**	**TT4**	**FT4**	**TT3**	**FT3**	**TG**	**TD**	**Hypo**	**Hyper**	**OC**
1. Bloom et al., 2010	U.S., Subgroup of sportfish anglers	**Study Design:* Cross-sectional **Study Size*: n=31-38 **Study Population Age*: 31-45 years **Exposure Range (Geo Mean)*: 1.3 ng/ml	—		—							
2. Chan et al., 2011	CAN, Cases – hypothyroxemic pregnant women; Controls – nonhypothyroxemic pregnant women	**Study Design:* Matched case-control **Study Size*: n=271 **Study Population Age*: Adults **Exposure Range (Median)*: Cases -3.9, Controls – 3.6 ng/ml										—[a]
3. de Cock et al., 2014	The Netherlands, Mother-child pairs	**Study Design:* Cross-sectional **Study Size*: n=83 pairs **Study Population Age*: Newborns **Exposure Range (Median)*: 0.9 ng/ml		[1]↑-								
4. Emmett et al., 2006b	U.S., Highly exposed community	**Study Design:* Cross-sectional **Study Size*: n=371 **Study Population Age*: 2.5-89 years **Exposure Range (Median)*: 354 ng/ml	—						—			
5. Jain 2013	U.S., General population	**Study Design:* Cross-sectional **Study Size*: n=1,540 **Study Population Age*: > 12 years **Exposure Range (Median)*: 4.1 ng/ml	↑	—	—	↑	—	—				
6. Ji et al., 2012	South Korea, Recruited from cohort study	**Study Design:* Cross-sectional **Study Size*: n=633 **Study Population Age*: > 12 years **Exposure Range (Median)*: 2.7 ng/ml	—	—								
7. Kim et al., 2011	South Korea, Mother-Infant pairs	**Study Design:* Prospective birth cohort **Study Size*: Mothers – n=44, Pairs - n=26 **Study Population Age*: > 25 years **Exposure Range (Median)*: Maternal prepartum blood– 1.5, Cord Blood- 1.2 ng/ml	[3]↑-	—		—						
8. Knox et al., 2011	U.S., Highly exposed community	**Study Design:* Cross-sectional **Study Size*: n=50,113 **Study Population Age*: >20 years **Exposure Range (Mean)*: 86.6 ng/ml	—	↑-		↓-						

Citation	Study Population	Study Details	TSH	TT4	FT4	TT3	FT3	TG	TD	Hypo	Hyper	OC
9. Lin et al., 2013	Individuals with abnormal urinalysis results from population-based screening program in Taiwan	**Study Design:* Cross-sectional **Study Size*: 664 (246 w/ elevated blood pressure and 398 w/ normal blood pressure) **Study Population Age*: 12-30 years **Exposure (GM)*: 2.7 ng/ml	—	—								
10. Lopez-Espinosa et al., 2012	U.S., Highly exposed community	**Study Design:* Cross-sectional and birth cohort **Study Size*: n=10,725 **Study Population Age*: 1-17 years **Exposure Range (Median)*: 29 ng/ml	—	—					↑-	↑-	—	
11. Melzer et al., 2011	U.S., General population	**Study Design:* Cross-sectional **Study Size*: n=3,974 **Study Population Age*: ≥ 20 years **Exposure Range (Geo Mean)*: Men=4.9 and Women=3.8 ng/ml							[1]↑-			
12 Olsen et al., 1998	U.S., Male, Occupational	**Study Design:* Cross-sectional **Study Size*: 111 (1993) and 80 (1995) **Study Population Age*: Not presented **Exposure Range (Range)* 0 to 80,000 (1993) and 0 to 115,000 (1995) ng/ml	—									
13. Olsen et al., 2003	Belgium & U.S., Occupational	**Study Design:* Cross-sectional & Longitudinal **Study Size*: n=421 & 174 **Study Population Age*: Adults **Exposure Range (Mean)*: 1220-1990 ng/ml	—	—	—	↑						
14. Olsen and Zobel 2007	Belgium & U.S., Occupational - male	**Study Design:* Cross-sectional **Study Size*: n=506 **Study Population Age (Mean)*: 37-41 **Exposure Range (Median)*: 2210 ng/ml	—	—	↓-	↑-						
15. Shrestha, et al., 2015	U.S., Community-based	**Study Design:* Cross-sectional **Study Size*: n=87 **Study Population Age*: 55-74 years **Exposure Range (Median)*: 9.3 ng/ml	—	—	—	—						

Citation	Study Population	Study Details	TSH	TT4	FT4	TT3	FT3	TG	TD	Hypo	Hyper	OC
16. Steenland et al., 2015	U.S., Occupational	**Study Design:* Retrospective cohort **Study Size*: n=3,713 **Study Population Age*: Adult (mean year of birth 1951) **Exposure Range (Median)*: 113 ng/ml							—			
17. Wang et al., 2014	Taiwan, pregnant women recruited from cohort	**Study Design:* Cross-sectional and prospective birth cohort **Study Size*: n=285 women and n=116 neonates **Study Population Age*: Adult and newborns **Exposure Range (Median)*: maternal=2.4 ng/ml	—	—	—	—						
18. Webster et al., 2014	Canada, Euthyroid pregnant women recruited from study	**Study Design:* Prospective cohort study **Study Size*: n=152 **Study Population Age*: >18 years **Exposure Range (Median)*: 1.7 ng/ml	[4]↑-	—	—							
19. Wen et al., 2013	U.S., General population	**Study Design:* Cross-sectional **Study Size*: n=1,180 **Study Population Age*: > 20 years **Exposure Range (Geo Mean)*: 4.2ng/ml	—	—	—	[1]↑-	—	—		[1]↑-	[2]↓-	
20. Winquist and Steenland, 2014b	U.S., Highly exposed community and Occupational	**Study Design:* Retrospective cohort, prospective analyses **Study Size*: Resident, n=28,541; Worker, n=3,713 **Study Population Age*: > 20 years **Exposure Range (Median)*: 26.1 ng/ml							[1]↑-	[2]↑-	↑-	

↑ = statistically significant increased association, ↓ = statistically significant decreased association, ↑- = inconsistent positively associated finding (findings from different models resulted in both statistically and non-statistically significant associations), ↓- = inconsistent negatively associated finding, — = not statistically significant, [statistical significant determined at α=0.05]
TSH=thyroid stimulating hormone, TT4=total thyroxine, FT4=free thyroxine, TT3=total triiodothyronine, FT3= free triiodothyronine, TG=thyroglobulin, TD=thyroid disease, Hypo=hypothyroidism, Hyper=hyperthyroidism, OC=other thyroid conditions (a. Maternal hypothyroxemia)
1. In girls/women only. 2. Men only 3. Outcome is fetal thyroid concentrations. 4.Sensitivity analysis indicates association in High TPOAb (marker of autoimmune hypothyroidism) only

Table 6D. Summary of findings from epidemiologic studies of PFOA and uric acid				
Citation	**Study Population**	**Study Details**	**Uric Acid**	**Hyper**
1. Costa et al., 2009	Italy, Occupational: Cases – Male workers engaged in the PFOA production department; Controls – Male workers never exposed to PFOA	**Study Design:* Exposure case-control & Cross-sectional **Study Size*: n=160 **Study Population Age*: 20-63 years **Exposure Range (Median)*: 4400 – 5700 ng/ml	↑	
2. Geiger et al., 2013	U.S., General population	**Study Design:* Cross-sectional **Study Size*: n=1,772 **Study Population Age*: ≤ 18 years **Exposure Range (Median)*: 4.3 ng/ml	↑	↑
3. Gleason et al., 2015	U.S., General population	**Study Design:* Cross-sectional **Study Size*: n=4,333 **Study Population Age*: ≥ 12 years **Exposure Range (Median)*: 3.7 ng/ml	↑	
4. Lin et al., 2013	Taiwan, Individuals with abnormal urinalysis results from population-based screening program in Taiwan	**Study Design:* Cross-sectional **Study Size*: 664 (246 w/ elevated blood pressure and 398 w/ normal blood pressure) **Study Population Age*: 12-30 years **Exposure (Median)*: 3.5 ng/ml	—	
5. Sakr et al., 2007a	U.S., Occupational	**Study Design:* Cross-sectional **Study Size*: n=840 **Study Population Age*: Adults **Exposure Range (Median)*:189 ng/ml	↑	
6. Shankar et al., 2011	U.S., General population	**Study Design:* Cross-sectional **Study Size*: n=3,883 **Study Population Age*: > 20 years **Exposure (Median)*: Women, 3.5; Men 4.6 ng/ml	↑	↑
7. Steenland et al., 2010b	U.S., Highly exposed community	**Study Design:* Cross-sectional **Study Size*: n=54,591 **Study Population Age*: > 20 years **Exposure (Median)*: 27.9 ng/ml	↑	↑

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

Table 6E. Summary of findings from epidemiologic studies of PFOA and antibody concentrations (following vaccination)

Citation	Study Population	Study Details	Tetanus	Diphtheria	Rubella	Measles	Mumps	Influenza
1. Grandjean et al., 2012	Faroe Islands, singleton births following through age 7 years	**Study Design:* Prospective birth cohort **Study Size*: n=656 → 587 **Study Population Age*: Birth to 7 years **Exposure Range (Median)*: Children (age 5) 4.1 ng/ml	↓	↓				
2. Granum et al., 2013	Norway, Sub-cohort recruited from cohort	**Study Design:* Prospective birth cohort **Study Size*: n=99 → 56 **Study Population Age*: Birth to 3 years **Exposure Range (Median)*:1.1 ng/ml	—		↓	—		[1]—
3. Kielsen et al., 2015	Denmark, recruited from hospital staff	**Study Design:* Prospective cohort **Study Size*: n=12 **Study Population Age*: 23-66 years **Exposure Range (Median)*: 1.7 ng/ml	—	—				
4. Looker et al., 2014	U.S., Highly exposed community	**Study Design:* Prospective cohort **Study Size*: n=411 **Study Population Age*: Adult **Exposure Range (Median)*: 31.5 ng/ml						[2]↓-
5. Stein et al., 2016	U.S., General population	**Study Design:* Cross-sectional **Study Size*: n=1,191 **Study Population Age*: 12-19 years **Exposure Range (Geo Mean)*: 4.1 ng/ml			↓-	—	↓-	

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

Tetanus=tetanus antibody concentrations; diphtheria=diphtheria antibody concentrations; Rubella = rubella antibody concentrations; Measles = measles antibody concentrations; Influenza= Influenza antibody titer

1. Haemophilus influenza type B (HiB) antibody concentrations 2. Influenza A H3N2 virus and an increased risk of not attaining the antibody threshold considered to offer long-term protection, other virus studied included Influenza Type B and Influenza Type A H1N1.

HEALTH EFFECTS - ANIMAL TOXICOLOGY

Overview

The toxicological database for PFOA includes studies of numerous effects in non-human primates and rodents. As discussed in the Introduction, a literature search was performed to identify publications relevant to health effects of PFOA including toxicological studies. The Health Effects Subcommittee's review focused on toxicological endpoints identified as sensitive and potentially relevant for use in risk assessment. The effects selected for detailed review were hepatic toxicity, developmental effects, immune system toxicity, and carcinogenicity. As discussed in the Epidemiology section, effects relevant to these endpoints have been associated with PFOA in humans. Studies related to these endpoints were summarized in summary tables and/or individual study tables, as described in the sections on each endpoint below. Additionally, information is presented on general toxicity in non-human primates, as well as thyroid, neurobehavioral, and male reproductive effects.

General issues

In reviewing the toxicology data, it should be kept in mind that female rats excrete PFOA much more quickly than males. Therefore, assuming that both genders are equally sensitive based on internal dose, effects are expected from lower administered doses of PFOA in male rats than female rats.

Another general issue for some of the animal toxicology studies is that, using sensitive analytical methods, measurable levels of PFOA were detected in the serum of animals in the untreated control groups. This exposure was likely due to a combination of two factors. First, there is likely some level of unavoidable background exposure to PFOA in laboratory animals, just as in the general human population, due to the ubiquitous presence of PFOA at low levels in the environment. Second, in some studies, the controls may have experienced some level of inadvertent exposure to the PFOA used to dose the treated animals. Serum levels of PFOA in control groups from the some of the studies discussed below were: Butenhoff et al. (2004a) cynomolgus monkey – 134 ng/ml; Loveless et al. (2006) rat and mouse – 100-400 ng/ml; DeWitt et al. (2008) mouse – 25-600 ng/ml; Abbott et al. (2007) mouse developmental: adults – 28-131 ng/ml, pups – 17-29 ng/ml; Macon et al. (2011) low dose mouse developmental: pups – 4-23 ng/ml.

Such low level exposure in laboratory control groups from sources such as diet and bedding also occurs for many other chemicals that are ubiquitous environmental contaminants. However, this issue is of particular relevance to PFOA because, unlike most other environmental contaminants, the dose-response curves for several of the associations seen in human epidemiology studies appear to be steepest at serum levels below about 40 ng/ml (discussed in Human Studies, above). Quantitation levels for serum PFOA in animal toxicology studies (e.g. 5 ng/ml, Reiner et al., 2009) are higher than in studies of human populations (e.g. 0.1 ng/ml in NHANES; CDC, 2015),

and serum levels in the control groups in some animal studies are within or above the range in which associations are found in the general population. Thus, the shape of the dose-response curve within the lower range of serum levels (at which associations were observed in some epidemiology studies) cannot be determined in these animal studies.

Finally, it should be noted that ammonium perfluorooctanoate (APFO, the ammonium salt of PFOA) was administered in some studies. Because APFO dissociates to PFOA in the body, results of studies of APFO are applicable to the evaluation of PFOA's toxicity.

Acute Toxicity

Oral LD_{50} values of 680 mg/kg (95% CI 399-1157 mg/kg) and 430 mg/kg (95% CI 295-626 mg/kg) were reported for male and female albino rats, respectively, after a single gavage doses of APFO and observation for 14 days (Griffith and Long 1980). In this study, five rats per sex per dose group were administered 100, 215, 464, 1000, or 2150 mg/kg. One or more deaths occurred in all groups except the 215 mg/kg group, and all animals in the 2150 mg/kg group died on day 1. Ptosis, piloerection, hypoactivity, decreased limb tone, ataxia, and corneal opacity were reported. These signs were intermittent, and there was no apparent dose-response relationship for them. Changes in the lungs (congestion, pitting, red foci) and the stomach (distension, hyperemic and thickened mucosa) were commonly seen in animals that died during the study, and many survivors had mottled kidneys and stomach changes similar to those in the animals that died. Other oral acute toxicity studies, as well as dermal and inhalation studies, are summarized by Kennedy et al. (2004).

Subchronic Studies in Non-Human Primates

90-day rhesus monkey study (Goldenthal, 1978)

Goldenthal (1978) dosed rhesus monkeys (2/sex/group) with 0, 3, 10, 30 or 100 mg/kg/day APFO by gavage for 90 days (13 weeks). All 3 and 10 mg/kg/day animals survived the study. Soft stools, diarrhea, and frothy emesis were seen in the 3 mg/kg/day group, and one monkey in the 10 mg/kg/day group was anorexic during week 4, had a pale and swollen face in week 7, and had black stools for several days in week 12. In the 3 and 10 mg/kg-day groups, body weight gains were similar to controls, and no treatment related hematology or clinical chemistry changes were reported. Serum PFOA data are not provided in this study.

Mortality occurred in the two higher dose groups. In the 30 mg/kg/day group, 3 of 4 monkeys died during the study, one male during week 7 and the two females during weeks 12 and 13. All 30 mg/kg/day animals showed decreased activity, beginning in week 4. One monkey in this group had emesis and ataxia, swollen face, eyes, and vulva, as well as pallor of the face and gums. Beginning in week 6, two monkeys in this group had black stools, and one monkey had slight to moderate dehydration and ptosis of the eyelids.

All monkeys in the 100 mg/kg/day group died by week 5, with the first death occurring during week 2. These animals showed signs and symptoms that first appeared during week 1 including

anorexia, frothy emesis, pale face and gums, swollen face and eyes, decreased activity, prostration and trembling. Weight loss occurred in the two higher dose groups after week 1.

No treatment related gross pathological lesions were reported. Histopathological examination revealed effects on the adrenal gland, bone marrow, spleen, and lymph nodes in the two higher dose groups. Notably, all animals that died during the study had marked diffuse lipid depletion in the adrenal glands. All animals (including the one 30 mg/kg/day survivor) in the two higher dose groups had slight to moderate hypocellularity of the bone marrow and moderate atrophy of lymphoid follicles in the spleen, and one 30 mg/kg/day female and all 100 mg/kg/day animals had moderate atrophy of the lymphoid follicles in the lymph nodes.

Statistically significant changes in absolute or relative organ weights at either 3 or 10 mg/kg/day in either males or females (but not both) were reported for heart, brain, and pituitary. The biological significance of these changes was stated to be unknown.

Relative liver weight data are of interest because this endpoint was affected by PFOA in cynomolgus monkeys and rodents (see below). Since each dose group consisted of only two animals, a large change would be needed to reach statistical significance for this effect. Although there were no statistically significant effects on this endpoint, the data presented in Table 7 suggests that PFOA caused increased relative liver weight at 30 and 100 mg/kg/day.

Table 7. Relative Liver Weight (compared to controls) in 90 Day Rhesus Monkey Study (Goldenthal, 1978)

	Relative Liver Weight	
Dose (mg/kg/day)	Males (2 per group)	Females (2 per group)
0	1	1
3	1.1	1
10	1.1	1
30	1.4 (1.5)	(1.9)
100	(1.6)	(1.4)

(Numbers in parentheses include dead animals)

The LOAEL for this study was 3 mg/kg/day based on the toxicity described above in this group, and no NOAEL was identified.

Because serum PFOA levels were not measured in this study, it is not possible to compare the internal dose at which effects occurred in the rhesus monkeys with the results from cynomolgus monkeys and rodents (detailed below).

4 week cynomolgus monkey study (Thomford, 2001a)

In a range finding study, the toxicity of APFO was studied in male cynomolgus monkeys (young adult to adult) dosed daily by capsule for 4 weeks (Thomford, 2001a). Dose levels were 0 (n=2), 2 mg/kg/day (n=3), and 20 mg/kg/day (n=3). Parameters evaluated in blood (prior to treatment and on Day 30) included serum PFOA (although data were not reported in Thomford et al., 2001a), hematology, clinical chemistry (also evaluated on Day 2, about 24 hours after first dose), and hormones (estradiol, estrone, estriol, thyroid stimulating hormone, total and free triiodothyronine, and total and free thyroxin). Of the parameters measured in blood, estrone was notably lower in both treated groups

All animals survived until the end of the study, no clinical signs of toxicity were observed in the treated groups, and body weight was not affected by treatment. Low or no food consumption occurred in one animal dosed with 20 mg/kg-day. Microscopic evaluation of the adrenals, liver, pancreas, spleen, and testes found minimal mineralization in the adrenals of one animal given 2 mg/kg/day and immature testes in one animal in each of the two dosed groups (2 of 6 dosed animals). The authors stated that these findings were not related to PFOA treatment. There was no evidence of peroxisome proliferation (assessed by palmitoyl CoA oxidase activity; PCO) in the liver, or increased cell proliferation (assessed by the proliferatingn cell nuclear antigen; PCNA) in the liver, testes, and pancreas of treated monkeys. The authors concluded that NOAEL was 20 mg/kg, and no LOAEL was established.

6 month (26 week) cynomolgus monkey study (Thomford, 2001b; Butenhoff et al., 2002)

The toxicity of APFO was studied in male cynomolgus monkeys dosed daily by capsule for 6 months (Thomford, 2001b; Butenhoff et al., 2002). This study is described in detail because it has been used as the partial or entire basis for several earlier final and draft PFOA risk assessments (USEPA, 2005a; MDH, 2008; Post et al., 2009a; Tardiff et al., 2009; NC SAB, 2010; ATSDR, 2015), and because it is considered in evaluating the human relevance of hepatic effects of PFOA (See Mode of Action section, below). Concerns related to use of this study as the basis for risk assessment and the reasons why the data do not support Benchmark Dose modeling based upon it (Butenhoff et al., 2004a) are discussed in detail in Appendix 3 of this document. A table summarizing this study, with a focus on hepatic effects, is included in Appendix 5. Data for increased liver weight from this study are included in Table 10.

In this study as originally designed, male monkeys, age 3 to 9 years, were to be administered doses of 0, 3, 10, or 30 mg/kg/day by capsule, 7 days per week for 26 weeks. Six animals were dosed per group, except for the 3 mg/kg/day group which had four animals. However, as detailed below, the high dose (30 mg/kg/day) was reduced to 20 mg/kg/day due to overt toxicity. Additionally, only three of the four low dose (3 mg/kg/day) and two of the six high dose (30 mg/kg/day) monkeys tolerated the administered dose well enough to complete the study, while all six animals in the mid-dose group (10 mg/kg/day) completed the study. Two animals from the control and 10 mg/kg/day groups were observed for delayed effects for 13 weeks following the 6 months of dosing.

One low dose (3 mg/kg/day) monkey was sacrificed on day 137 (week 19) after loss of about 10% of its weight in one week accompanied by low food consumption, few feces, hind-limb paralysis, ataxia, and lack of response to pain. No specific organ pathology or clinical chemistry changes explaining the morbidity were found, and the study authors stated that it was unclear whether or not these effects resulted from exposure to PFOA. It is notable that the liver-to-body weight ratio in this animal, 2.44, was much higher than for other animals in this group and was comparable to the ratios in the high dose group. Also, the liver-to-brain weight ratio in this animal, 1.66, was the highest of any animal in the study. However, the serum and liver PFOA levels in this animal were not higher than for other animals in the same treatment group. These findings suggest that this animal may have been particularly susceptible to the hepatic effects of PFOA.

Dosing of the high dose (30 mg/kg/day) group was stopped on day 12 due to toxicity in the first week, including low food consumption, weight loss, and few or no feces. The dosing of this group was restarted at 20 mg/kg/day on day 22, after a 10-day break to allow for recovery from the toxicity in first week.

One high dose monkey was sacrificed on day 29 due to decreased body weight, lack of eating, hypoactivity, and coldness to touch. The liver of this animal had lesions including mid-zonal and centrilobular hepatocellular degeneration and necrosis, diffuse hepatocellular vacuolation, and centrilobular hepatocyte basophilia indicative of liver regeneration. Other effects observed in this monkey included necrosis, erosions, and ulcerations of the esophagus and stomach, and involution of the thymus. This animal had increased serum enzymes indicative of substantial liver and muscle injury, and a marked decrease in serum cholesterol to about 10% of control levels. The study authors state that the liver lesions were likely related to treatment.

Dosing of three high dose (30/20 mg/kg/day) monkeys was stopped on days 43, 66, and 81 due to low or no food consumption, dramatic weight loss (18-23% of body weight), and few or no feces. These three monkeys were monitored without dosing for the rest of the study, and their body weights increased to above pre-dosing levels by the end of the study.

In the study, serum PFOA levels were analyzed every 2 weeks, including in animals for which dosing was stopped. Steady state was reported to have been reached after 4-6 weeks. The complete set of serum data was reported in a separate publication (Butenhoff et al., 2004a). Means and standard deviations for serum levels in the control, 3, 10, and 30/20 mg/kg/day groups, excluding animals for which dosing was stopped, were reported as 134±113 ng/ml, 80,000±40,000 ng/ml, 99,000±49,000 ng/ml, and 155,000±103,000 ng/ml. It is evident from the data that there was a wide variation in serum levels within the same treatment group, and that serum levels did not increase proportionally with dose. The ratios between mean serum levels at steady-state in the 3, 10, and 30/20 mg/kg/day groups were approximately 1:1:2 (i.e. serum

levels were similar in the 3 and 10 mg/kg/day groups), and the mean serum levels at 3 mg/kg/day and 10 mg/kg/day did not differ significantly at the $p<0.05$ level (Butenhoff et al., 2002).

Hepatic PFOA concentrations at sacrifice did not differ between the 3 and 10 mg/kg/day groups (15,000±3600 ng/g and 14,000±7600 ng/g, respectively) and were very disparate in the two high dose (20/30 mg/kg/day) animals that completed the study (16,000 and 83,000 ng/g). It is notable that the highest liver concentration by far (154,000 ng/g) was found in the high dose animal sacrificed due to treatment-related morbidity at week 5.

Average body weight gains during the dosing period in the 0, 3, and 10 mg/kg/day groups were 19, 20, and 20%, while the weight changes in the two 30 mg/kg/day animals that completed the study were –8% and 18%.

Serum cholesterol was not affected by PFOA treatment in this study, while triglycerides were significantly increased compared to controls at several time points in the mid- and high dose group. These observations are notable, since decreases in serum cholesterol have been observed in rodent studies with PFOA, consistent with the effects of other PPAR-alpha activators, and since human PFOA exposure has been associated with increases in cholesterol (see Epidemiology section, above). As discussed above, the dose-response curves for increased cholesterol in humans appears steepest at serum PFOA levels below about 40 ng/ml, with a much flatter dose-response between about 40 and 350 ng/ml PFOA in serum (Nelson et al., 2010; Steenland et al., 2009b). Since the mean serum PFOA level in the control monkeys was 134 ng/ml, the shape of the dose response curve for cholesterol at serum levels relevant to effects observed in humans is not known.

In the high dose group, including the three monkeys in which treatment was stopped, total neutrophils, total serum protein, and albumin were decreased. In the two monkeys in this group that were dosed until the end of the study, the changes were not significant but were consistent over time. In one of the monkeys whose dosing was stopped, serum liver enzyme levels and elevated serum bile acids were greatly increased three days prior to the stopping of the dosing. Additionally, the high dose monkey that was sacrificed in moribund condition on Day 29 had markedly elevated liver enzymes and markedly low cholesterol.

Changes in thyroid hormone levels occurred in treated groups; these are described in detail in the section on thyroid effects below. Although estradiol levels in the high dose group appeared to decrease with treatment, the authors did not attribute these findings to treatment.

Absolute liver weight and liver-to-body weight ratios were increased in all treated groups (Butenhoff et al., 2002). The increase in absolute liver weight was statistically significant ($p<0.01$) in all groups, but was only significant for relative liver weight in the high dose group. However, this analysis does not include the animals that were sacrificed in moribund condition during the study. The absolute and relative liver weight of the sacrificed low dose monkey was

far higher than the others in its group, and inclusion of data for this animal increases the mean value for these parameters (Table 10). Liver-to-brain weight ratio is considered to be a reliable measure of effects on liver weight, because brain weight tends to remain stable when body weight changes. This ratio was increased in all treated groups compared to controls, but did not increase with administered dose or serum level (Butenhoff et al., 2004c). In the control, 3, 10, and 30/20 mg/kg/day groups, the liver-to-brain weight ratios were 0.934, 1.34, 1.30, and 1.22, respectively.

No gross or microscopic pathological changes were seen in the organs examined from animals that completed the study. However, as mentioned above, multiple pathological changes were found in the high dose monkey sacrificed in moribund condition on Day 29. These included edema and inflammation of the esophagus and stomach (attributed to dosing injury); liver lesions including mid-zonal and centrilobular hepatocellular degeneration and necrosis, diffuse hepatocellular vacuolation, and hepatocyte basophilia in centrilobular areas indicative of liver regeneration; involution of the thymus (noted as a common stress response); and degeneration and necrosis of the heart (noted as likely an agonal change).

Two monkeys from the control group and two from the 10 mg/kg/day group were observed for 13 weeks following the 6-month treatment period. Weight gain during the recovery period was lower in the 10 mg/kg/day animals than in the controls. During the recovery period, the control group monkeys gained 10 and 11% of their body weight, while one treated monkey gained 5% and the other lost 3%.

Studies of hepatic biochemical markers in this study are discussed in the Mode of Action section, below.

The LOAEL in this study is 3 mg/kg/day based on mortality possibly related to treatment (25%) and increased liver weight, and the NOAEL is unknown. Mean serum levels at this dose were reported by Butenhoff et al. (2004a) as 80,600 $\pm$ 40,000 ng/ml. It is important to note that 6 months represents less than 2% of the lifespan of about 30 years in this species of monkey. It is not known whether additional or more severe effects would have occurred with continued dosing of the monkeys that tolerated dosing for the full 6 months of the study. Additionally, this study did not include female monkeys and, because exposure was initiated at age 3 to 9 years, effects specific to exposures during development or at younger ages would not be evident.

Comparison of the results of this study with chronic rat studies discussed below suggests that cynomolgus monkeys are more sensitive to the overt toxicological effects of PFOA than are rodents. As discussed above, four of the six high dose monkeys exhibited severe toxicity that necessitated removal from the study. The serum PFOA level in the high dose monkey that was sacrificed on Day 29 with severe multi-organ toxicity was 822,000 ng/ml. The serum levels of the other three high dose monkeys removed from the study on days 43, 66, and 81 due to overt toxicity (low or no food consumption, dramatic weight loss of 18-23%, and few or no feces)

were 102,000 ng/ml, 467,000 ng/ml, and 235,000 ng/ml, respectively, at the times when dosing was stopped. These severe toxic effects occurred after dosing for about 0.3-0.7% of the monkeys' lifespans. In contrast, survival was unaffected or increased compared to controls, and no overt toxicity was observed, in male rats with lifetime (2 year) exposure to PFOA doses that were estimated to result in serum PFOA levels of almost 600,000 ng/ml (Sibinski 1987; Biegel et al., 2001; USEPA, 2005a). Thus, severe toxicity resulted from relatively short exposures to PFOA in primates at serum levels below those that were well tolerated chronically by rats.

Hepatic Effects in Rodents and Non-Human Primates

PFOA causes liver toxicity in experimental animals, including rodents and non-human primates (reviewed by Kennedy et al., 2004; Lau, 2012). As discussed in the Epidemiology section, increased liver enzymes are associated with exposure to PFOA in humans.

Increased liver weight is a sensitive toxicological endpoint for PFOA that has been observed in many toxicological studies. Although studies of PFOA that report increased liver weight do not always include evaluation of other hepatic endpoints, numerous studies of PFOA have demonstrated that increased liver weight co-occurs with and/or progresses to more severe hepatic effects including increased serum liver enzymes, hepatocellular necrosis, fatty liver, and/or hyperplastic nodules. Additionally, recent studies show that cellular damage indicative of liver toxicity persists until adulthood following developmental exposure to PFOA.

This section first reviews data indicative of liver damage from toxicological studies of PFOA. This is followed by a presentation of data on increased relative liver weight from PFOA. Numerous toxicological studies of PFOA have evaluated liver weight, and a separate discussion of each of these is beyond the scope of this document. Because studies of increased liver weight that include relatively low doses of PFOA are most relevant to quantitative risk assessment, they are reviewed in detail and are summarized in Table 10.

Chronic exposure to PFOA also caused hepatic adenomas in rats (See Chronic Studies, below). The mode of action for hepatic effects, particularly as related to human relevance, is discussed in detail in the Mode of Action section.

Histopathological changes in the liver

Hepatocellular hypertrophy is a consistently reported histopathological finding for PFOA that accompanies increased absolute and relative liver weight. A number of studies in both non-human primates and rodents also report other histopathological changes that are indicative of liver injury and/or lipid accumulation in the liver. These effects occurred at doses similar to those causing increased liver weight. Notably, prenatal exposure to a very low dose (0.01 mg/kg/day) of PFOA caused liver toxicity that persisted until adulthood at doses below those which caused increased liver weight (Quist et al., 2015).

International harmonization of diagnostic criteria and terminology for histopathologic lesions has recently been developed to provide consistency and to reflect current knowledge of the biochemical and cellular processes involved in changes in the liver and other organs (Mann et al., 2012). The terminology and current definitions that are recommended by the international harmonization group to describe hepatic damage were applied where appropriate to describe the histopathological findings discussed below.

Information from some studies that reported histopathological effects indicative of liver damage is summarized below. It should be noted that this summary does not represent a complete compilation of all studies such effects. The significance of these effects as related to the mode of action of PFOA is discussed in the Mode of Action section.

As discussed above, the high dose (30/20 mg/kg/day) male cynomolgus monkey sacrificed in moribund condition on Day 29 of the 90-day study had liver lesions including mid-zonal and centrilobular hepatocellular degeneration and necrosis, diffuse hepatocellular vacuolation, and centrilobular hepatocyte basophilia indicative of liver regeneration (Butenhoff et al., 2002).

In a 28-day study of male and female Chr-CD albino rats with dietary exposure to 30-1000 ppm APFO, degeneration and/or necrosis of hepatocytes and focal bile duct proliferation occurred in all treated groups (Kennedy et al., 2004). The dose from 30 ppm in the diet was estimated as 1.5 mg/kg/day. Similar changes were seen in livers of rats dosed with 3 to 1000 ppm in the diet for 90 days, and were stated to be more frequent in males, and most pronounced at the highest dose (Griffith and Long, 1980).

Individual cell and focal necrosis, increased hepatocellular mitotic figures, fatty changes, and bile duct hyperplasia were observed in male CD-1 mice dosed by gavage with linear APFO for 29 days (Loveless et al., 2008). The LOAEL for individual cell and focal necrosis was reported by the authors as 1 mg/kg/day, and the NOAEL as 0.3 mg/kg/day. Focal necrosis occurred in one of ten mice at 0.3 mg/kg/day but was stated by the authors to not be related to PFOA treatment. Increased mitotic figures, fatty changes, and bile duct hyperplasia occurred at $\geq$ 10 mg/kg/day.

Multiple histopathological changes were observed in the livers of three strains (wild type, PPAR-alpha null, and humanized PPAR-alpha) of male Sv/129 mice dosed with 1 or 5 mg/kg/day PFOA for 6 weeks (Nakagawa et al., 2011). PFOA caused a dose-dependent increase in macrovesicular steatosis in the PPAR-alpha null mice, while this effect was not seen in the wild type mice. Additionally, microvesicular steatosis occurred at both doses in the PPAR-alpha null and humanized PPAR-alpha mice. Lobular inflammation was observed only in the PPAR-alpha null mice treated with PFOA. Single cell necrosis occurred in all three strains of PFOA treated mice and appeared to be most severe in the wild type mice. Hydropic degeneration of hepatocytes was seen in the PPAR-alpha null and humanized PPAR-alpha strains treated with PFOA, but not the wild type strain.

Adverse histological changes including deranged liver architecture, severe edema, vacuolar degeneration, focal necrosis, and obvious inflammatory infiltration were observed in livers of male Kunming (KM) mice dosed by gavage with 2.5 to 10 mg/kg/day PFOA for 14 days (Yang et al., 2014). These changes were more severe with increasing dose and were not seen in the control mice.

Bile duct injury, which was much more severe in PPAR-alpha null mice than in wild type mice (male 129S4 strain), occurred after 4 weeks of gavage dosing with 4.4, 10.8, or 21.6 mg/kg/day APFO (Minata et al., 2010). Microvesicular steatosis was also more prominent in treated PPAR-alpha null than similarly treated wild type mice, and focal necrosis occurred at the highest dose in the null mice.

Focal and multi-focal hepatic necrosis was observed at sacrifice on PND 109-120 in F1 male offspring in the 3, 10, and 30 mg/kg/day dose groups (but not at 1 mg/kg/day) in a two-generation rat reproductive developmental study that is discussed in detail below (Butenhoff et al., 2004b).

Histopathological changes occurred in the livers of male Sprague-Dawley rats sacrificed one day after the end of dietary exposure to 300 ppm APFO for 1, 7, or 28 days (Elcombe et al., 2010). This study was conducted twice, and the daily doses were estimated as 19 mg/kg/day and 23 mg/kg/day in the two studies. In both studies, PFOA caused periportal glycogen depletion after 1, 7, and 28 days of exposure to PFOA, hepatocellular hypertrophy after 7 and 28 days, and hepatocellular hyperplasia in all treated rats after 28 days.

In a chronic rat study discussed in detail below (Sibinski, 1987; Butenhoff et al., 2012), focal hepatocellular necrosis occurred in 6/15 high dose (14.2 mg/kg/day) males but not in any of the 15 controls at the one-year sacrifice. (The low dose group was not evaluated at one year). At the two-year sacrifice, the incidence of focal hepatocellular necrosis was similar in control and treated groups, but the incidence of hepatic hyperplastic nodules was increased in the high dose males compared to the control and low dose groups. Butenhoff et al. (2012) concluded that the increased incidence of focal necrosis and vacuolation at one year, but not at later time points, may have been due to the higher background incidence of these changes in older rats.

Additionally, PFOA caused hepatocellular adenomas in chronically exposed male rats in one of the two chronic rat studies that have been conducted (Biegel et al. 2001). Although these tumors were not increased in the other chronic rat study (Sibinski et al., 1987), the EPA Science Advisory Board (2006) concluded that the increased in hepatic hyperplastic nodules in Sibinski et al. (1987) may have been part of the continuum of proliferative lesions in the hepatic carcinogenic process. More recently, Butenhoff et al. (2012) suggested that the observations at one year and two years in Sibinski et al. (1987) suggest a progression of lesions “from hepatocellular hypertrophy to fatty degeneration to necrosis followed by regenerative

hyperplasia." Butenhoff et al. (2012) also note that the diagnostic criteria for these nodules, which indicate a regenerative process, have changed since the study was evaluated in 1986.

PFOA caused hepatic triglyceride accumulation (fatty liver) in male Wistar rats after 7 days of dietary exposure (Kawashima et al., 1995). Hepatic triglyceride levels were significantly increased at the lowest dose (25 ppm) and were 3.5 times control levels in the 100 ppm dose group.

Several studies have evaluated hepatic effects of PFOA in mice that were fed diets containing specific types of lipids or higher general fat content. Kudo and Kawashima (1997) studied the effect of PFOA (2.5 - 10 mg/kg/day by intraperitoneal injection for 7 days) on hepatic triglyceride accumulation in male mice that had been fed diets containing soy bean oil, perilla oil, or fish oil for 4 weeks. Fish oil, but not the other two types of oil, was known to generally decrease hepatic triglycerides. PFOA caused a dose-dependent increase in hepatic triglycerides in mice exposed to perilla oil and soybean oil, but had no effect in mice exposed to fish oil.

Additionally, necrotic cell death, lipid droplet accumulation, and inflammatory cell infiltration were found in male C57BL/6N mice dosed with 5 mg/kg/day PFOA in a liquid diet for 3 weeks (Tan et al., 2013). These effects of PFOA were more severe in mice receiving a high fat diet than a regular diet. PFOA caused increased hepatic triglyceride levels in mice receiving regular diets or high fat diets, and relative white adipose tissue weight was decreased by PFOA treatment.

Finally, PFOA (5, 10, or 20 mg/kg/day by gavage for 14 days) caused lipid accumulation in the livers of male Balb/C mice fed either a regular diet or a high fat diet (Wang et al., 2013). Consistent with Tan et al. (2013), PFOA caused decreased relative weight of adipose tissue in mice receiving either diet. The authors concluded that lipids were both being released from the adipose tissue and accumulating in the liver. Evaluation of hepatic ultrastructural changes showed that PFOA caused a dose-dependent increase in the accumulation of lipid droplets in the nucleus of hepatic cells; the dose-response for this effect was similar in the regular and high fat diet groups. In the regular diet mice dosed with 10 and 20 mg/kg/day, dilation of the endoplasmic reticular was observed. More severe changes were seen in high fat diet mice at these doses including mitochondrial swelling, irregular nuclei, and condensed chromatin suggesting apoptosis.

Two recent studies found persistent hepatic damage in female mouse offspring after maternal gestational exposure to low doses of PFOA. Quist et al. (2015) evaluated hepatic effects in female CD-1 mouse offspring from dams dosed with 0.01 to 1 mg/kg/day PFOA on GD 1-17. Livers from offspring were evaluated on PND 21 (weaning) and PND 91. On PND 21, relative liver weight was significantly increased at 0.3 and 1 mg/kg/day, with no effect at 0.01 or 0.1 mg/kg/day, and PFOA treatment did not cause hepatocellular hypertrophy at any dose at this time point. In contrast, hepatocellular hypertrophy was significantly increased ($p<0.01$) at PND 91 in all dosed groups (0.01 mg/kg/day; NOAEL not identified), although the increased relative

liver weight observed at higher doses on PND 21 had been resolved. Additionally, dose-related hepatic periportal inflammation occurred in treated offspring on PND 21 and 91, and was more severe on PND 21 than on PND 91. On PND 21, the severity score for this effect was significantly ($p<0.05$) increased at all doses ($\geq$ 0.01 mg/kg/day) in a dose-related fashion, with no NOAEL identified. In this study, periportal inflammation was a more sensitive indicator of toxicity from prenatal exposure to PFOA than increased liver weight in female CD-1 mice.

Quist et al. (2015) also evaluated ultrastructural hepatic changes in control and highest dose (1 mg/kg/day) mice using transmission electron microscopy. Mitochondrial proliferation and abnormal mitochondrial morphology occurred in PFOA-treated mice at both PND 21 and PND 91 and was more severe at the later time point. Peroxisomes were present only in a single liver section from one treated animal and were closely associated with areas of mitochondrial proliferation in this section.

In summary, hepatocellular hypertrophy was observed by Quist et al. (2015) at low doses ($\geq$ 0.01) on PND 91, a time point when PFOA had almost completely been eliminated from the body and the transient increased relative liver weight observed at the higher PFOA doses on PND 21 had resolved. Although hepatocellular hypertrophy from PFOA in adult rodents has typically been associated with proliferation of peroxisomes or smooth endoplasmic reticulum, these changes were not seen in adult (PND 91) female mice after prenatal exposure to PFOA (1 mg/kg/day). Instead, hepatocellular hypertrophy was associated with mitochondrial proliferation and abnormal mitochondria. The significance of these observations is further discussed in the Mode of Action section.

Filgo et al. (2015) evaluated histopathological effects in the liver at age 18 months in female offspring of dams dosed during gestation. The study evaluated three strains of mice: CD-1, 129/Sv wild type, and 129/Sv PPAR-alpha null. CD-1 dams were dosed with PFOA at 0, 0.01, 0.1, 0.3, 1, and 5 mg/kg/day, and 129/Sv wild type and PPAR-alpha null dams were dosed with 0, 0.1, 0.3, 1, and 3 mg/kg/day. Findings related to non-neoplastic hepatic changes in CD-1 mice included significant dose-related trends for increased incidence of centrilobular hepatocyte hypertrophy, Ito cell hypertrophy, and oval cell hyperplasia, and for increased severity of chronic active inflammation. In the 129/Sv strains, non-neoplastic hepatic changes caused by PFOA were primarily observed in the PPAR-alpha null mice. These included increased incidence of bile duct hyperplasia, hyaline droplet accumulation in the bile duct, hematopoietic cell proliferation, and centrilobular hepatocyte hypertrophy. In the wild type 129/Sv mice, only severity of centrilobular hepatocyte hypertrophy was increased by PFOA treatment.

The incidence of liver tumors was also evaluated by Filgo et al. (2015) because of the unexpected finding of hepatic tumors in some animals that died before the scheduled end of the study. However, the authors emphasize that the study was not designed or intended to be a carcinogenicity bioassay. In CD-1 mice, single or multiple hepatocellular adenomas were found in one or more animals in each of the treated groups (n=21 to 37 per group) except for at the

lowest dose (0.01 mg/kg/day), but were not found in controls (n=29). In total, adenomas occurred in 4.9% (7 of 144) treated CD-1 mice, compared to a historic control incidence of 0.4% in untreated female CD- mice. Hepatocellular carcinomas occurred in two treated CD-1 mice (0.3 and 5 mg/kg/day) but not in controls. In 129/Sv wild type mice, hepatocellular adenomas did not occur in control or treated groups (n=6 to 10 per group). In PPAR-alpha null mice of this strain, in contrast, there were no adenomas in the controls (n=6), one adenoma in the 0.1, 0.3, and 1 mg/kg/day groups (n=9 or 10), and two adenomas at 3 mg/kg/day (n=9). These tumors occurred in 13.2% of all treated PPAR-alpha null mice. The significance of these findings is further discussed in the Mode of Action section below.

Serum liver enzymes and bile acids

Most toxicology studies of PFOA's effects on liver weight and histopathology did not assess serum levels of hepatic enzymes or bile acids. However, several studies in rodents have reported that these parameters were increased by PFOA, and these data are summarized below. As above, this summary does not represent a complete compilation of all data on these endpoints. These endpoints are of interest because they are indicative of hepatic damage, and because increased serum liver enzymes are associated with PFOA in human studies of the general population, communities with drinking water exposure, and workers (see Epidemiology section, above).

In the 90 day cynomolgus monkey study, ALT and AST were greatly elevated (about 10 to 50 times control levels) in two of the high dose animals that experienced toxicity from PFOA prior to the end of the scheduled dosing period (Butenhoff et al., 2002).

The liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were increased in all dose groups ($\geq$ 0.49 mg/kg/day) in a study of mice dosed with PFOA in drinking water for 21 days (Table 8). These increases were statistically significant ($p<0.05$) at $\geq$2.64 mg/kg/day for AST and $\geq$17.62 mg/kg/day for ALT (Son et al., 2008).

Table 8. Liver enzymes (relative to control) in serum of mice exposed to PFOA in drinking water for 21 days (Son et al., 2008)

Dose (mg/kg/day)	ALT	AST
0	1	1
0.49	1.5	2.0
2.64	2.9*	1.5
17.62	4.2*	3.3*
47.21	5.2*	4.0*

**p $\leq$ 0.05.*
Numerical data for liver enzymes (mean $\pm$ SD) are presented in Son et al. (2008).

PFOA caused dose-related increases in serum levels of four liver enzymes (ALT, AST, alkaline phosphatase, and lactic dehydrogenase [LDH]), as well as total bile acids, in male mice (Yang et al., 2014). The LOAEL for increased ALT was 2.5 mg/kg/day (the lowest dose in the study), while the LOAEL for increases in the other liver enzymes and total bile acids was 5 mg/kg/day. All parameters were increased to several-fold above control levels at 5 and/or 10 mg/kg/day.

Dose-related increases in ALT, AST, total bilirubin, and total bile acids occurred in both wild type and PPAR-alpha null mice treated with PFOA (Minata et al., 2010). For all of these parameters, the maximum increase at the highest dose was much greater in the PPAR-alpha null mice than in the wild type mice.

Nakagawa et al. (2011) also reported small but statistically significant increases in ALT in all three strains of mice (wild type, PPAR-alpha knockout, humanized PPAR-alpha) studied. The greatest increase was 2.3-fold in wild type mice given 5 mg/kg/day PFOA, with smaller increases in the PPAR-alpha null and humanized PPAR-alpha strains.

Liver enzymes (AST, ALT, GGT, and LDH) were significantly elevated on GD 18 in pregnant ICR mice dosed with 10 mg/kg/day PFOA on GD 1-17. AST and ALT were elevated at 5 mg/kg/day, but this effect was not significant, while no changes were observed at 1 mg/kg/day. (Yahia et al., 2010).

In a chronic rat study discussed below, ALT, AST, and alkaline phosphatase were increased in males in both low (1.3 mg/kg/day) and high dose (14.2 mg/kg/day) groups between 2 and 18 months of dosing, and in the high dose group after 24 months of dosing (Sibinski, 1987; Butenhoff et al., 2012). Butenhoff et al. (2012) conclude that these increases may result from the hepatic hypertrophy and/or "borderline chronic liver toxicity" caused by PFOA.

<u>Detailed review of selected data for increased relative liver weight</u>

Numerous studies have consistently reported that PFOA causes increased absolute and relative liver weight in laboratory animals. Increased relative liver weight is a sensitive endpoint for PFOA that co-occurs and/or can progress to more severe manifestations of hepatic toxicity. Data on increased relative liver weight from rodent studies that used relatively low doses (1 mg/kg/day or less), as well as the 90 day cynomolgus monkey study (Butenhoff et al., 2002), are summarized in Table 10. This group of studies includes five studies that provide data on serum PFOA levels at the end of the dosing period period (Butenhoff et al., 2002; Lau et al., 2006; Loveless et al., 2006; Perkins et al., 2004; Macon et al., 2011) and six additional studies that do not include such serum data. Studies providing serum PFOA data at the end of the dosing period are most appropriate for dose-response evaluation in risk assessment, because serum levels are highest at this time point and thus represent the maximum internal doses that could have caused the observed effect. Detailed individual study tables are found in Appendix 5 for the five studies that provide relevant serum PFOA levels.

Rodent data on increased relative liver weight

Three studies (Perkins et al., 2004; Loveless et al., 2006; Loveless et al., 2008) evaluated effects in male rats exposed to APFO. Perkins et al. (2004) and Loveless et al. (2006) provide serum PFOA data useful for dose-response modeling, while Loveless et al. (2008) does not include serum PFOA levels. Individual study tables for the two rat studies that include serum data (Perkins et al., 2004 and Loveless et al., 2006) are found in Appendix 5.

APFO formulations containing differing isomeric compositions (linear/branched, linear, or branched) were used in the three male rat studies (Perkins et al., 2004; Loveless et al., 2006; Loveless et al., 2008). Both linear/branched and linear PFOA were produced industrially, while information on effects of the branched form, which was not produced industrially, contributes to the understanding of the toxicology and mode of action of PFOA. Perkins et al. (2004) evaluated effects of linear/branched PFOA for 4, 7, and 13 weeks, while Loveless et al. (2006) studied the effects of 2 weeks of exposure to the linear isomer, branched isomers, or a mixture of linear/branched isomers. Both of these studies evaluated absolute and relative liver weight, hepatic palmitoyl CoA oxidase activity (PCO; a marker of peroxisome proliferation), and serum PFOA levels at sacrifice. The third male rat study, Loveless et al. (2008) evaluated the linear isomer of PFOA and reported relative liver weight data after exposure for 29 days, but did not report serum PFOA levels or PCO activity. (Both Loveless et al., 2006, and Loveless et al. , 2008, also evaluated male mice, discussed below.)

In Perkins et al. (2004), serum PFOA levels in rats reached steady state by 4 weeks. The dose-response curves for increased liver weight relative to body weight were almost identical at all three time points (4, 7, and 13 weeks), indicating that relative liver weight did not continue to increase over time with exposures longer than 4 weeks.

In the study evaluating the three PFOA formulations with differing isomer content (Loveless et al., 2006), serum PFOA levels from branched PFOA were lower than from the same doses of linear/branched or linear PFOA. This difference is likely to result from the more rapid excretion of the branched isomers (Benskin et al., 2009; DeSilva et al., 2009). The serum level LOAELs for male rats after 2 weeks of exposure to linear/branched PFOA reported by Loveless et al. (2006) are consistent with those from the longer exposure periods in Perkins et al (2004). The serum level dose response curves for increased relative liver weight from linear/branched PFOA in these two male rat studies with differing exposure durations are also consistent at the lower serum PFOA levels (less than 75,000 ng/ml) and are generally similar at the higher serum PFOA levels. Additionally, the dose-response curves for male rats based on administered doses of linear PFOA in the 2-week study (Loveless et al., 2006) and the 29-day study (Loveless et al., 2008) are generally consistent; serum PFOA data are not provided in the 29-day study.

Effects in male mice from exposure for 2 weeks to the three isomeric formulations of PFOA were also studied by Loveless et al. (2006). The same parameters that were evaluated in rats were studied in the mice, including absolute and relative liver weight, hepatic PCO activity, and

serum PFOA levels at sacrifice. The dose-response curves for male mice based on administered doses of linear PFOA in the the 2-week study (Loveless et al., 2006) and the 29-day study (Loveless et al., 2008) were generally similar. However, as above, serum PFOA data are not provided in the 29-day study.

The LOAEL for increased in liver weight in male mice was 0.3 mg/kg/day (the lowest dose in the study) for the linear form and the branched form. The serum levels at this dose were 13,000 ng/ml for the linear form and 14,000 ng/ml for the branched form. These are the lowest serum level LOAELs that were identified for increased relative liver weight. The relative liver weight at this dose was 1.17 for the linear form and 1.19 for the branched form, compared to the controls. For linear/branched PFOA, the serum level at 0.3 mg/kg/day was 10,000 ng/ml, and relative liver weight was 1.19 compared to the controls (similar to the increase at 0.3 mg/kg/day for the other isomeric forms). However, this change was not reported to be statistically significant, and the authors reported 0.3 mg/kg/day as the NOAEL and 1 mg/kg/day as the LOAEL for the linear/branched mixture.

PFOA increased hepatic peroxisome proliferation (as indicated by PCO activity) in rats in Perkins et al. (2004) and in rats and mice in Loveless et al. (2006). Evaluation of these PCO data reveals that, in the standard strains of rodents used in these experiments, increased liver weight did not correlate well with this biochemical marker of hepatic peroxisome proliferation, particularly in mice. Additionally (as mentioned above), PFOA caused increased hepatic peroxisome proliferation (as indicated by PCO activity) in primates as well as rodents. These PCO data are informative in evaluating the role of PPAR-alpha activation in the increased liver weight caused by PFOA and are discussed in detail in the Mode of Action section below.

In another study that includes serum PFOA data from the end of the dosing period, pregnant mice were dosed on GD 1-17 with 1, 3, 5, 10, 20, or 40 mg/kg/day PFOA (Lau et al, 2006; Table 10; Appendix 5A). At sacrifice on GD 18, the serum level LOAEL for increased liver weight was 22,000 ng/ml (from an administered dose of 1 mg/kg/day) and a NOAEL was not identified (Lau et al., 2006). (Numerical data are not shown in publication and were provided by the investigator.)

A second study of pregnant mice evaluated liver weight and serum PFOA levels on PND 21 in wild type and PPAR-alpha null mice dosed throughout gestation (Abbott et al., 2007). The NOAELs and LOAELs for increased liver weight based on serum PFOA from this study are not comparable to those from the other studies because serum PFOA was evaluated 3 weeks after dosing ended.

Two additional studies in male (Son et al., 2008) or female (DeWitt et al., 2008) adult mice exposed to PFOA in drinking water for 15 or 21 days that did not report serum PFOA data are also included in Table 10. As shown in the table, LOAELs based on administered dose from

these studies are generally consistent with the other mouse studies discussed above. NOAELs were not identified in these studies.

Three studies included in Table 10 evaluated increased liver weight in mouse pups after developmental exposures to PFOA. One of these studies (Macon et al., 2011) evaluated liver weight in offspring on PND 1, 4, 7, 14, and 21 after maternal exposure on GD 10-17 (late gestation). This study provides serum PFOA data from PND 1, one day after gestational exposure ended. As shown in the detailed table for this study in Appendix 5B and in Table 12, delayed mammary gland development (LOAEL – 0.01 mg/kg/day) was a much more sensitive endpoint than increased liver weight (LOAEL – 1 mg/kg/day) in this study. In an additional component of this study with full gestational exposure (GD 1-17) to PFOA, serum PFOA levels were not measured. The LOAEL for increased liver weight on PND 7 was lower with the longer exposure period (GD 1-17) than for the late gestational (GD 10-17) exposure discussed above, consistent with the greater total dose from longer exposure.

Another study (Tucker et al., 2015) assessed relative liver weight on PND 21 and later time points in CD-1 and C57/Bl mouse pups after maternal exposure to 0.01 – 1 mg/kg/day PFOA on GD 1-17. As in Macon et al. (2011), delayed mammary gland development was a more sensitive endpoint than increased liver weight in this study.

Finally, Abbott et al. (2007) evaluated relative liver weight in wild type and PPAR-alpha null offspring, as well as maternal relative liver weight (above), on PND 21. In this study, the LOAEL for increased relative liver weight in wild type pups (0.1 mg/kg/day) was 10-fold lower than the maternal LOAEL (1 mg/kg/day), while the LOAEL for this effect in PPAR-alpha null mice was the same in pups and dams (3 mg/kg/day).

Cynomolgus monkey data on increased relative liver weight

Liver weight (absolute and relative to body weight) was increased in all treated groups in the 90-day study of cynomolgus monkeys (Appendix 5; Butenhoff et al., 2002). The increase in absolute liver weight was statistically significant ($p<0.01$) in all groups, but relative liver weight was significantly increased only in the high dose group. However, this analysis did not include the animals sacrificed during the study due to overt toxicity. The absolute and relative liver weight in the sacrificed low dose monkey was far higher than the others in its group, and inclusion of data for this animal increases the mean value for these parameters in the low dose group. Liver-to-brain weight ratio is considered to be a reliable measure of effects on liver weight, because brain weight tends to remain stable when body weight changes. This ratio was increased in all treated groups compared to controls, with statistical significance at the two lower dose levels, but there was no dose-response based on either administered dose or serum level. As discussed above, serum PFOA levels did not differ significantly between the low and mid dose groups (Butenhoff et al., 2004c).

The data from Butenhoff et al. (2002) are not informative as to the dose-response curve or the NOAEL for PFOA's effects on increased liver weight in this species of monkey, because this effect did not increase with either administered or internal dose. Additionally, mortality of one of four animals at the lowest dose was possibly treatment-related, and four of the six high dose monkeys did not complete the study because of overt toxicity or mortality.

Comparison of the relative liver weight and serum PFOA level data from the 90 day cynomolgus monkey study and the rat studies (Perkins et al., 2004; Loveless et al., 2006) reveals similar increases in liver weight at comparable serum levels in both species. Additionally, comparable increases in PCO activity occurred at similar serum PFOA levels in monkeys and rats (discussed further in Mode of Action section). These data suggest that cynomolgus monkeys and rats have similar sensitivity to the hepatic effects of PFOA.

Immune system effects in rodents and non-human primates

PFOA causes suppression of the immune response in experimental animals. As discussed in the Epidemiology section, decreased response to vaccinations has been associated with PFOA in humans. Toxicological studies that evaluated effects of oral exposure to PFOA on the immune system in rodents are summarized in Table 13. Sixteen publications of such studies were identified. Of these, 14 publications report only studies of mice, one publication reports only studies in rats, and one publication includes studies in both species. Both of the rat studies, and 11 of the 15 mouse studies, were conducted in males, while two of the mouse studies evaluated adult females, and two of the mouse studies evaluated effects of developmental exposures on offspring. Additionally, effects on the immune system were reported in the 90-day oral study of rhesus monkeys (Goldenthal, 1978). Studies of effects on the immune system from dermal exposure to PFOA are not reviewed in this document. Toxicological effects on the immune system are of particular interest because PFOA and other PFCs have been associated with decreased vaccine response in humans (see Epidemiology section).

Non-human primates

In the 90-day rhesus monkey study (Goldenthal, 1978), histopathological changes related to the immune system occurred in all animals in the two higher dose groups (30 and 100 mg/kg/day). All animals in these two dose groups had slight to moderate hypocellularity of the bone marrow and moderate atrophy of lymphoid follicles in the spleen, and one 30 mg/kg/day female and all 100 mg/kg/day animals had moderate atrophy of the lymphoid follicles in the lymph nodes. These changes were not seen in the lower dose groups (3 and 10 mg/kg/day). Because serum PFOA levels are not provided in this study, it cannot be used for dose-response in risk assessment.

Rodents

PFOA consistently suppressed the immune system in studies of mice (Table 13). Effects in mice include decreased absolute and relative spleen and thymus weights, decreased thymocyte and splenocyte counts, decreased immunoglobulin response, and changes in total numbers and/or

specific populations of lymphocytes in the spleen, thymus, peripheral blood, and bone marrow. The available data indicates that rats are less sensitive than mice to immunotoxic effects of PFOA, since immune system effects were not observed in the two studies that have been conducted in rats. These rat studies included doses higher than those which generally caused immune effects in mice.

Relative liver weight was evaluated along with immune parameters in nine of the 13 studies of adult mice. In all of these nine studies, the LOAEL for increased relative liver weight was the same or lower than the LOAEL for immune system effects. As shown in Table 13, the lowest dose at which immune effects were clearly demonstrated in mice is 0.49 mg/kg/day in a 21-day drinking water study, with no NOAEL reported (Son et al., 2009). Increased liver weight also occurred at this dose in this study, as reported in the accompanying paper (Son et al., 2008). Therefore, the data available at this time suggest that increased relative liver weight is an endpoint that is as sensitive as or more sensitive than immune system effects in rats and mice. For this reason, immune system effects from toxicology studies were not used as the basis for the dose-response in this risk assessment, and individual study tables are not provided for these studies.

Reproductive and Developmental Effects

Overview

As discussed in the Pharmacokinetics section (above), PFOA exposures in the developing human fetus are similar to those experienced by the mother, and neonatal exposure from breast-feeding or consuming formula prepared with contaminated drinking water is much higher than in the mother or other older individuals using the same drinking water source. As discussed in the Epidemiology section, prenatal exposure to PFOA is associated with decreased birth weight in human epidemiology studies (Johnson et al., 2014).

Reproductive or developmental effects of PFOA have not been studied in non-human primates. Prior to 2006, the reproductive and developmental effects of PFOA had been studied only in rats and rabbits. These species are not the most appropriate models for evaluation of the potential for human reproductive and developmental effects of PFOA because the half-life of PFOA in female rats and female (as well as male) rabbits is only a few hours (see Table 4 in Toxicokinetics section). Because of this rapid elimination, serum levels from a given dose of PFOA in females of these species are much lower than in other species with long half-lives, such as mice and humans, and PFOA does not reach steady state in females of these species with daily dosing by gavage.

Beginning in 2006, the reproductive and developmental toxicity of PFOA has been studied in mice. The mouse is a more appropriate species for evaluating the potential human effects of PFOA on reproduction and development, since the female mouse excretes PFOA slowly and steady state is achieved with continued dosing. As discussed in detail below, effects observed in mice include full litter resorptions, decreased postnatal survival and growth, delayed

development, and accelerated sexual maturation in males. More recent studies have found that delayed mammary gland development is caused by developmental exposure to doses as low as 0.01 mg/kg/day.

In this section, the mouse studies of developmental and reproductive effects from gestational dosing with PFOA are presented in Table 12 and discussed in the text. This is followed by a detailed discussion of effects of developmental exposures to PFOA on mammary gland development, the most sensitive developmental endpoint with serum data appropriate for dose-response modeling. Summaries of additional studies of effects of pre-pubertal exposure to PFOA on reproductive organs in female mice, and of reproductive effect in male mice, are then presented. Finally, the studies of developmental and reproductive effects in rats and rabbits that were conducted prior to the mouse studies are summarized.

Mouse developmental studies

Summary of study designs

Table 12 summarizes data on reproductive and developmental effects from gestational and/or lactational exposure to PFOA in mice. Sixteen publications reporting such studies are included in the table. Several of these publications include multiple studies with different exposure protocols, in one case, different aspects of the same study are described in two publications (Wolf et al., 2007; White et al., 2009). In total, 18 separate studies, three of which used multiple strains of mice, are reported. Most but not all of the studies assessed both maternal/reproductive endpoints and developmental endpoints in the offspring. The table includes the six publications that evaluated developmental effects of PFOA on mammary gland development in the dam and/or the female offspring, as well as other endpoints. Effects on mammary gland development are further discussed in detail in a separate section below.

Several additional studies of effects of gestational dosing with PFOA are not included in the Table 12 because they did not evaluate standard reproductive or developmental endpoints and/or because they used non-standard exposure protocols. These papers are discussed elsewhere in this document. These include two studies of histopathological changes in the livers of mice in adulthood after low dose developmental exposures (Filgo et al., 2015; Quist et al., 2015), a study of intestinal tumorigenesis in wild type and genetically susceptible strains of mice (Ngo et al., 2014), and three studies of neurobehavioral effects at age 5-8 weeks in offspring of dams dosed during gestation (Johansson et al., 2008; Onishchenko et al., 2011; Sobolewski et al., 2014).

Most (11 of 16) of the studies in Table 12 used CD-1 mice, three used C57Bl/6 mice, one study (Tucker et al., 2015) used both strains, and one study used ICR mice (Yahia et al., 2010). Two studies included strains of mice with differingnt PPAR-alpha status (wild type, null, and/or humanized) and are discussed in more detail in the Mode of Action section.

In most of the studies (13), dosing was by oral gavage, while one study used drinking water exposure (Hu et al., 2010), one study used drinking water exposure with or without additional

gavage exposure in some, but not all, dose groups (White et al., 2011b), and one study used dietary exposure (van Esterik et al., 2016).

In most of the studies (10), the dams were dosed with PFOA throughout gestation, and postnatal development of the offspring was assessed. However, other protocols were used in some studies. In two studies (Hu et al., 2012; van Esterik et al., 2016), maternal dosing began before mating and continued until weaning. In other studies, the dosing period was shorter than the full period of gestation. Fenton et al. (2009) was primarily a pharmacokinetic study of a single dose of PFOA administered on GD 17 and is included because offspring body weight was assessed. In several other studies, PFOA was administered for only a portion of gestation (Hu et al., 2010, GD 6-17; Macon et al., 2011 "late gestation study", GD 10-17; Suh et al., 2011, GD 11-16; White et al., 2007 "restricted exposure study", GD 1-17, 8-17, and 12-17; Wolf et al., 2007, "restricted exposure study", GD 7-17, 10-17, 13-17, and 15-17). Two studies, one with exposure on GD 8-17 (White et al., 2009) and one with exposure on GD 1-17 (Wolf et al., 2007; White et al., 2009) used a cross-fostering protocol in which offspring were exposed during gestation and/or lactation. In these studies, treated dams were dosed during gestation. The dams that were not dosed but fostered pups from dosed dams were exposed to PFOA via grooming of the pups and ingestion of excreted pup urine and feces. One study (White et al.; 2011b) was a multi-generation study in which effects on three generations were evaluated (P0, F1, and F2). Effects on P0 dams and F1 offspring are shown in Table 12; complete information on the study is presented in the section on mammary gland development (below).

Most of the studies (15) assessed both maternal/reproductive endpoints and endpoints of postnatal developmental in the offspring, although the specific endpoints that were evaluated differed among studies. Only two of the studies evaluated malformations at birth. These were Lau et al. (2006), which also evaluated postnatal development, and Yahia et al. (2010). Two studies (Macon et al., 2011, and Tucker et al., 2015) assessed effects in the offspring, but not maternal or reproductive effects. Several studies focused on specific effects in addition to standard developmental endpoints or mammary gland development. Hines et al. (2009) evaluated effects in female offspring of developmental exposures on body weight and hormones (insulin and leptin) in adulthood. Hu et al. (2010) and Hu et al. (2012) assessed effects of developmental exposures on immune endpoints at age 7 to 9 weeks and are also included in the summary table of immune effects (Table 13). Finally, Suh et al. (2011) focused on placental toxicity and other reproductive endpoints, and did not evaluate the offspring.

Two additional studies assessed the effects of pre-pubertal exposure to PFOA on the reproductive system in female mouse pups (Dixon et al., 2012; Yao et al., 2014). These are not included in the table and are discussed in the text later in this section.

Maternal and reproductive effects

PFOA caused reproductive effects in mice including increases in full litter resorptions (Abbott et al., 2007), increased litter loss, prenatal loss per live litter, or fetal resorptions/dead fetus (Abbott

et al., 2007; Lau et al., 2006; Suh et al., 2011; White et al., 2007; White et al., 2011b), and decreased number of live fetuses per litter or decreased litter size (Lau et al., 2006; Suh et al., 2010; van Esterik et al., 2016; Wolf et al., 2007; White et al., 2011b). In all of these studies, one or more of these reproductive effects occurred at doses below those which caused decreased maternal weight gain, an indicator of general maternal toxicity.

PFOA caused toxicity to the placenta, including decreased placental weight, decreased fetal/placental weight ratio, and decreased expression of genes for prolactin family hormones (hormones that support fetal growth and nutrition), after treatment on GD 11-16 at the lowest dose tested, 2 mg/kg/day (Suh et al., 2011). Higher doses caused placental necrosis and reduced numbers of placental trophoblast cells. These results suggest that placental toxicity may contribute to the increased number of dead fetuses/decreased number of live fetuses and decreased fetal growth observed in this and other developmental studies of PFOA.

Effects on maternal relative liver weight and maternal mammary gland development are discussed below.

Effects in Offspring: Fetal through Weaning

Fetal teratology

Two studies evaluated fetal teratology. In CD-1 mice, gestational exposure to PFOA caused reduced ossification of phalanges, limb and tail defects, and microcardia at doses below those which affected maternal weight gain (Lau et al., 2006). Reduced ossification of proximal phalanges of both the forelimb and the hindlimb was significantly increased at the lowest dose used (1 mg/kg/day) but was not significantly increased in some higher dosed groups. This effect represents a delay in timing of development rather than a permanent structural change, since the phalanges developed normally in mice treated with PFOA that were not sacrificed prior to delivery in this study (personal communication with C. Lau). Ossification at other sites (caudal vertebrae, metacarpals, and metatarsals) was delayed only at a much higher dose (20 mg/kg/day) which also caused maternal and reproductive toxicity.

In ICR mice, gestational exposure to PFOA caused increased incidence of cleft sternum, delayed ossification of phalanges, and delayed incisor eruption. The LOAEL and NOAEL for increased incidence of cleft sternum, delayed ossification of phalanges, delayed incisor eruption were 10 mg/kg/day and 5 mg/kg/day, respectively (Yahia et al., 2010). In this study, maternal weight gain, fetal weight, and pup survival until PND 4, were significantly decreased at dose(s) below the LOAEL for increased cleft sternum, delayed ossification, and delayed incisor eruption. No pups survived until PND 4 at 10 mg/kg/day, the LOAEL for delayed ossification.

Birth weight, and growth and postnatal development until weaning

PFOA caused decreased body weight at birth, postnatal mortality, reduced postnatal growth until weaning, and delayed development (as indicated by day of eye opening) in mice. As shown in Table 12, one or more of these effects was reported by Abbott et al. (2007), Hines et al. (2009),

Hu et al. (2010), Hu et al. (2012), Lau et al. (2006), Tucker et al. (2015), van Esterik et al. (2016), White et al. (2007), White et al. (2009), Wolf et al. (2007), White et al. (2011b), and Yahia et al. (2010). Exposure during only the latter part of gestation (GD 15-17) was sufficient to cause decreased body weight at birth, increased postnatal mortality, and decreased postnatal growth (Wolf et al., 2007). Furthermore, decreased postnatal growth resulted from either gestational or lactational exposure, as shown by the results of the cross-fostering study by White et al. (2009).

Effects on relative liver weight and mammary gland development during the period from birth until weaning are discussed below.

Effects in Offspring: Post-Weaning

In studies which continued to assess offspring during the post-weaning period, effects at these later time points were observed in some studies (summarized below) but not in others (e.g. no effect on body weight in Abbott et al., 2007, or on immune parameters in Hu et al., 2012).

In the restricted exposure component of Wolf et al. (2007), developmental exposure to 5 mg/kg/day PFOA beginning on GD 10 caused decreased body weight in male offspring until age 10-11 weeks. In the cross fostering study component of the same publication, body weight remained decreased until PND 36 in male offspring after *in utero* and lactational exposure to 5 mg/kg/day PFOA, and until PND 85 in females exposed to the same dose *in utero* even without postnatal exposure from breastmilk.

Markers of sexual maturation (vaginal opening and first estrus) were delayed in female CD-1 mouse offspring with gestational exposure to PFOA. However, sexual maturation (preputial separation) in male offspring was accelerated by PFOA exposure, despite the fact that PFOA caused decreased body weight at 6.5 weeks of age (Lau et al., 2006). Notably, acceleration puberty in males did not follow a typical dose-response curve. The greatest acceleration occurred at the lowest dose (1 mg/kg/day) with a smaller effect at each increasing dose. At the highest dose (20 mg/kg/day), puberty was delayed rather than accelerated.

Body weight and hormone (insulin and leptin) levels were increased in early adulthood (20-29 weeks) in CD-1 mice with gestational exposure to low doses (0.01 to 0.1 mg/kg/day for obesity; 0.01 to 0.3 mg/kg/day for hormones) but not at higher doses. In this study, body weight at birth or early in life was not affected by the low doses that caused increased body weight later in life (Hines et al., 2009). In contrast, Ngo et al. (2014) found no effect on body weight at age 12-20 weeks in male or female wild type or Min/+ (a strain susceptible to intestinal tumorigenesis) C57Bl/6 mice offspring of dams dosed with 0.01, 0.1, and 3 mg/kg/day PFOA during gestation. These studies are of interest because several epidemiology studies evaluated associations of prenatal and/or early life PFOA exposure with parameters associated with increased body weight in childhood or adulthood. Three studies found associations of maternal serum PFOA levels during pregnancy with increased risk of overweight/obesity and changes in metabolic hormones

in young women (Halldorsson et al., 2012), more rapid weight gain in girls (Maisonet et al., 2012), or greater adiposity and more rapid increase in BMI in childhood (Braun et al., 2016), while two studies did not find such an association with prenatal or early childhood exposure to PFOA (Andersen et al., 2013; Barry et al., 2014).

Splenic T cells were decreased at age 6 weeks in C57Bl/6 mice exposed gestationally to 2 mg/kg/day PFOA, with no effect at 0.2 mg/kg/day (Hu et al., 2012). The NOAEL and LOAEL for this effect were the same as for decreased body weight gain on PND 1-21, suggesting that the immune system effect from developmental exposure was not a more sensitive endpoint than other developmental effects in this study.

Effects on relative liver weight and mammary gland development during the post-weaning period are discussed below.

Relative liver weight

Increased relative liver weight is a sensitive toxicological endpoint for PFOA. This effect was evaluated in dams and/or offspring in 12 of the 18 mouse developmental studies summarized in Table 12. Maternal relative liver weight was assessed at delivery in three studies, between delivery and weaning in two studies, and at weaning in five studies. In offspring, relative liver weight was evaluated at birth in two studies, between birth and weaning in three studies, at weaning in five studies, and post-weaning in five studies.

The NOAELs and LOAELs for increased relative liver weight in dams and/or offspring and for other reproductive and developmental effects are presented in Table 12. (*Note*: Fenton et al., 2009, is not included in this table because no effects were seen at the single dose used.) In all studies except one (Hu et al., 2010), the LOAEL and/or NOAEL for increased relative liver weight in the dam and/or the offspring is the same or lower than the LOAEL and/or NOAEL for reproductive developmental effects (other than delayed mammary gland development). In Hu et al. (2010), offspring liver weight was evaluated on PND 48 and PND 63; these are post-weaning time points at which PFOA was almost totally eliminated from the body. These data indicate that increased relative liver weight (maternal and/or offspring) is an endpoint for PFOA toxicity in mice that is as sensitive or more sensitive than most of the other reproductive and developmental effects that were evaluated, with the exception of delayed mammary gland development which is discussed below.

Mammary gland development

Background information on mammary gland development as a toxicological endpoint

Because the developmental patterns of the mammary gland are similar in humans and rodents, rodents provide a good model for studying the effects of environmental contaminant on human mammary gland development (Rudel and Fenton, 2009; Fenton et al. 2012; Rudel et al., 2011; Fenton and Birnbaum, 2015; Osborne et al., 2015). The development of the mammary gland in

these species involves a complex series of events that is regulated by a balance of hormones, growth factors and stromal factors (Osborne et al., 2015; Gore et al., 2015). Agents that affect any of these steps can interfere with the normal process of mammary gland development, and this process is particularly sensitive to effects of endocrine disrupting chemicals, including environmental contaminants such as bisphenol A, atrazine, and dioxin (Gore et al., 2015; Fenton and Birnbaum, 2015; Fenton et al., 2012; IOM 2011; Rudel et al., 2011). As reviewed in detail below, adverse effects on mammary gland development are sensitive toxicological endpoints for PFOA, and a NOAEL for these effects has not been identified.

The mammary gland is most sensitive to the effects of toxic substances during critical periods when development occurs including fetal, neonatal, puberty, and pregnancy (Gore et al., 2015; Osborne et al., 2015), while the mammary glands of non-pregnant adult females may not be affected by the same exposures. Adverse effects on the mammary gland from early life exposures can include accelerated or delayed development (Macon and Fenton, 2013; Osborne et al., 2015), and effects of the same chemical on mammary gland development may vary depending on the life stage when exposure occurs (Osborne et al., 2015). These disruptions of mammary gland development may result in adverse effects later in life including impaired lactation and increased cancer risk (Fenton 2006; IOM, 2011; Rudel et al., 2011; Fenton et al., 2012).

The mammary gland is distinct from other tissues in that it undergoes a significant portion of its development postnatally; in addition to the fetal/neonatal period, puberty and pregnancy are critical periods of mammary gland development (Osborne et al., 2015). The mammary gland grows and differentiates slowly during embryonic and juvenile life and does not mature until after puberty. Mammary gland development in rodents has been reviewed by Sakakura (1987) and Daniel and Silberstein (1987) and in humans by Russo and Russo (1987) and Howard and Gusterson (2000).

The mammary gland is an organ that is unique to the class *Mammalia*, and its embryonic, postnatal, and adult development is highly conserved between species. Mammary gland development in humans and rodents takes place at a similar biological pace, although the absolute timeframes differ (Table 12 and Figure 10, both from Fenton, 2006). The description of mammary gland development below is taken from concise summaries provided by Osborne et al. (2015) and Fenton et al. (2012).

In rodents and humans, mammary gland development begins with the formation of the mammary, or milk line, during embryonic development. This structure separates into individual placodes (areas of thickening of the ectodermal layer), each of which develops into a ductal tree that embeds in a fat pad to form the mammary bud. Although there are slight timing differences in mammary gland development among species, ductal branching begins during the prenatal period in both rodents and humans. Subsequent to the fetal/neonatal period, there is little epithelial growth until puberty. During puberty, exponential growth of the female mammary

gland occurs for several weeks in rodents and several years in humans. During puberty, the fat pad rapidly fills with epithelial cells to become the adult form of the gland. The epithelium develops bundles of ducts, which then form club-like structures, called terminal end buds (TEB) in humans. Each TEB cleaves into alveolar buds and sprouts into ductules, forming a structure called the terminal ductal lobular unit. In rodents, TEBs are the sites of future ductal branching and disappear as the gland differentiates, and they are the structures considered to be most functionally equivalent to the terminal ductal lobular unit in humans. TEBs are particularly susceptible to the effects of carcinogens, and it has been suggested that "factors that lengthen the period when TEBs are present lengthen the period during which the mammary gland is susceptible to carcinogens (Osborne et al., 2015)." After puberty, the mammary gland remains in a resting state until pregnancy occurs. During pregnancy, the gland undergoes another period of rapid differentiation, involving branching and the development of lobulo-alveoli to prepare for lactation.

Table 9. Developmental events in human and rodent mammary tissue (Fenton, 2006)

Developmental Event	Human	Rodent
Milk Streak Evident	EW4-6	GD10-11 (mice)
Mammary Epithelial Bud Forms	EW10-13	GD12-14 (mice), GD14-16 (rat)
Female Nipple and Areola Form	EW12-16	GD18 (mice), GD20 (rat)
Branching and Canalization of Epithelium	EW20-32	GD16 to birth (mice, GD18 to birth (rat)
Secretion is Possible	EW32-40 (ability lost Postnatally)	At birth, with hormonal stimuli
Isometric Development of Ducts	Birth to Puberty	Birth to Puberty
TEBs Present (Peripubertal)	8 to 13 yr old girls	23-60 d old (rat)
Formation of Lobular Units	EW32-40, or within 1-2 yr of first menstrual cycle	Puberty and into Adulthood

EW – embryonic week; GD – gestation day

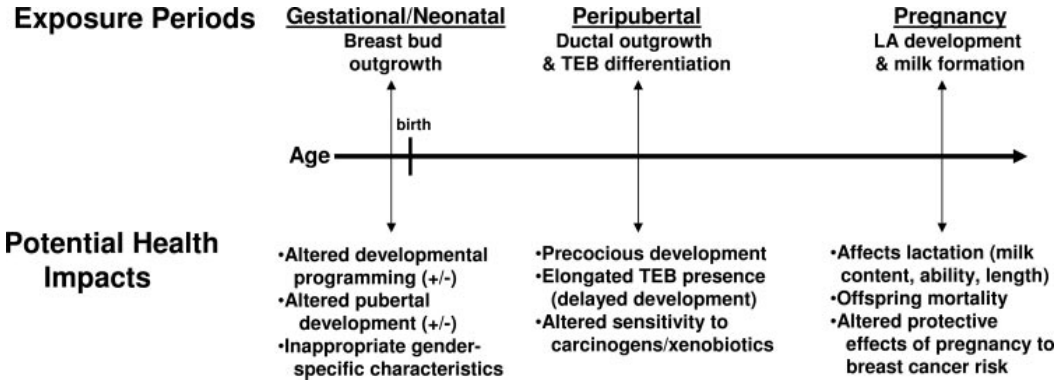


Figure 10. Timeline of critical periods of mammary gland development and potential effects of endocrine disrupting compounds on mammary gland development (Fenton, 2006).

Effects on mammary gland development in rodents are commonly assessed by evaluation of "whole mounts". In this approach, the entire fourth and/or fifth abdominal mammary gland fat pad is mounted flat on a slide, fixed, stained, defatted, and permanently affixed to the slide.

Whole mounts are assessed microscopically for parameters such as numbers of mammary terminal ductal structures (i.e., TEBs, terminal ducts, alveolar buds, and lobules), extension of the epithelial cells through the fat pad, and branching patterns and density at different times during development branching (Rudel et al., 2011). Whole mounts were evaluated in the nine studies of the effects of PFOA on mammary gland development in mice described below, and tissue sections stained with hematoxylin and eosin (H&E) were also examined in some of these studies. Effects on mammary gland development are reported as overall age-adjusted developmental scores based on a number of parameters and/or as quantitative values for specific parameters.

Effects of PFOA on mammary gland development in mice

Delayed mammary gland development in mice is a sensitive toxicological endpoint that is considered relevant to humans because developmental patterns are similar in both species. Nine publications have reported the effects of PFOA on mammary gland development in mice, and some of these publications include multiple studies. These studies are summarized in Table 16, and details of each study are provided in an individual study table for each publication in Appendix 5. Information related to the mode of action of PFOA's effects on mammary gland development is reviewed in the Mode of Action section, below.

Studies of effects of maternal and fetal/neonatal exposure to PFOA

Six publications evaluated effects of PFOA exposure during gestation and/or lactation on mammary gland development. Studies presented in these publications are summarized in Table 16A, and details for each study are provided in Appendix 5. Some of these publications include multiple studies, and, in total, ten separate studies were reported. Nine of these studies reported delayed mammary gland development, and one reported no effect on mammary gland development. Five of these publications report studies in CD-1 mice (White et al., 2007, three studies; White et al., 2009, two studies; Macon et al., 2011, two studies; White et al., 2011b; Tucker et al., 2015), and one of these also includes C57Bl/6 mice (Tucker et al., 2015). The sixth publication evaluated wild type, PPAR-alpha null, and PPAR-alpha humanized mice of the Sv/129 genetic background (Albrecht et al., 2013). Nine of the ten studies evaluated female offspring, and four of the studies (White et al., 2007; White et al., 2009; White et al., 2011b) evaluated pregnant and/or lactating dams.

Effects on structure of the mammary gland development

PFOA exposure during critical developmental periods (fetal, neonatal, pregnancy, and lactation) caused delayed mammary gland development in both lactating dams (White et al., 2007; White et al., 2009; White et al., 2011b) and pups (White et al., 2007; White et al., 2009; Macon et al., 2011; White et al., 2011b; Tucker et al., 2015), while even a high dose (5 mg/kg/day) did not affect mammary gland development in non-pregnant adult female mice (White et al., 2007).

As shown in the comparison of LOAELs and NOAELs for mammary gland development and other endpoints in Table 12, mammary gland development was a more sensitive endpoint for PFOA than other effects found in dams and pups in mouse developmental studies including

reproductive endpoints (number of fetuses per litter, prenatal loss, prenatal survival), pubertal markers (day of vaginal opening or day of first estrus; Tucker et al., 2015), estrogen or progesterone levels (Tucker et al., 2015), body weight, or liver weight (Macon et al., 2011; Tucker et al., 2015).

Lactating dams

Delayed mammary gland development occurred in lactating dams after dosing with PFOA during gestation (White et al., 2007; 2009; 2011b) or from exposure via treated pups (White et al., 2009). At the end of pregnancy just prior to initiation of nursing (GD 18; White et al., 2007) and on PND 1 after one day of nursing (White et al., 2009), mammary glands of treated dams were not saturated with milk filled alveoli, as is normally seen, but rather exhibited stunted, immature development. On PND 10, normally the peak of lactation, mammary glands from treated mice were delayed in development and resembled those normally seen earlier in lactation (White et al., 2007; 2009). On PND 20-22, mammary glands from treated dams had milk-filled alveoli and resembled normal mammary glands at the peak of lactation on PND 10, instead of the normal involution that occurs at weaning, indicating delayed development of up to 10 days (White et al., 2007; 2011b).

Female offspring

Delayed mammary gland development occurred in female pups in both CD-1 and C57Bl/6 strains (Tucker et al., 2015). In CD-1 mice, the mammary gland developmental score was significantly decreased compared to controls at all doses including the lowest dose (0.01 mg/kg/day). In C57Bl/6 mice, the developmental score was decreased compared to controls at all doses at both timepoints assessed (PND 21 and PND 61). However, this difference was not statistically significant at the two lowest doses (0.01 and 0.1 mg/kd/day), but was significant at 0.3 and 1 mg/kg/day. Serum PFOA levels in C57Bl6 pups were lower than in CD-1 pups at the same administered dose. Lack of statistical significance in C57Bl/6 offspring at the two lowest doses may be due to the lower serum PFOA levels and/or small number of animals in the two lower dose groups for this strain (n=2-5 per dose group) as compared to CD-1 mice (n=8-22 per dose group), rather than differences in intrinsic sensitivity to effects of PFOA on mammary gland development in the two strains mice (Tucker et al., 2015). It should be noted that the LOAELs in female offspring for other well-established developmental effects of PFOA (decreased body weight and increased relative liver weight, both on PND 21) were also higher in C57Bl/6 mice than in CD-1 mice in this study, possibly due to the factors mentioned above.

PFOA is found in breast milk after maternal exposure in both rodents and humans, resulting in lactational exposure to offspring (reviewed in Post et al., 2012; also Mogensen et al., 2015). Cross-fostering studies in which pups with no prenatal exposure were exposed via breast milk from exposed dams, and pups with prenatal exposure were fostered by untreated dams, show that delayed mammary gland development results from exposure during gestation, lactation, or both. Significant delays in mammary gland development occurred as early as PND 1 in non-gestationally exposed pups nursing from treated dams for only 12-24 hours, and as early as PND

3 in non-gestationally exposed dams nursing treated pups. The exposure in these dams was only through maternal behavior such as ingestion of treated pups' waste and grooming of treated pups (White et al., 2009).

In White et al. (2009), effects on the mammary gland persisted in exposed offspring until 18 months of age, long after PFOA had been eliminated from the body; these effects are considered to be permanent. Although the study was not designed to quantitatively evaluate mammary gland development in older animals, epithelial density appeared to be reduced in the mammary glands of exposed animals at 18 months. An increase in the number of unusual darkly staining foci per gland of approximately 5-fold was also seen at 18 months in the mammary glands of exposed mice. According to the authors, these foci appeared to result from hyperplasia of ductal epithelium, infiltration of inflammatory cells into ductal regions, increased stromal density surrounding the ducts, and/or inappropriate differentiation of the ductal epithelium. Gore et al. (2015) notes that abnormalities of this type can be associated with increased breast cancer risk.

Mammary gland development, as assessed on PND 21 by overall developmental score, number of terminal end buds, and other measures of mammary gland development, was delayed in a dose-related fashion in CD-1 mouse pups after late gestational exposure (GD 10-17) to doses lower than those used in earlier studies (Macon et al., 2011). The LOAEL was 0.01 mg/kg/day, with no NOAEL identified. These effects occurred at serum PFOA levels of 285 ng/ml or below, lower than the mean serum level (371 ng/ml) in a community exposed to highly contaminated drinking water (Emmett et al., 2006a).

In a multi-generation study of CD-1 mice exposed to 5000 ng/L (5 μg/L) PFOA in drinking water, mammary gland development was delayed in both F1 dams (PND 22) and F1 female pups (PND 22, 42, and 63) at serum levels relevant to human environmental exposures (White et al., 2011b). Pups were significantly affected at serum levels as low as 21.3 ng/ml on PND 22 (compared 0.6 ng/ml in controls at this time point). This serum level is below the mean serum level of 28 ng/ml in the six Ohio and West Virginia communities with contaminated drinking water that comprise the C8 Health Study population, and is within about 10-fold of the mean and 4-fold of the 95th percentile serum levels in the U.S. general population (CDC, 2015). This serum level would be expected in humans with ongoing exposure to drinking water concentrations of approximately 200 ng/L (0.2 μg/L), based on the serum:drinking water ratios discussed above.

In contrast to the delays in mammary gland development observed in CD-1 and C57Bl/6 mice in the five studies discussed above, no significant effects on mammary gland development were found in wild type, PPAR-alpha null, or humanized PPAR-alpha Sv/129 mouse pups exposed gestationally to 3 mg/kg/day PFOA (Albrecht et al., 2013). This is the only study of mammary gland development in these strains. Albrecht et al. (2013) report that postnatal lethality occurred in the wild type mice, but not in the PPAR-alpha null or humanized PPAR-alpha mice. They conclude that the study confirms the PPAR-alpha dependent postnatal lethality of PFOA

previously reported by Abbott et al. (2007). However, several problematic issues with this study limit the consideration of its results:

- Although postnatal lethality in wild type mice treated with 3 mg/kg/day (the only dose used in the study) on PND 20 was reported as statistically significant ($p<0.05$), this conclusion appears to be based on an inappropriate statistical comparison. In evaluating postnatal lethality, the number of pups per litter on PND 20 in the control and PFOA-treated groups of wild type mice were compared. However, this comparison does not appear to be valid because the control and PFOA treated litters initially had different numbers of pups on PND 0. The appropriate evaluation of this parameter is a comparison of the number of pups within the same litter on PND 0 and PND 20 (i.e. percent mortality within the litter between PND 0 and PND 20). In wild type pups, 96% of controls and 70% of PFOA-treated survived from PND 0 to PND 20. From the analysis presented, it is unclear whether postnatal lethality is actually significantly increased by PFOA in wild type pups. For this reason, the basis for the conclusion that wild type, but not humanized PPAR-alpha, mice are sensitive to developmental effects of PFOA is uncertain.

- An important concern is that Albrecht et al. (2013) state that elevated PFOA levels (up to > 1000 ng/ml) were found in liver and serum from some control fetuses, pups, and dams. However, no further information such as which groups of animals these samples came from, how many samples had elevated PFOA concentrations, or statistical data for serum levels in the control samples is provided. Importantly, data from control animals with elevated PFOA exposures do not appear to have been excluded in the comparisons of endpoints of toxicity in control and treated groups. Inclusion of these data from the control animals could have affected the results of these comparisons, especially since serum levels in some of the treated groups were only a few fold higher than those in some of the controls.

- Developmental effects observed in the same strain of mice (SV/129) in another study (Abbott et al., 2007) at lower doses (0.6 and 1 mg/kg/day) were not observed at the higher dose (3 mg/kg/day) used by Albrecht et al. (2013). Abbott et al. (2007) observed significantly increased postnatal lethality in wild type pups exposed gestationally to 0.6 and 1 mg/kg/day PFOA. Additionally, eye opening was significantly delayed in the 0.6 and 1 mg/kg/day wild type pups in Abbott et al. (2007), but not at 3 mg/kg/day in Albrecht et al. (2013).

- Although both studies used SV/129 mice, Albrecht et al. (2013) obtained them from NIH and Abbott et al. (2007) obtained them from Jackson Laboratories. Albrecht et al. (2013) suggest that pharmacokinetic differences in the wild type mice from the two different sources may explain the differences in effects of PFOA in these mice in the two studies. However, a close review of the data from the two studies (Table 3 of Abbott et al., 2007; Figure 10 of Albrecht et al., 2013) indicates that the serum levels in wild type pups in Albrecht et al. (2013) at which no developmental effects occurred were higher than the

serum levels in wild type pups at which delayed eye opening and postnatal mortality were reported by Abbott et al. (2007). Furthermore, the serum PFOA data for wild type dams on PND 20 appear to be inconsistent within the publication. Maternal serum levels in wild type dams on PND 20 are stated to range from 2066 – 6812 ng/ml, and no statistical parameters (e.g. median, mean, S.D.) are provided. However, the estimated serum level from the bar graph of maternal serum levels is 6700±3600 in the wild type dams (higher than what would be expected from the range provided in the text).

In summary, no effect of PFOA on mammary gland development was reported in pups from the three strains on PND 20; this is the only study that evaluated PFOA's effect on this endpoint in these strains. It is possible that these strains are less sensitive to this effect than are the other strains in which effects were reported. However, the general issues with this study create uncertainty about its conclusions related to mammary gland development. As discussed above, the inclusion of data from controls with elevated PFOA exposures may have affected the ability to observe effects in treated groups. Pertinent data on serum levels is not presented or appears to be presented inconsistently. The differences in developmental effects in wild type pups in Albrecht et al. (2013) versus Abbott et al. (2007) cannot be explained on the basis of pharmacokinetic differences in the two studies, since effects occurred at pup serum levels in Abbott et al. (2007) that are lower than the pup serum levels in Albrecht et al. (2013).

Effects on milk quality and quantity

In dams exposed to PFOA during pregnancy, the morphological delays observed in mammary glands during lactation suggest that milk production and/or composition may be impacted. Potentially relevant to this issue, maternal PFOA exposure was associated with shorter duration of breast feeding in both of the two human studies that evaluated this effect (Fei et al., 2010; Romano et al., 2016). However, the available toxicological information is not sufficient to make conclusions about the effects of developmental exposure to PFOA on lactational function, as only one study (White et al., 2007) evaluated effects on the composition of milk, and only one study (White et al., 2011b) evaluated the amount of milk produced. Evaluation of effects on growth of offspring is complicated by the fact that PFOA itself can cause decreased postnatal growth in mouse pups from only *in utero* exposure in a cross-fostering study (Wolf et al., 2007). Therefore, effects on postnatal growth could result from the intrinsic toxicity of PFOA and/or from decreased lactational function.

In the only toxicological study of effects on milk composition, expression of genes for four milk proteins (beta-casein, EGF, alpha-Lac, and LactoF) in mammary gland tissue were altered in dams exposed to 5 mg/kg/day PFOA during gestation. For example, peaks in LactoF expression normally seen early and late in lactation were delayed, consistent with the observed structural delays in mammary gland development at these time points (White et al., 2007).

The only toxicological data on effects of prenatal and early life exposure to PFOA on milk production in adulthood comes from the two-generation study in CD-1 mice (White et al.,

2011b). The design of this study is shown in Figure 11. As part of this study, a lactational challenge experiment was conducted in F1 dams and their F2 litters on PND 10, the time point at which lactation is at its peak. Except for the dose group receiving 5000 ng/L (5 µg/L) PFOA in drinking water throughout the experiment, the F1 dams that were evaluated for lactational function were exposed prenatally and in neonatal life until weaning through breast milk, but not after weaning or while pregnant and lactating. Milk production was evaluated by weighing a litter of 10 F2 pups before and after a nursing period of 30 minutes. There were no statistically significant effects on milk production, as measured by weight gain in the litter, or on time to initiate nursing behavior. However, high variability, the small number of animals assessed, and the lack of sensitivity of this assay may have limited the ability to detect effects on lactational function. Additionally, postnatal survival and body weight were not affected in the F2 pups, indicating that the ability of the F1 dams to provide nutritional support was not decreased. The authors note that it is not known whether deficits in lactational function were present, but were compensated for by increased frequency or longer duration of nursing events, since these parameters were not assessed.

Possibley relevant to this issue, the two available human studies both suggest that maternal exposure to PFOA may be related to shorter duration of breastfeeding. A study of 1400 Danish women from the general population found that serum PFOA concentration during early pregnancy was associated with shorter duration of breastfeeding among multiparous, but not nulliparous, women (Fei et al., 2010). Because women who breastfed previously were more likely to so again, and because longer duration of lactation would result in decreased serum PFOA due to excretion via breast milk, reverse causality could not be ruled out. A second study evaluated 336 U.S. women from a community with median serum PFOA levels about twice those in the U.S. general population, possibly due to past exposure to contaminated drinking water (Romano et al., 2016). In contrast to Fei et al. (2010), this study controlled for prior breast feeding history. Notably, serum PFOA levels during pregnancy were associated with shorter duration of breast feeding even after adjustment for previous breast feeding.

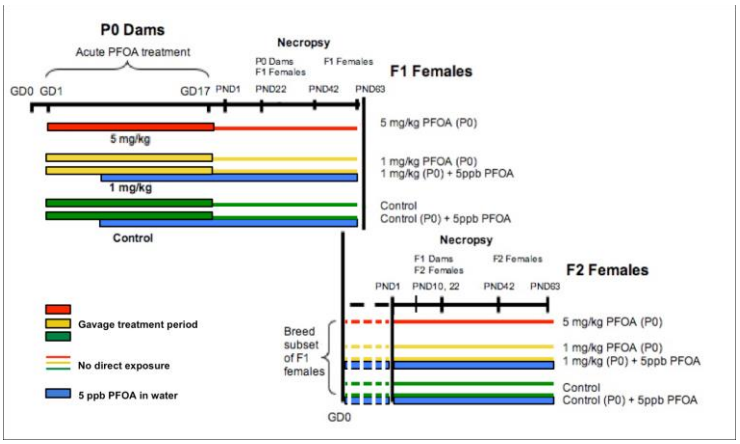


Figure 11. White et al. (2011b) study design and experimental timeline. Bar color denotes dose – green, 0 mg PFOA/kg body weight/day; yellow, 1 mg PFOA/kg body weight/day; red, 5 mg PFOA/kg body weight /day; blue, 5

ppb PFOA in drinking water – and bar thickness denotes timing of treatment – thick bars denote on-going direct treatment, thin bars denote only group identity subsequent to treatment.

Studies of effects of peripubertal exposure to PFOA

Three studies reported effects of peripubertal (during periods between 3 and 7 weeks of age) exposure to PFOA on mammary gland development in female mice. Studies presented in these publications are summarized in Table 16B, and details for each study are provided in Appendix 5. Two studies used C57Bl/6 and Balb/C mice (Yang et al., 2009a; Zhao et al, 2012). One of these studies (Zhao et al., 2012) and an additional study (Zhao et al., 2010) evaluated C57Bl/6 PPAR-alpha null mice. Data on serum PFOA levels from all three of these studies are presented in Zhao et al. (2012). Interpretation of the combined results of these three studies is problematic because each PFOA dose level was used in each strain in only one of the three studies, and any dose-response interpretations must be made based on combining data from different studies. Because conditions (e.g. animals, housing conditions, time) may vary during different studies, dose-response curves based on combining data from different studies are difficult to interpret and conclusions based on such dose-response curves are highly uncertain.

Yang et al. (2009a) reported stimulation of mammary gland development in C57Bl/6 mice at 1 and 5 mg/kg/day PFOA, but complete inhibition at 10 mg/kg/day. In contrast, mammary gland development was inhibited at these three doses in a dose-related manner in Balb/c mice in this same study (Yang et al., 2009a).

A subsequent study (Zhao et al. 2012) attempted to further elucidate the dose response for mammary gland effects in these two strains by using a single dose in between those used in the first study in each strain (2.5 mg/kg/day in Balb/c; 7.5 mg/kg/day in C57Bl/6). Mammary gland development was inhibited at these doses in both strains. However, the doses used in the Yang et al. (2009) were not repeated in Zhao et al. (2012), and, importantly, the stimulatory effects reported at 1 and 5 mg/kg/day in C57Bl/6 mice by Yang et al. (2009a) were not replicated in Zhao et al. (2012).

It is important to note that the stimulation of mammary gland development in C57Bl/6 mice exposed to 5 mg/kg/day by Yang et al. (2009a) was not replicated in a second study. Additionally, this observation does not contradict the findings of delayed mammary development in this strain after gestational exposure (Tucker et al., 2015), since effects on mammary gland development may differ depending on life stage of exposure.

In C57Bl/6 PPAR-alpha null mice, mammary gland development was not affected by 7.5 mg/kg/day PFOA (Zhao et al., 2012), and was reported to be stimulated at 5 mg/kg/day, although quantitative data are not shown by Zhao et al. (2010). As was the case for the studies of C57Bl/6 wild type mice and Balb/C mice discussed above, each dose was used in PPAR-alpha null mice in only one of the studies (Zhao et al., 2010; Zhao et al., 2012). The effects on mammary gland development in the PPAR-alpha null mice therefore were similar to those in wild type mice of

the same strain at 5 mg/kg/day and differed at 7.5 mg/kg/day. As above, interpretation of these data is problematic because only one dose was used in each study.

Effects of pre-pubertal PFOA exposure on reproductive organs in female mice

Two studies evaluated effects of pre-pubertal exposure to PFOA on reproductive organs in female mice. Groups of female CD-1 mouse pups (8 per group) to 0.01, 0.1, or 1 mg/kg/day were exposed to PFOA by gavage for three days starting on PND 18 (Dixon et al., 2012) and sacrificed one day after the last dose. Uterine weight (absolute and relative) was significantly increased (about 1.5-fold) only at 0.01 mg/kg/day, the lowest dose, while body weight was not affected by any dose of PFOA. As expected in this model, administration of 17-beta-estradiol greatly increased uterine weight by about 10-fold, and PFOA had no effect on uterine weight in the estradiol treated animals. In mice treated with PFOA alone, histopathological changes in the uteri in some, but not all, sections included minimal to mild endometrial and myometrial edema, and hyperplasia of the mucosal and endometrial glandular epithelia and smooth muscle layers. There was also focal minimal stromal edema of the cervix and focal areas of mucification of the vagina in some, but not all, sections from PFOA treated mice. The severity scores for these changes were statistically significant ($p<0.05$) only in the low dose (0.01 mg/kg/day) and may have contributed to the increased uterine weight in this dose group, indicating a non-monotonic dose response curve for this effect. In reproductive organs from mice treated with estradiol, histological changes that are known to result from estradiol were observed and these changes were more severe than those seen in PFOA treated mice. The authors note that PFOA did not appear to have anti-estrogenic effects in this study, since it did not decrease the uterine weight gain induced by estradiol, and that more research is needed regarding the mechanism for the observed histopathologic effects of PFOA.

In a second study of similar design, groups of 15 female CD-1 mouse pups were dosed with 0.005, 0.01, 0.02, 0.05, 0.1, or 1 mg/kg/day PFOA for 3 days beginning on GD-18 (Yao et al., 2014). Another group of 15 female mouse pups was dosed with 17-beta-estradiol (0.5 mg/kg/day) for the same 3-day time period. Mice were sacrificed one day after the last dose. In this study, PFOA did not affect relative uterine weight at any dose, while estradiol caused the expected increase in relative uterine weight. Body weight was not affected by PFOA or estradiol treatment. In histological examination of the uterus, cervix, and vagina from 5 mice from each treatment group, there were no differences in types or severity of observations between controls and PFOA treated groups, while estradiol treatment produced the expected histopathological changes. In uterine tissue from the other 10 mice per treatment group, expression of estrogen receptor target genes in the uterus was not changed in PFOA-treated mice. Additionally, the human estrogen receptor was not activated by PFOA *in vitro* studies. Based on these results, the authors concluded that PFOA does not activate the mouse or human estrogen receptor.

Reproductive effects in male mice

Adverse reproductive effects were observed in four studies of male mice.

Li et al. (2011) studied the effects of 6 weeks of oral exposure to 0, 1, and 5 mg/kg/day APFO in wild type, PPAR-null, and humanized PPAR-alpha 129/sv mice. Serum testosterone was significantly decreased and the percentage of abnormal sperm was significantly increased in a dose-dependent manner in wild type and humanized PPAR-alpha mice, but not in PPAR-alpha null mice. Histopathological examination of the testis found increased vacuolated cells in the seminiferous tubules of wild type and humanized PPAR-alpha at both doses, while no obvious effects occurred in the PPAR-alpha null mice.

Male Balb/C mice (10 per group) were dosed with PFOA by gavage for 28 days with 0, 0.31, 1.25, 5, and 20 mg/kg/day (Zhang et al., 2014). Sperm parameters were evaluated in five mice from the control and 5 mg/kg/day groups, histopathological studies were performed on testes from three mice from each dose group, and testes from the remaining animals were used in mode of action studies not described here. Relative testes weight was not affected by PFOA treatment, and absolute testes weight was decreased only at the highest dose (20 mg/kg/day). Sperm numbers, sperm motility, and sperm progression were significantly decreased, and percent teratosperm was markedly and significantly increased, by treatment with 5 mg/kg/day PFOA. Histopathological examination found no differences in testes in the two lower dose groups (0.31 and 1.25 mg/kg/day) as compared to controls, while the seminiferous tubules in the testes of the two higher dose groups (5 and 20 mg/kg/day) were severely damaged.

In a second study from this research group, fertility of male Balb/C mice (6-8 weeks old) was significantly reduced after 28 days of gavage dosing with 5 mg/kg/day PFOA (Lu et al., 2015). The number of mated females and the number of pregnant females per male mouse were significantly decreased by treatment of the males with PFOA. Further studies found that the blood-testis barrier was disrupted after 28 days of exposure to 1.25 or 5 mg/kg/day.

Finally, Liu et al. (2015), reported testicular toxicity in Kunming mice dosed with 2.5, 5, or 10 mg/kg/day PFOA for 14 days. Testicular damage including atrophy of seminiferous tubules, disrupted arrangement of spermatogenic cells, depletion of spermatagonial cells, detachment of germ cells from seminiferous epithelium, and decreased sperm production occurred in all treated groups, with severity increasing with dose. Epididymal sperm count was decreased in a dose-related fashion, with no NOAEL identified. Other parameters evaluated in this study are discussed in the <u>Mode of Action</u> section.

These adverse reproductive effects in male mice are in contrast to the results of the two generation study in rats (Butenhoff et al., 2004b, see below). In this study, mating, fertility, and sperm parameters in F0 and F1 male rats were unaffected by PFOA at up to 30 mg/kg/day. Additionally, Cui et al. (2009) did not observe distinct histopathological changes in the testes of male Sprague-Dawley rats treated with 5 or 20 mg/kg/day of PFOA for 28 days and found that testes weight relative to body weight was significantly increased at both doses. As discussed below, PFOA increased the incidence of testicular Leydig cell tumors in two chronic studies of male rats (Biegel et al., 2001; Butenhoff et al., 2012). In the chronic study of male rats dosed

with 13.6 mg/kg/day PFOA in the diet for 24 months (Biegel et al., 2001), absolute testes weight was significantly increased by PFOA at 24 months but not at 21 months or earlier time points.

Reproductive and developmental studies in rats and rabbits

As discussed above, the rat and the rabbit are not the most appropriate animal models for human reproductive and developmental effects of PFOA because of their rapid excretion of this chemical in female rats and both male and female rabbits. Studies of reproductive and developmental effects in these species are summarized below.

Rabbit developmental study

Gortner (1982, cited in USEPA, 2005a) studied developmental effects in New Zealand white rabbits (18/dose group) given 0, 1.5, 5, or 50 mg/kg/day PFOA on gestation days 6-18. Maternal body weight was decreased in treated groups on GD 6-9 but returned to control levels on GD 12-29. Parameters such as number of resorptions and implantations, and fetal viability, sex ratio, and weight, were not affected by treatment. A dose related increase in incidence of the skeletal variation, extra ribs or 13th rib, was observed, with incidence of 16, 20, 30, and 38% in the 0, 1.5, 5, and 50 mg/kg/groups. This increase was statistically significant in the highest dose group.

Rat studies

Rat developmental study

Gortner (1981, cited in USEPA, 2005a) gave Sprague-Dawley rats (22/dose group) 0, 0.05, 1.5, 5, and 150 mg/kg/day APFO on gestation days 6-15. The maternal NOAEL was 5 mg/kg/day and the LOAEL was 150 mg/kg/day, based on decreased body weight, ataxia, and mortality seen only in the high dose group. Parameters including number of resorptions and implantations, or fetal viability, sex ratio of offspring, and pup weight, were not affected by treatment. A significantly increased incidence of missing sternebrae occurred in the high dose group. Since this effect was also seen in the control and lower dose groups at lower frequency, the study authors did not believe that it was treatment related.

Two-generation rat reproductive study

A two-generation reproductive study in Sprague-Dawley rats using gavage doses of 0, 1, 3, 10, and 30 mg/kg/day APFO was conducted by York (2002) and was also reported by Butenhoff et al. (2004b). Various parameters related to reproduction and development, as well as general toxicology endpoints, were evaluated in each generation (F0, F1, F2). Because of the design of the study, some observed effects may have been due to developmental/reproductive toxicity and others due to adult toxicity (USEPA, 2005a).

The parental (F0) generation (30 per sex per group) were dosed for at least 70 days prior to cohabitation beginning at age 6 weeks and until after mating in males and through pregnancy and lactation until weaning of pups for females. Males of the F0 generation were sacrificed after mating, and females were sacrificed at weaning of offspring on PND 22.

Mean serum levels on the day of sacrifice in the F0 males in the control, 10, and 30 mg/kg/day groups were 34 ng/ml, 51,500 ng/ml and 45,300 ng/ml, with no increase in serum levels with increasing dose. In females, serum levels were much lower, < 5 ng/ml, 370 ng/ml, and 1020 ng/ml in the control, 10 and 30 mg/kg/day groups. Serum levels were not measured in the 1 and 3 mg/kg/day groups.

In the F0 males, there were no effects on mating, fertility, or sperm parameters. There were dose-related statistically significant decreases in body weight and body weight gain at 3, 10, and 30 mg/kg/day of 6, 11, and 25%, respectively, although relative food consumption was increased in these groups. Liver weights (absolute, relative to body weight, and relative to brain weight) were increased significantly in all dose groups (1 mg/kg/day and above). Kidney weights were also increased relative to brain and liver weights in all dose groups. Livers and kidneys were not examined histologically. Reproductive and endocrine organs were examined histologically, and hypertrophy and/or vacuolation of the zona glomerulosa of the adrenal glands were seen in 20% of the 10 mg/kg/day group and 70% of the 30 mg/kg/day group.

In F0 females, there were no effects on estrous cyclicity, mating, fertility, pup sex ratio, pup viability, pup birth weight, or other related parameters. Relative kidney weights were reduced at 30 mg/kg/day, and relative liver weight was reduced at 3 and 10 mg/kg/day. This decrease in relative liver weight is in contrast to findings in many other studies in which increased liver weight increases occurred. Histological examination was not performed on the kidneys or livers. Lau et al. (2006) concluded that the profile of maternal effects in the rat study differed from those seen in similar studies in mice because of the more rapid excretion in the adult female rat compared to the adult female mouse.

Dosing of F1 offspring with the same dose levels that their parents had received began at weaning (PND 22). Most of the F1 animals were sacrificed after sexual maturation (about 5 weeks in females and 7 weeks in males). From the F1 generation, 30 pairs per dose group were selected to be bred to produce the F2 generation. These F1 males were sacrificed at about 133 days of age, after 113 days of treatment, and F1 females were sacrificed at weaning of the pups, at 13 to 15 weeks of age.

The weight of the F1 pups (males and females combined) was reduced compared to controls through lactation on a per litter basis in the 30 mg/kg/day group. During the post-weaning period, signs of toxicity in the F1 males included increased incidence of annular constriction of the tail at all doses (significant at 1, 10, and 30 mg/kg/day), significant increases in the number of emaciated pups at 10 and 30 mg/kg/day, and significantly increased urine-stained fur, decreased motor activity, and abdominal distention at 30 mg/kg/day. Deaths were significantly increased in the 30 mg/kg/day males during the post-weaning period. Sexual maturation, as indicated by day of preputial separation, was significantly delayed in the high dose group (52.2 days) compared to controls (48.5 days).

No effects were observed on mating, fertility, or sperm parameters in the F1 males. There was a significant dose-related reduction in body weight gain at all doses (1 mg/kg/day and above) during the post-weaning period, although relative food consumption was significantly increased. At sacrifice (day 113 of dosing), body weights were reduced by 6, 6, 11, and 22% in the 1, 3, 10, and 30 mg/kg/day groups compared to controls. Absolute and relative liver weights were increased at all doses, and hepatocellular hypertrophy and necrosis were seen in livers of some animals in the 3, 10, and 30 mg/kg/day groups. Kidney weight relative to body and brain weight was increased in all treated groups, but histopathological examination was not performed. Hypertrophy and vacuolation of the adrenal cortex was seen in 70% of high dose animals, but not in other groups.

In F1 females, there was a significant increase (6/60) in mortality in the high dose group 2-8 days post-weaning. Sexual maturation, as indicated by day of vaginal opening, was significantly delayed in the 30 mg/kg/day group, from 34.9 days in controls to 36.6 days in treated females. Significant reductions in body weight were seen at several time points during post-weaning, gestation, and lactation, although relative food intake was not decreased and body weight was not decreased at terminal sacrifice. No effects were seen in F1 females on mating or fertility parameters, or number of implantations, number of stillborn pups, or length of gestation.

The F2 pups were followed until weaning at PND 22. In the F2 generation, no treatment-related effects were seen on pup viability until weaning, percentage of male pups, litter size, average pup body weight on days 1, 5, 8, 15, or 22, or anogenital distance. Because these pups were sacrificed at weaning, post-weaning effects were not assessed.

Several LOAELs and NOAELs were identified in this study for males and females at different life stages, and the serum PFOA levels at these NOAELs and LOAELs were modeled (USEPA, 2005a). The LOAEL for adult males in this study was 1 mg/kg/day (modeled serum concentration – 42,000 ng/ml) with no NOAEL identified, based on increased liver weight in the F0 and F1 generations and decreased body weight in the F1 generation. For pregnant females, the LOAEL was 10 mg/kg/day, based on decreased body weight in F1 pups during post-weaning, and the NOAEL was 3 mg/kg/day (modeled serum concentration – 3500 ng/ml). For the male rat pups during post-weaning, the LOAEL was 10 mg/kg/day and the NOAEL was 3 mg/kg/day (modeled serum concentration - 8400 ng/ml at week 4), based on decreased body weight in the F1 generation. For the female rat pups during post-weaning, the LOAEL was 30 mg/kg/day and the NOAEL was 10 mg/kg/day (modeled serum concentration -13,000 ng/ml) at week 7, based on decreased body weight in the F1 generation.

Thyroid effects

Effects of PFOA on the thyroid in animal toxicology studies are of interest because there have been many human epidemiological studies of PFOA and thyroid hormones and/or thyroid disease. However, only a few toxicology studies have evaluated effects of PFOA on thyroid function.

In the 4-week study of male cynomolgus monkeys (Thomford et al., 2001a), there were no effects on thyroid hormones (thyroid stimulating hormone, TSH; total and free triiodothyronine, T3; total and free thyroxin, T4) after 29 days of exposure to 0, 2, or 20 mg/kg/day APFO. It should be noted that the number of animals per dose group in this study was very small (controls, n=2; treated, n=3).

Changes in thyroid hormones were observed in PFOA-treated male cynomolgus monkeys in the 6-month study (Butenhoff et al., 2002). This study is described in detail above. At the beginning of the study, there were 6 monkeys in the control and high dose group, and 4 in the other two groups. Some animals in the treated groups did not complete the study because of mortality or overt toxicity (3 mg/kg/day, n=3; 30/20 mg/kg/day, n=2 at end of study.) Thyroid hormones (total and free triiodothyronine, T3; total and free thyroxin, T4) and TSH (thyroid stimulating hormone) were measured before dosing, and after 5, 10, 14, and 27 weeks of dosing with 0, 3, 10, 30/20 mg/kg/day APFO. Interpretation of the data is complicated by the fact that comparisons for these parameters are made both to the pretreatment values for the same animals and to the control animals at the concurrent time point. Statistically significant changes were reported for all hormones at one or more time points in one or more dosed groups.

TSH was significantly increased at week 35 compared to pretreatment values at 3 and 10 mg/kg/day. Total T4 was significantly decreased compared to concurrent controls in all dosed groups (3, 10, and 30/20 mg/kg/day) at week 35, at all time points during treatment (weeks 5 to 35) at 10 mg/kg/day, and at week 10 and week 35 at 30/20 mg/kg/day. Free T4 was significantly lower than in concurrent controls at 10 mg/kg/day at weeks 5, 10, and 27, and at 30/20 mg/kg/day at weeks 5 and 27. Total T3 was significantly increased compared to pretreatment values at two time points (weeks 10 and 14) at 3 mg/kg/day, and was decreased compared to concurrent controls in all dosed groups at 30/20 mg/kg/day; this change was not significant at 10 weeks. Finally, free T3 was significantly decreased compared to concurrent controls in the 30/20 mg/kg/day group at weeks 5, 10, and 35.

The authors reported that all thyroid hormone values were within normal range, and that no relevant histological observations were observed. They also state that there were no relevant changes in TSH or T4, despite the changes in levels of these hormones noted above. It is stated, in the 3 high dose monkeys that were removed from the study due to toxicity, the decreased T3 observed during treatment trended upward after treatment ceased. The authors state that the observed changes in thyroid hormone levels were likely due to stress or normal variation rather than a direct effect of APFO (PFOA).

Thyroid hormones (total T4, free T4, and total T3) were measured in male Sprague-Dawley rats one day after dosing for 1, 3, or 5 days with a high dose (20 mg/kg/day) of PFOA (Martin et al., 2007). Levels of free and total T4 in PFOA treated animals were significantly reduced to several fold below control levels at all three time points. Total T3 was also significantly decreased at all

three time points, although the magnitude of the decreases was smaller than for T4. The effects were similar or greater than those caused by PFOS at 10 mg/kg/day in the same study. Unfortunately, thyroid hormones have not been evaluated in longer term, lower dose rodent studies of PFOA.

Neurobehavioral and Central Nervous System Effects

Several studies have found neurobehavioral effects, particularly increased activity, in rodents exposed to low doses of PFOA during development. Although human studies on these effects are not reviewed in this document, toxicological studies of neurobehavioral effects are of interest because some human studies have reported associations of PFOA and other PFCs with behavioral effects, particularly attention deficit hyperactivity disorder, in children (Hoffman et al., 2010; Ode et al., 2014).

Johannson et al. (2008) found significant behavioral effects in adult male NMRI mice given a single gavage dose of 0.58 or 8.7 mg/kg PFOA at 10 days of age. Behavioral tests were conducted at 2 and 4 months of age. These behavioral tests included spontaneous behavior (locomotion, rearing, total activity) over three consecutive 20-minute time periods (an indicator of habituation over time), nicotine-induced motor activity (locomotion, rearing, total activity), and behavior in an elevated plus-maze (a measure of anxiety). These single doses of PFOA did not affect weight gain or cause any overt signs of toxicity. At 2 and 4 months, some or all measures of spontaneous behavior were affected in both the low and high dose groups, with greater effects in the high dose group (8.7 mg/kg) and at the later time point (4 months). Habituation was greatly decreased at both 2 and 4 months in the high dose group compared to controls. This effect increased with age of the treated mice. Lack of effect in the elevated plus-maze test indicated that these effects were not likely to be caused by anxiety.

Responses over three 20 minute periods following an injection of 80 μg/kg nicotine, a measure of the susceptibility of the cholinergic system, was also significantly altered in high and low dose mice at 4 months. Control mice showed an increase in activity from the nicotine, followed by a decrease to baseline behavior. Low dose mice also showed increased activity after receiving nicotine, but less so than controls, and this increased activity was followed by greater activity than in controls during the last 20-minute time period. In contrast, high dose mice showed decreased activity during the first 20 minutes after receiving nicotine but were hyperactive during the later time period. The authors concluded that neonatal PFOA exposure caused deranged spontaneous behavior such as lack of habituation and hyperactivity that worsened with age in adult mice, and that PFOA exposure also affected the cholinergic system.

Significant effects were seen at both doses in this study. Therefore, the LOAEL was 0.58 mg/kg, and no NOAEL was identified. Serum levels were not measured in this study and have not been measured in neonatal mice administered a single dose of PFOA. A single oral dose of 1 or 10 mg/kg to adult CD-1 mice resulted in maximum serum concentrations of about 10,000 and 100,000 ng/ml (Lou et al., 2009). From these data, the serum concentrations from 0.58 and 8.7

mg/kg can be estimated as 5800 and 87,000 ng/ml, assuming that kinetics in these neonatal mice are similar to in adult mice.

The authors stated that the effects caused by PFOA in this study are similar to those seen with PCBs and PBDEs, known developmental neurotoxicants, and that PFOA should be classified as a developmental neurotoxicant along with these other persistent chemicals. It is noted that studies from this laboratory using the same dosing protocol (a single dose given to 10 day old mice) and the same behavioral tests are the basis for the chronic USEPA IRIS Reference Doses developed in 2008 for two PBDEs, 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) and 2,2',4,4',5-pentabromodiphenyl ether (BDE-99) (USEPA 2008b,c). Because the effects persisted into adulthood and were permanent, these effects were regarded by USEPA IRIS as chronic, not acute, even though only a single dose was given.

A second study in this laboratory (Johansson et al., 2009*)* examined the effects of a single gavage dose of 8.7 mg/kg PFOA to 10 day old mice on proteins important for neuronal growth and synaptogenesis in the developing brain. The mice were sacrificed 24 hours after dosing. Rapid brain development, the "brain growth spurt," occurs in mice during this time period. Levels of two proteins known to be involved with neuronal survival, growth, synaptogenesis, and other aspects of neuronal development were significantly increased in the hippocampus but not the cerebral cortex of treated mice, and two other proteins were increased in both areas of the brain. PBDEs, which are known to affect behavior after developmental exposures, had previously been found by these researchers to cause similar effects. The authors conclude that these changes may relate to some of the observations in their earlier behavioral study.

Onishchenko et al. (2010) found sex-related behavioral changes in offspring of C57Bl/6 mice exposed to 0.3 mg/kg/day PFOA throughout gestation. Liver weights were significantly increased in treated pups that were sacrificed at birth. PFOA exposure did not affect locomotor behavior in 5-8 week old mice tested individually, but significant sex-related effects were seen on circadian activity when these mice were housed in social groups. Initially, PFOA treated males were more active than controls, while treated females were less active than controls. After habituation, activity in both light and dark phases was increased during the remainder of the first 24 hours in exposed males, while no effects were seen in exposed females. During the second 24-hour period when the mice were more adapted to the test environment, the number of inactive periods during the light phase was decreased in both male and female PFOA-treated mice, and also in the dark phase in PFOA-treated males. No effects of PFOA were seen in other tests, including the elevated plus-maze test for anxiety-like behavior, the forced swimming test for depression-like behavior, or muscle strength in the hanging wire test. Serum PFOA levels in this study were not measured, but they are not expected to be below the serum levels at the LOAELs and NOAELs in some other studies which evaluated other effects. The NOAEL for these effects is not known.

Finally, Sobolewski et al. (2014) studied behavioral effects of developmental exposures to PFOA in C57Bl/6 mice. Three other unrelated environmental contaminants and a mixture of all four chemicals were also evaluated; only results for PFOA alone are reported here. PFOA (0.1 mg/kg/day) was administered to dams in puffed wheat cereal on GD7 through weaning. Behavioral effects were evaluated in male and female offspring (one or two per sex per litter) beginning at age 60 days.

PFOA treatment caused behavioral effects, which differed between males and females. Locomotor behavior was increased in males treated with PFOA, while no effect was seen in females. Horizontal movement and ambulatory movements were increased and resting time was decreased during one or more of three test sessions, while vertical activity was unaffected in males. Behaviors related to novel object exploration and recognition were altered in both PFOA-treated males and females to a highly significant degree, although the specific parameters affected differed between sexes. Fixed interval reinforcement schedule-controlled behavior was not affected by PFOA treatment in males or females.

Chronic toxicity and carcinogenicity

As discussed in the Epidemiology section, PFOA has been associated with increased incidence of kidney and testicular cancer in humans exposed through drinking water, after adjustment for smoking and other relevant factors. The chronic toxicity and carcinogenicity of PFOA has been evaluated in two dietary studies in rats. One study (Sibinski et al., 1987; Butenhoff et al., 2012) included male and female rats, while Biegel et al. (2001) studied only males. As such, chronic toxicity and carcinogencity have been studied only in the rat, a species in which PFOA is rapidly excreted by females. Chronic studies in another species in which PFOA is persistent in both sexes, such as the mouse, would provide important information specific to females. Furthermore, the chronic studies did not assess effects including carcinogenicity which might result from exposures during the critical developmental stages now known to be sensitive periods for PFOA toxicity.

Two year chronic/carcinogenicity study in male and female rats

Sprague-Dawley rats (30 per sex per group) were dosed with APFO through diets containing 0, 30, or 300 ppm APFO for two years (Sibinski, 1987; Butenhoff et al., 2012). Additional animals (15 per sex) were included in the control and high dose groups for evaluation at sacrifice after one year. From food consumption data, mean APFO doses were determined to be 1.3 and 14.2 mg/kg/day in males, and 1.6 and 16.1 mg/kg/day in females, for the 30 and 300 ppm groups, respectively.

Body weight gains were decreased in both sexes in the high dose group, with maximum differences between the high dose group and controls of 21% in males at 6 weeks and 11% in females at 92 weeks. Smaller decreases were seen in low dose males, and decreases were not consistently seen in low dose females. Decreases in weight gain were treatment-related, since food consumption on a body weight basis was increased in treated males, by about 13% in the

high dose group. Mortality was not increased by APFO treatment, and 2-year survival rates were 70%, 72% and 88% in males and 50%, 48% and 58% in females in the 0, 30 ppm, and 300 ppm groups, respectively.

The incidence of ataxia was increased in female rats treated with PFOA and occurred most frequently in moribund animals. Ataxia occurred in 3%, 18% and 23% of rats in the control, low- and high-dose groups (including animals sacrificed at one year), respectively.

Clinical chemistry changes occurred in high and low dose males, but not in females. The liver enzymes alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase were increased in both low dose and high dose males between 2 and 18 months, but only in the high dose group at 24 months. Albumin was significantly increased in the male low dose group at 3 and 6 months, and until 24 months in the high dose group. Butenhoff et al. (2012) concluded that the elevations in liver enzymes in both high and low dose males may represent "borderline chronic liver toxicity".

Hematological parameters including red blood cell counts, hemoglobin concentrations and hematocrit were significantly decreased in high and low dose males and females at different time points. In high dose males, these erythrocyte-related hematological parameters were decreased from 3 to 18 months. Additionally, leukocytes were increased in both dose groups of males during the first year. Statistically significant changes included increases in lymphocytes and neutrophils at 3 and 12 months in both dose groups, and in lymphocytes and 6 and 18 months in the low dose group.

In females, hematological parameters were significantly changed with equal frequency in the high and low dose groups. At 3 months, red blood cells were decreased in the low dose group and hematocrit was decreased in the high dose group. At 6 months, erythrocytes, hemoglobin, and hematocrit were decreased only in the low dose group, while at 12 months, these three parameters were decreased only in the high dose group.

At the one-year interim sacrifice, statistically significant changes in organ weights in high dose males included increased liver weight and decreased pituitary weight (absolute and relative to body or brain weight), increased kidney weight (only relative to body weight), decreased adrenal and heart weight (absolute and relative to brain weight). No organ weight changes were observed in high dose females sacrificed at one year.

At the one-year interim sacrifice, histopathological changes in the livers of high dose males included diffuse hepatocellular hypertrophy in 12/15 (compared to 0/15 controls); portal mononuclear cell infiltration in 13/15 (compared to 7/15 controls); and focal hepatocellular necrosis in 6/15 (compared to 0/15 controls). In high dose females, hepatocellular vacuolation occurred in 11/15 (compared to 5/15 controls). Testicular tubular atrophy and marked aspermatogenesis was found in 2/15 high dose males but in none of the controls.

At the two-year sacrifice, slight non-significant increases in liver weight occurred in all treated groups of males and females. Relative kidney weight was slightly increased in male and female high dose rats, and this change was significant in females.

Several non-neoplastic liver lesions were also increased in treated animals at two years. Hepatocellular hypertrophy did not occur in controls but was found in 12% and 80% of the males, and 2% and 16% of the females, in the low and high dose groups, respectively. Hepatic cystoid degeneration occurred in 8%, 14% and 56% of the control, low, and high-dose males. However, hepatocellular necrosis or hepatocellular vacuolation was not increased in treated animals compared to controls, although an increase of focal hepatocellular necrosis in high dose males and hepatocellular vacuolation in high dose females was observed at the one-year sacrifice.

Hepatocellular carcinomas were found in the control (6%), low dose (10%), and high dose (2%) males, and occurred in females only in the high dose group (2%). The incidence of hepatic hyperplastic nodules was 6% in high-dose males, and they were not found in the other groups of males. In females, these nodules were found in 2% of the control and high dose animals, but not in the low dose group. Butenhoff et al. (2012) state that diagnostic criteria for these nodules, which represent a regenerative process, have changed since the histopathological evaluation for this study was performed. The USEPA SAB (2006) concluded that these nodules may have been part of the continuum of proliferative lesions in the liver carcinogenic process. Butenhoff et al. (2012) further conclude that the increased incidence of focal necrosis and vacuolation in treated animals, which was observed at the one year sacrifice but not at the two year sacrifice (because of the higher background incidence of these changes in older rats), suggests a progression of lesions "from hepatocellular hypertrophy to fatty degeneration to necrosis followed by regenerative hyperplasia."

The incidence of neoplastic lesions of the testes differed significantly between control and treated rats. Leydig cell adenomas of the testes were found in 0, 4, and 14% of the control, low, and high dose males, respectively, with a significantly increased incidence in the high dose group. Additionally, vascular mineralization of the testes occurred in 0, 6, and 18% of control, low, and high dose males, respectively, and reached statistical significance in the high-dose group.

The incidence of mammary gland fibroadenomas was 22%, 42%, and 48% in the control, low dose, and high dose females, and the increase in the high dose group was significant. Mammary gland adenocarcinomas were present in 15, 31, and 11% of the control, low, and high dose females, respectively. A subsequent Pathology Working Group reevaluation of the mammary gland slides concluded that the incidence of mammary gland proliferative lesions was not increased by PFOA in this study (Hardisty et al., 2010).

A statistically significant, dose-related increase in the incidence of ovarian tubular hyperplasia was found in female rats at the two-year sacrifice, with incidence of 0%, 14%, and 32% in the

control, low, and high dose groups. The authors of the study report stated that the biological significance of this effect was unknown, as there was no evidence of progression to tumors. Slides of the ovaries were later re-evaluated (Mann and Frame, 2004) using more recent pathology criteria. In the reevaluations, no statistically significant increases in hyperplasia (total number), adenomas, or hyperplasia/adenoma combined were seen in treated groups compared to controls. Although the size of stromal lesions was increased in the high dose group, the incidence of ovarian adenomas was higher in the controls than in the treated groups.

USEPA (2005a) concluded that "based on these toxic effects, the high dose selected in this study appears to have reached the Maximum Tolerated Dose. Based on a decrease in body weight gain, increase in liver and kidney weights and toxicity in the hematological and hepatic systems, the LOAEL for male rats is 300 ppm and the NOAEL is 30 ppm. The LOAEL for female rats is 300 ppm based on a decrease in body weight gain and hematologic effects and the NOAEL is 30 ppm."

However, as noted above, a significant increase in the incidence of ataxia, as well as hematological effects equivalent to those in the high dose group, occurred in low dose females in this study. Also, in males in this study, there were significant changes in several clinical chemistry parameters at multiple time points in the low dose group. Thus, statistically significant effects occurred at multiple time points in the low dose males and female in this study. It should be noted that the study authors concluded that the elevation of the three liver enzymes, alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase, in both dosed groups of males, in conjunction with liver weight changes and histopathology observations, suggested that PFOA affected hepatocytes in both the low dose and the high dose groups of male rats.

The serum level in the low dose (1.6 mg/kg/day) females, considered to be the NOAEL by USEPA (2005a), was estimated at 1800 ng/ml, based on average AUC (USEPA, 2005a; Post et al., 2009a). The serum levels in the low dose (1.3 mg/kg/day) males were much higher, and can be estimated at 55,000 ng/ml from the kinetic data presented by USEPA (2005a).

<u>Chronic mechanistic study in male rats</u>

The second chronic study (Biegel et al., 2001) was designed to investigate the mode of action for testicular tumors observed in male rats in the first chronic study (Sibinski et al.,1987; Butenhoff et al., 2012). Male Sprague-Dawley rats were given 300 ppm PFOA in the diet (mean dose of 13.6 mg/kg/day) for up to two years. Serum levels at this dose were estimated as 572,000 ng/ml (USEPA, 2005a). Two controls groups were used, an *ad libitum* fed (AL) group and a pair-fed (PF) group in which the food intake was controlled to match the food intake of the PFOA exposed group. Another group of rats was treated with Wyeth 14,643 (WY), a model peroxisome proliferator. There were 156 rats in each of the control and dosed groups.

At interim time points of 1, 3, 6, 9, 12, 15, 18, and 21 months, the liver and testes from six rats per treatment group were weighed and evaluated for cell proliferation. At each time point,

peroxisome proliferation (PCO) was evaluated in 6 additional rats per group, and serum hormone levels (estradiol, testosterone, LH, FSH, and prolactin) were measured in 10 rats from each group.

Liver-to-body weight ratios were increased in PFOA treated animals at all time points compared to controls. This increase was greatest at the earliest time point (1 month) and decreased over time. At 24 months, there was only a slight difference in liver-to-body weight ratio between the treated and control animals. Similarly, the increase in peroxisomal beta-oxidation (PCO), a measure of peroxisome proliferation, in the liver was greatest at 1 month, and the magnitude of the increase over controls decreased with time. WY also increased liver weight and peroxisomal beta-oxidation (PCO). Liver cell proliferation was increased by WY but not by PFOA.

In contrast to the liver, relative organ weight, PCO activity, and cell proliferation in testes were not affected in PFOA or WY treated rats except for increased relative testicular weight at 18 and 24 months in PFOA treated animals and at 24 months in WY treated animals. In the pancreas, PFOA increased acinar cell proliferation at 12, 16, 18, and 21 months, while WY did not have this effect.

Estradiol levels were significantly increased by PFOA at the 1, 3, 6, 9, and 12-month time points. WY also increased serum estradiol. Testosterone, prolactin, LH, and FSH were not changed compared to pair-fed controls.

In this study, there was a significant increase of three types of tumors (Leydig cell adenomas, hepatic adenomas, and pancreatic acinar cell adenomas) in the PFOA treated group at the 24-month sacrifice. The incidence of these tumors in the ad libitum control (AL), pair fed control (PF), and treated groups was: Leydig cell adenomas (AL – 0/80; PF – 2/78, treated – 8/76), hepatic adenomas (AL – 2/80, PF – 1/79, treated –10/76), and pancreatic acinar cell adenomas (AL – 0/80, PF- 1/79, treated – 7/76). These same three types of tumors also occurred at a higher rate in the WY treated animals. As discussed above, the incidence of testicular Leydig cell adenomas was also increased by PFOA in a dose-related fashion in the first chronic study (Sibinski et al., 1987; Butenhoff et al., 2012).

<u>Information relevant to carcinogenicity from developmental exposure</u>
Carcinogenicity studies using prenatal or perinatal exposure protocols have not been reported for PFOA. Very limited information is available that is relevant to carcinogenicity after developmental exposure to PFOA.

As discussed in the section on hepatic effects above, Filgo et al. (2015) evaluated hepatic histopathological effects, including tumor incidence, at age 18 months in female offspring of dams dosed on GD 1-17. The study evaluated three strains of mice - CD-1, 129/Sv wild type, and 129/Sv PPAR-alpha knockout. CD-1 dams were dosed with 0, 0.01, 0.1, 0.3, 1, and 5 mg/kg/day, and 129/Sv wild type and PPAR-alpha null dams dosed with 0, 0.1, 0.3, 1, and 3 mg/kg/day.

The authors emphasize that the study was not designed or intended to be a carcinogenicity bioassay. Rather, the incidence of liver tumors was evaluated by Filgo et al. (2015) because of the unexpected finding of liver tumors in some treated animals that died before the scheduled end of the study. In CD-1 mice in this study, single or multiple hepatocellular adenomas were found in one or more animals in each of the treated groups (n=21 to 37 per group) except for at the lowest dose (0.01 mg/kg/day), but not in controls (n=29). In total, adenomas occurred in 4.9% (7 of 144) treated animals, compared to a historic control incidence of 0.4% in untreated female CD-mice. Hepatocellular carcinomas occurred in two treated mice (0.3 and 5 mg/kg/day) but not in controls.

In 129/Sv mice, hepatocellular adenomas did not occur in control or treated groups (n=6 to 10 per group). In PPAR-alpha null mice of this strain, in contrast, there were no adenomas in the controls (n=6), one adenoma in the 0.1, 0.3, and 1 mg/kg/day groups (n=9 or 10), and two adenomas at 3 mg/kg/day (n=9). These tumors occurred in 13.2% of all treated PPAR-alpha null mice.

Possibly because of the small numbers of animals per dose-group, tumor incidences were not significantly increased in PFOA-treated groups compared to control in this study, with the exception of the CD-1 mice dosed with 0.3 mg/kg/day in which adenomas were found in 4 of 26 animals. The results of this study are suggestive of the potential for developmental exposures to PFOA to cause tumors in mice later in life, and also suggest that these tumors may occur through a PPAR-alpha independent pathway. An additional study with larger numbers of animals and designed to detect increased tumor incidence is needed to further evaluate these questions.

Ngo et al. (2014) evaluated the incidence of intestinal tumors and other endpoints in wild type and Min/+ C57Bl/6 mice offspring of dams dosed with 0.01, 0.1, and 3 mg/kg/day PFOA during gestation. The Min/+ strain has a mutation of a tumor suppressor related to intestinal adenomatous polyps and is a sensitive model for chemical-induced intestinal tumorigenesis. PFOA did not cause increased intestinal tumorigenesis at sacrifice at age 11 weeks in either wild type or Min/+ mice.

Discussion

Leydig cell testicular tumors were the only tumor type that were increased by PFOA in the chronic study of Butenhoff et al. (2012), and the incidence of these tumors was also increased by PFOA in Biegel et al. (2001). In Biegel et al. (2010), the incidence of hepatic and pancreatic tumors was also increased to a similar degree as testicular tumors in male rats treated with PFOA.

Although hepatic tumors were not significantly increased in treated animals in Butenhoff et al. (2012), both the USEPA SAB (2006) and Butenhoff et al. (2012) point out that the incidence of hepatic hyperplastic nodules was increased in the high dose males in comparison to the control and low dose groups in this study. Butenhoff et al. (2012) state that diagnostic criteria for these nodules, which represent a regenerative process, have changed since the histopathological

evaluation for this study was performed. The EPA SAB (2006) concluded that these nodules may have been part of the continuum of proliferative lesions in the liver carcinogenic process, and Butenhoff et al. (2012) further conclude that the increased incidence of focal necrosis and vacuolation found at the one year sacrifice but not at the two year sacrifice (because of the higher background incidence of these changes in older rats) suggests a progression of lesions "from hepatocellular hypertrophy to fatty degeneration to necrosis followed by regenerative hyperplasia."

In regard to pancreatic tumors, Frame and McConnell (2003) reevaluated the slides of the pancreas from both studies and found that different diagnostic criteria and nomenclature were used by the pathologists in Sibinski et al. (1987) and Biegel et al. (2001). Frame and McConnell (2003) concluded that the incidence of pancreatic focal acinar lesions, which can progress to adenomas, was increased by 300 ppm PFOA in the diet in both studies and that these lesions were larger, more frequent, and had a greater tendency to progress to adenomas in Biegel et al. (2001).

PFOA has been shown to promote liver carcinogenesis in rats initiated with diethylnitrosamine as well as with a more complex initiating protocol (Abdellatif et al.,1991; Nilsson et al., 1991). PFOA also promoted hepatocarcinogenicity in rainbow trout, a model species for human liver cancer (discussed in Mode of Action section, below).

An increased incidence of liver tumors from prenatal exposure to PFOA in CD-1 and PPAR-alpha null mice is suggested by the results of Filgo et al. (2015). However, this study is not definitive, and additional research on carcinogenicity later in life after developmental exposures to PFOA is needed.

Information related to the mode of action for carcinogenicity is discussed in the Mode of Action section below.

Summary of Conclusions of Toxicology Studies

The Subcommittee's review of the toxicology data identified increased relative liver weight and delayed mammary gland development from developmental (perinatal) exposure as the most sensitive systemic toxicological endpoints with data appropriate for dose-response modeling. Delayed mammary gland development in mice is the most sensitive systemic endpoint with data appropriate for dose-response modeling. Increased liver weight is a more sensitive endpoint than most other systemic effects, and it co-occurs with and/or progresses to more severe hepatic effects including increased serum liver enzymes, hepatocellular necrosis, fatty liver, and/or hyperplastic nodules. PFOA also causes other types of toxicity including reproductive effects in both males and females, delayed growth and development, immune system toxicity, and neurobehavioral effects. PFOA caused tumors in two chronic rat studies, and one of these studies provides data for testicular tumors that is appropriate for dose-response modeling. All of these toxicological effects are considered relevant to humans for the purposes of risk assessment, as discussed in the Mode of Action section.

Table 10. Summary of Increased Relative Liver Weight Data from Rodents Studies Using Doses ≤ 1 mg/kg/day, and 90 Day Non-Human Primate Study

TABLE 10A: STUDIES PROVIDING SERUM PFOA DATA AT END OF DOSING PERIOD*

Citation	***Species***	***Life-stage***	***Doses*** *(mg/kg/day)*	***Dura-tion***	***NOAEL*** *(mg/kg/day)*	***Serum NOAEL*** *(ng/ml)*	***LOAEL*** *(mg/kg/day)*	***Serum LOAEL*** *(ng/ml)*	***Comments***
Lau et al. (2006)	Female mouse	Preg-nant GD 18	0, 1,3, 5, 10, 20, 40	GD 1-18	----	----	1	22,00	• Data for absolute liver weight are presented in bar graph in publication. Numerical data were obtained from the investigator. • Data on relative liver weight are based on comparisons with body weight after gravid uterus was removed. These data are not shown in the publication and were obtained from the investigator.
Loveless et al. (2006)	Male rat	Adult	0, 0.3, 1, 3, 10, 30	2 weeks	Linear/ Branched 0.3	19,000	1	51,000	• Three formulations of PFOA of differing isomer compositions were tested. • This study also provides data on peroxisomal beta-oxidation. In mice, increased relative liver weight does not correlate with increased peroxisomal beta oxidation among formulations with differing isomer compositions, indicating PPAR-alpha independent effects on liver weight.
					Linear 0.3	20,000	1	65,000	
					Branched 0.3	16,000	1	48,000	
	Male mouse	Adult	0, 0.3, 1, 3, 10, 30	2 weeks	Linear/ Branched 0.3	10,000	1	27,000	
					Linear ---	---	0.3	13,000	
					Branched ---	---	0.3	14,000	
Macon et al. (2011)	Female mouse	Female pup PND 1	0, 0.01, 0.1, 1	GD 10-17	0.1	2300	1	16,000	• LOAEL for increased relative liver weight is 100 fold higher than LOAEL for delayed mammary gland development. • PFOA serum levels were measured on PND 1. • With longer GD 1-17 exposure, LOAEL for increased relative liver weight was 0.3 mg/kg/day in male and female pups on PND 7. PFOA serum levels were not measured in this component of the study.
Perkins et al. (2004)	Male rat	Adult	0, 1,10, 30, 100 ppm in diet	4, 7, 13 weeks	0.07 (1 ppm) at 4 weeks	6500	0.71 (10 ppm) at 4 weeks	55,000	Steady-state serum levels were reached by 4 weeks. Dose-related increases in relative liver weight were similar at 4, 7, and 13 wks (see individual study table in Appendix 5).
Thomford et al. (2001); Butenhoff et al. (2002)	Male cynomol-gus monkey	Adult	0, 3, 10, 20/30	26 weeks	----	---	3	72,000	• PFOA serum levels did not significantly differ at 3 and 10 mg/kg/day. • NOAEL was not identified.

*LOAELs are based on statistical significance at $p<0.05$. Effects that were not statistically significant occurred at doses below the NOAELs in some studies.

TABLE 10B: STUDIES NOT PROVIDING SERUM PFOA DATA AT END OF DOSING PERIOD*									
Citation	***Species***	***Life-stage***	***Doses*** *(mg/kg/day)*	***Duration***	***NOAEL*** *(mg/kg/day)*	***Serum NOAEL*** *(µg/L)*	***LOAEL*** *(mg/kg/day)*	***Serum NOAEL*** *(µg/L)*	***Comments***
Abbott et al. (2007)	Mouse (PND 21)	WT** dam	0, 0.1, 0.3, 0.6, 1.0	GD 1-17	0.3	NA	1	NA	• Serum data are not presented in this table because they are from PND 21 and do not reflect maximum exposure at end of dosing period. • Liver weight was assessed in offspring on PND 21 and later time points. No data are available from earlier time points closer to the end of dosing.
		WT pup			--		0.1		
		PPAR-α null dam	0, 0.1, 0.3, 1.0, 3.0		1		3		
		PPAR-α null pup			1		3		
DeWitt et al. (2008)	Female mouse	Adult	0, 0.94, 1.88, 3.75, 7.5	15 days	----	NA	0.94	NA	Serum PFOA data are not available for the two lowest doses in this study.
Loveless et al. (2008)	Male mouse	Adult	0, 0.3, 1, 10, 30	29 days	0.3	NA	1	NA	• Linear PFOA was used in this study. • Relative liver weight increased in a dose-related manner at all doses below the LOAELs, but changes were not statistically significant.
	Male rat	Adult			1		10	NA	
Quist et al. (2015)	Female mouse (PND 21)	Pup	0, 0.01, 0.1, 0.3, 1 (On control or high fat diet from PND 35-77)	GD 1-17	0.1 (Note: No NOAEL was identified for other hepatic effects including chronic active inflammation on PND 21 and hepatocellular hypertrophy on PND 91)	NA	0.3 (Note: LOAEL was 0.01 mg/kg/day for chronic active inflammation on PND 21 and hepatocellular hypertrophy on PND 91)	NA	• Relative liver weight was increased on PND 21, but not on PND 91. • Frequency and severity of hepatocellular hypertrophy increased on PND 91 in all dosed groups, in the absence of increased liver weight. Statistical analysis was not presented. • Increased severity of chronic active hepatic periportal inflammation on PND 21 was significantly increased in all dosed groups. This effect occurred at doses below those that caused increased liver weight on PND 21. • Increased severity of periportal inflammation also occurred on PND 91, but was less severe than on PND 21. • Hepatic mitochondrial abnormalities occurred at 1 mg/kg/day on PND 21 and PND 91; they were more severe on PND 91. This effect was not evaluated at other doses. • Hepatic peroxisome proliferation was not observed at 1 mg/kg/day on PND 91; other doses were not evaluated.
Son et al. (2008)	Male mouse	Adult	0. 0.49, 2.64, 17.63, 47.21	4 weeks	-----	NA	0.49	NA	• PFOA serum data are not available from this study. • Significantly increased levels of one or both liver enzymes, ALT and AST, occurred at all doses (data shown in Table 8).
Tucker et al. (2015)	Female mouse	Pup PND 21	0, 0.01, 0.1, 0.3, 1	GD 1-17	CD-1 0.3	NA	1	NA	• Serum data are not presented here because they are from PND 21 and do not reflect maximum exposure at end of dosing period. • Liver weight was assessed in offspring on PND 21 and later time points. No data are available from earlier time points closer to the end of dosing. Increased liver weight was not observed at time points later than PND 21. At these time points, PFOA body burdens would be lower than on PND 21. • Delayed mammary gland development occurred at lower doses than liver effects in both strains.
					C57/Bl 1	NA	-----	NA	
		WT pup			--		0.1		
		PPAR-α null dam	0, 0.1, 0.3, 1.0, 3.0		1		3		
		PPAR-α null pup			1		3		

*LOAELs are based on statistical significance at $p<0.05$. Effects that were not statistically significant occurred at doses below the NOAELs in some studies. **WT – Wild Type

Table 11. Summary of toxicological studies of effects of oral exposure to PFOA on the immune system

Citation	*Species & Strain*	*Lifestage for Dosing*	*Dose(s) (mg/kg/day)*	*Dosing Duration*	*Endpoint(s)**	*NOAEL** (mg/kg/day)*	*LOAEL** (mg/kg/day)*	*PFOA Serum Concentration (ng/ml)*	*Comments*
Botelho et al. (2015)	Male C57BL/6 (H-2)[b] mice	Adult	0, 20, 50, 100, and 200 ppm in diet (20 ppm estimated as 3 mg/kg/day from Qazi et al., 2013)	10 days	↓ complement activation (various parameters) at end of dosing period.	100 ppm (estimated as 15 mg/kg/day based on data for lower dose in Quazi et al., 2013)	200 ppm (estimated as 30 mg/kg/day based on data for lower dose in Quazi et al., 2013)	Estimated serum level at 200 ppm (LOAEL): 152,000 ng/ml (Qazi et al., 2009b)	Effects associated with PPAR-alpha occurred at doses below the LOAEL for ↓ complement activation; authors concluded that ↓ complement activation is PPAR-alpha independent. LOAEL for ↑ liver weight was lower than for immune effect.
DeWitt et al. (2008)	Female C57/BL6N mice	Adult	0, 3.75, 7.5, 15, and 30 mg/kg/day in drinking water.	15 days	↓ IgM, ↑ IgG after immunization with sheep red blood cells	1.88 mg/kg/day	3.75 mg/kg/day	74,913 ng/ml at 3.75 mg/kg/day (LOAEL), one day post-dosing	NOAEL and LOAEL for ↓ relative spleen and thymus weight were higher than for other effects.
			0, 0.94, 1.88, 3.75, and 7.5 mg/kg/day in drinking water.		↓ IgM, ↑ IgG after immunization with sheep red blood cells ↓ relative spleen weight	------	3.75 mg/kg/day		NOAEL and LOAEL for ↓ relative thymus weight were higher than for other effects. LOAEL for ↑ liver weight was lower than for immune effect.
DeWitt et al. (2009a)	Female C57/BL6N mice	Adult	0, 3.75, 7.5, and 15 mg/kg/day in drinking water	10 days	↓ IgM	7.5 mg/kg/day (sham operated) 3.75 mg/kg/day (adrenalectomized)	15 mg/kg/day (sham operated) 3.75 mg/kg/day (adrenalectomized)	Not assessed	Authors conclude that effects on immune system are not due to increased serum corticosterone.
Hu et al. (2010)	Female C57/BL6N mice	Pregnant	0, 0.5, and 1 mg/kg/day Gavage	GD 6-17	IgM (PND 49), IgG (PND 63) after immunization with sheep red blood cells. Absolute and relative spleen and thymus weight (PND 49 and 63) in offspring	1 mg/kg/day	------	Not assessed at end of dosing	

Citation	*Species & Strain*	*Lifestage for Dosing*	*Dose(s) (mg/kg/day)*	*Dosing Duration*	*Endpoint(s)**	*NOAEL** (mg/kg/day)*	*LOAEL** (mg/kg/day)*	*PFOA Serum Concentration (ng/ml)*	*Comments*
Hu et al. (2012)	Female C57/BL6N mice	Pregnant and lactating	0, 0.02, 0.2, and 2 mg/kg/day Gavage	Pre-pregnancy (10.1-15.7 days) through weaning	↓ percentage of splenic T regulatory cells in offspring (age - at least 6 weeks old)	0.2 mg/kg/day/---- (see Comments)	2 mg/kg/day/0.02 mg/kg/day (see Comments)	Not assessed	*Ex vivo* IL-10 production by splenic CD4+ T cells was ↓ at ≥ 0.02 mg/kg/day in male offspring; it was ↑ at 0.02 mg/kg/day but not other doses in females. No effect on two serum autoantibodies. For 3rd serum autoantibodies (anti-ssDNA), ↓ at 0.02 and 2, but not 0.2 mg/kg/day, in females only. Significance of changes at 0.02 mg/kg/day are not clear.
Iwai and Yamashita (2006)	Male Crj:CD (SD)IGS rats	Adult	0, 0.5, 5, and 50 mg/kg/day Gavage	14 days	Percent lymphocytes in peripheral blood Percent or number of lymphocytes subsets in peripheral blood Absolute or relative spleen weight	50 mg/kg/day	----------	Not assessed	Relative liver weight ↑ at NOAEL for immune effects (50 mg/kg/day).
Loveless et al. (2008)	Male Crj:CD (SD)IGS rats	Adult	0, 0.3, 1, 10, 30 mg/kg/day	28 days Gavage	Several immune parameters	30 mg/kg/day	-----	Not assessed	Other immune effects in mice occurred at doses above LOAEL for decreased relative spleen weight. LOAEL in mice was also 1 mg/kg/day for ↑ relative weight and necrosis in liver. Although authors concluded immune effects are secondary to ↑ corticosterone, corticosterone was not ↑ at LOAEL for ↓ relative spleen weight.
	Male Crj:CD (SD)IGS mice				↓ relative spleen weight	0.3 mg/kg/day	1 mg/kg/day		

Citation	*Species & Strain*	*Lifestage for Dosing*	*Dose(s) (mg/kg/day)*	*Dosing Duration*	*Endpoint(s)**	*NOAEL*** (mg/kg/day)*	*LOAEL*** (mg/kg/day)*	*PFOA Serum Concentration (ng/ml)*	*Comments*
Qazi et al. (2009a)	Male C57BL/6 (H-2)[b] mice	Adult	10 or 200 ppm in diet	10 days	Numerous parameters related to immune function	10 ppm (data not shown) Estimated as 1.5 mg/kg/day from Qazi et al. (2013).	200 ppm Estimated as <30 mg/kg/day from Qazi et al. (2013)	Estimated serum level at 200 ppm (LOAEL) was 152,000 ng/ml (Qazi et al., 2009b)	
Qazi et al. (2009b)	Male C57BL/6 (H-2)[b] mice	Adult	200 ppm in diet	10 days	↓ relative spleen and thymus weight. ↓ spleen and thymus cellularity. ↓ splenocyte and thymocyte cell populations. Histopathological changes in thymus.	---------	200 ppm Estimated as <30 mg/kg/day from Qazi et al. (2013)	152,000 µg/L	Relative liver weight also ↑ at LOAEL.
Qazi et al. (2010)	Male C57BL/6 (H-2)[b] mice	Adult	20 ppm in diet	10 days	Several parameters of hepatic immune status	-------	20 ppm Estimated as 3 mg/kg/day from Qazi et al. (2013).	87,600 ng/ml	↑ liver weight and histopathological changes in the liver also occurred at this dose.
Qazi et al. (2012)	Male C57BL/6 (H-2)[b] mice	Adult	10, 20, or 200 ppm in diet	10 days	↓ numbers of beta-lymphoid cells and beta-lymphoid cell subpopulations in bone marrow.	10 ppm Estimated as 1.5 mg/kg/day from Qazi et al. (2013).	20 ppm Estimated as 3 mg/kg/day from Qazi et al. (2013).	Estimated serum level at 20 ppm (LOAEL) was 87,600 ng/ml (Qazi et al., 2010)	Effects at 200 ppm may be secondary to decreased food consumption. Food consumption not decreased at LOAEL of 20 ppm. ↑ relative liver weight at NOAEL for immune effects of 10 ppm.

Citation	*Species & Strain*	*Lifestage for Dosing*	*Dose(s) (mg/kg/day)*	*Dosing Duration*	*Endpoint(s)**	*NOAEL*** (mg/kg/day)*	*LOAEL*** (mg/kg/day)*	*PFOA Serum Concentration (ng/ml)*	*Comments*
Qazi et al. (2013)	Male C57BL/6 (H-2)[b] mice	Adult	0.5 or 20 ppm in diet 0.07 or 3 mg/kg/day	10 days (high dose) 28 days (low dose)	↑ Concavalin A-induced liver damage. Changes in cytokine levels (both with and without response to Con A).	0.07 mg/kg/day (0.5 ppm) for 28 days	3 mg/kg/day (20 ppm) for 10 days	Estimated serum level at 20 ppm (LOAEL) was 87,600 ng/ml (Qazi et al., 2010)	
Son et al. (2009)	Male ICR mice	Adult	0.49, 2.64, 17.63, 47.21 mg/kg/day	21 days	↓ numbers of splenic T cell subpopulations (CD4-CD8+, CD4+CD8+).	---	0.49 mg/kg/day		These T cell subpopulations were not evaluated in other studies of PFOA. ↑ liver weight at LOAEL for immune effects in this study, as reported in accompanying paper (Son et al., 2008)
Yang et al. (2000)	Male C57BL/6 mice	Adult	200 ppm in diet	Up to 10 days	↓ absolute and relative thymus and spleen weight beginning at 5 days. ↓ thymocyte and splenocyte numbers, selective ↓ in immature thymocyte subpopulations, ↓ T and B cells in spleen, ↓ numbers of splenic T cell subpopulations (CD4 +, D4+CD8+), inhibition of thymocyte proliferation at 7 days (other time points not evaluated).	-----	200 ppm in diet Estimated as <30 mg/kg/day from Qazi et al. (2013	Estimated serum level at 200 ppm (LOAEL) was 152,000 ng/ml (Qazi et al., 2009b)	Relative liver weight also ↑ at LOAEL.

Citation	*Species & Strain*	*Lifestage for Dosing*	*Dose(s) (mg/kg/day)*	*Dosing Duration*	*Endpoint(s)**	*NOAEL** (mg/kg/day)*	*LOAEL** (mg/kg/day)*	*PFOA Serum Concentration (ng/ml)*	*Comments*
Yang et al. (2002a)	Male C57BL/6 mice	Adult	200 ppm in diet	10 or 16 days	↓ IgM and IgG response to horse red blood cell (HRBC) immunization.	-------	200 ppm in diet Estimated as <30 mg/kg/day from Qazi et al. (2013)	Estimated serum level at 200 ppm (LOAEL) was 152,000 ng/ml (Qazi et al., 2009b)	
Yang et al. (2002b)	Male C57BL/6 mice (wild type)	Adult	200 ppm in diet	7 days	*C57BL/6 wild type:* ↓ spleen and thymus weight. ↓ # of splenocytes and thymocytes. ↓ CD4+CD8+ thymocytes. ↓ T and B cells in spleen. ↓ % of proliferating thymocytes. ↓ proliferation of splenocytes ex vivo in response to proliferative stimulation.		200 ppm in diet Estimated as <30 mg/kg/day from Qazi et al. (2013)		Comparison of wild type versus PPAR-alpha null mice was between two different strains. Strain differences unrelated to PPAR-alpha status may have affected the results. Similar increase in relative liver weight occurred in both wild type C57BL/6 and Sv/129 PPAR-alpha null mice. Peroxisome proliferation only increased in wild type mice. Effects of PFOA on thymus occurred in PPAR-alpha null mice, to a lesser degree than in wild type mice. WY also caused these effects in PPAR-alpha null mice. Effects of PFOA on spleen seen in wild type mice did not occur in PPAR-alpha null mice.
	Male Sv/129 PPAR-alpha null mice				*Sv/129 PPAR-alpha null:* ↓ thymus weight and number of thymocytes (attenuated). ↓ CD4+CD8+ thymocytes (attenuated). ↓ % of proliferating thymocytes (attenuated).				

* IgG and IgM evaluations were based response to injection with sheep or horse red blood cells (RBC). In some studies, additional endpoints that are not shown in the table were evaluated; data for the most sensitive immune-related endpoint(s) are shown.

** NOAELs are defined as the highest dose that did not produce a statistically significant ($p<0.05$) effect, and LOAELs are defined as the lowest doses with statistically significant ($p<0.05$) effects.

Table 12: Summary of studies of effects of gestational/lactational exposure to PFOA in mice (most sensitive effect(s) in each study are shown in ***red italics***)*

Citation and Study Design				**Maternal/ Reproductive Effects**			**Effects in Offspring: Fetal through Weaning**			**Effects in Offspring: Post-weaning**			**Comments**
Citation	***Strain***	***Administered dose(s) and routes (mg/kg/day)***	***Duration***	***Effect***	***NOAEL***	***LOAEL***	***Effect***	***NOAEL***	***LOAEL***	***Effect***	***NOAEL***	***LOAEL***	
Abbott et al. (2007)	129S1/SvlmJ wild type	0, 0.1, 0.3, 0.6, 1, 5, 10, or 20 Gavage	GD 1-17	• Maternal weight gain • % FLR • Implants/litter • % litter loss (includes FLR) • Pups/litter (live + dead; excludes FLR) • **↑ relative liver weight on PND 22.**	10 1 20 0.3 1 0.6	-- 5 -- 0.6 -- 1	• Body weight at birth • Eye opening delay • Postnatal mortality • Neonatal growth • **↑ relative liver weight on PND 22.**	1 0.6 0.3 0.6 --	-- 1 0.6 1 ***0.1***	• Body weight at week 28 (M) & week 52 (F)	1	--	• No wild type live pups at ≥ 5 mg/kg/day. • FLR is PPAR-alpha independent. • ↑ pup liver weight is most sensitive endpoint in both strains.
	129S1/SvlmJ PPAR-α null	0, 0.1, 0.3, 1, 3, 5, 10, or 20 Gavage	GD 1-17	• Maternal weight gain • % FLR • Implants/litter • % litter loss (includes FLR) • Pups/litter (live + dead; excludes FLR) • **↑ relative liver weight on PND 22.**	20 3 20 3 1 1	-- 5 -- 5 -- ***3***	• Body weight at birth • Eye opening • Postnatal mortality • Neonatal growth • **↑ relative liver weight on PND 22.**	20 3 3 3 1	-- -- -- -- ***3***	• Body weight at week 28 (M) & week 52 (F)	3	--	

Citation and Study Design				Maternal/Reproductive Effects			Effects in Offspring: Fetal through Weaning (including fetal body weight and malformations)			Effects in Offspring: Post-weaning			Comments
Citation	***Strain***	***Administered dose(s) and routes (mg/kg/day)***	***Duration***	***Effect***	***NOAEL***	***LOAEL***	***Effect***	***NOAEL***	***LOAEL***	***Effect***	***NOAEL***	***LOAEL***	
Albrecht et al. (2013)	Sv/129 wild-type	0 or 3 Gavage	GD 1-17	• Maternal weight gain • Day of parturition • Implants/litter • % litter loss • Live fetuses/litter • Male/female ratio • **↑ relative liver weight on GD 18** • **↑ relative liver weight on PND 20**	3 3 3 3 3 3 -- --	-- -- -- -- -- -- ***3*** ***3***	• Body weight and crown-to-rump length on GD 18 • Postnatal mortality • Postnatal growth • Eye opening • **↑ relative liver weight on GD 18** • **↑ relative liver weight on PND 20** • **MG on PND 20**	3 3 3 3 -- -- 3	-- -- -- -- ***3*** ***3*** --	NA	NA	NA	• Issues related to this study are discussed in text and individual study table in Appendix 5. • No significant effect for any developmental endpoint in any strain. Not consistent with effects reported in Abbott et al. (2007). • ↑ maternal and pup liver weight is the most sensitive endpoint in all strains.
	Sv/129 PPAR-alpha null			• Maternal weight gain • Day of parturition • Implants/litter • % litter loss • Live fetuses/litter • Male/female ratio • **↑ relative liver weight on GD 18** • **↑ relative liver weight on PND 20**	3 3 3 3 3 3 -- 3	-- -- -- -- -- -- ***3*** --	• Body weight & crown-to-rump length on GD 18 • Postnatal mortality • Postnatal growth • Eye opening delay • **↑ relative liver weight on GD 18** • **↑ relative liver weight on PND 20** • **MG on PND 20**	3 3 3 3 3 3 3	-- -- -- -- -- -- --	NA	NA	NA	
	Sv/129 Human-ized PPAR-alpha			• Maternal weight gain • Day of parturition • Implants/litter • % litter loss • Live fetuses/litter • Male/female ratio • **↑ relative liver weight on GD 18** • **↑ relative liver weight on PND 20**	3 3 3 3 3 -- -- 3	-- -- -- -- -- ***3*** ***3*** --	• Body weight & crown-to-rump length on GD 18 • Postnatal mortality • Postnatal growth • Eye opening • **↑ relative liver weight on GD 18** • **↑ relative liver weight on PND 20** • **MG on PND 20**	3 3 3 3 -- 3 3	-- -- -- -- ***3*** -- --	NA	NA	NA	

MG – delayed mammary gland development.

Citation and Study Design				Maternal/Reproductive Effects			Effects in Offspring: Fetal through Weaning (including fetal body weight and malformations)			Effects in Offspring: Post-weaning			Comments
Citation	***Strain***	***Administered dose(s) and routes (mg/kg/day)***	***Duration***	***Effect***	***NOAEL***	***LOAEL***	***Effect***	***NOAEL***	***LOAEL***	***Effect***	***NOAEL***	***LOAEL***	
Fenton et al. (2009)	CD-1	0, 0.1, 1, or 5 Gavage	GD 17 Single dose	• Body weight on GD 17 and 18, and PND 1 -18 • **↑ relative liver weight on GD 18, PND 1,4, 8, 18**	5 5	-- --	Offspring body weight on GD 18 and PND 1-18	5	--	NA	NA	NA	• This was primarily a pharmacokinetic study and used a single dose.
Hines et al. (2009)	CD-1	0, 0.01, 0.1, 0.3, 1, 3 or 5	GD 1-17	• Live pups/litter	NA 5	NA --	↓ Body weight at PND 1 • ↓ Body weight at PND 22	1 0.3	5 1	• ↑ Body weight at 20-29 wks*** • ↓ Body weight at 20-29 wks • ↑ insulin & leptin at 21-33 wks*** (Only females evaluated)	--- 1 ---	***0.01*** 5 ***0.01***	• Effects at 20-29 weeks on body weight (0.01-0.3 mg/kg/day) and hormones (0.01-0.1 mg/kg/day) occurred at low doses but not higher doses. • Mortality occurred in all groups including controls after 36 weeks. • Effects at 18 months are not in this table.
Hu et al. (2010)	C57BL/6N	0, 0.5, 1 Drinking water	GD 6-17	• Maternal body weight • M/F ratio	1 1	-- --	• ↓ Body weight at PND 2 • ↓ Body weight at PND 7 & 14 • **↑ relative liver weight on GD 18**	-- 0.5 1	***0.5*** 1 --	• IgM on PND 48 • IgG on PND 63 • Spleen, adrenal, thymus weight, PND 48 & 63 • **↑ relative liver weight on PND 48 & 63**	1 1 1 1	-- -- -- --	See also summary table of studies of immune effects (Table 11).
Hu et al. (2012)	C57BL/6N	0.02, 0.2 or 2 Gavage	Pre-mating – PND 21; (mean 12.9 days pre-pregnancy)	• Maternal body weight (through weaning) • Pups/litter • M/F ratio	2 2 2	-- -- --	• ↓ Body weight at PND 1 - 21	0.2	***2***	• Body weight at 6 weeks. • ↓ T cells in spleen at ~ 6 wks (Only females evaluated).	2 0.2	-- ***2***	• *Ex vivo* and inconsistent *in vivo* immune effects observed at 0.02 mg/kg/day; see summary table for immune effects (Table 11).

.

Citation and Study Design				Maternal/ Reproductive Effects			Effects in Offspring through Weaning (including fetal body weight)			Effects in Offspring: Post-weaning			Comments
Citation	***Strain***	***Administered dose(s) and routes (mg/kg/day)***	***Duration***	***Effect***	***NOAEL***	***LOAEL***	***Effect***	***NOAEL***	***LOAEL***	***Effect***	***NOAEL***	***LOAEL***	
Lau et al. (2006)	CD-1	0, 1, 3, 5, 10, 20, or 40 Gavage	GD 1-17 (sacrificed GD 18) GD 1-18 (allowed to deliver)	• ↓ Maternal weight gain • FLR • # of implants • Live fetuses/litter • Prenatal loss/live litter **• ↑ relative liver weight on GD 18**	10 3 -- 10 10 --	20 5 40 20 20 ***1***	• ↓ fetal body weight • ↑ reduced ossification (several sites) • ↑ Limb & tail defects • ↑ Micro-cardia • ↑Postnatal mortality • ↓ Postnatal growth • Delayed eye opening	10 -- 3 10 3 1 3	20 ***1[a]*** 5[a] 10 5 3 5	• ↓ body weight in males at 6.5 weeks ($p<0.05$ for treatment effect) • Delay in vaginal opening. • Delay in first estrus. • Accelerated preputial separation.	Not sta-ted 20 3 -- .	Not sta-ted 10 5 ***1[a]***	• Reduced ossification and increased limb defects did not increase as dose increased. These effects were not significant at one or more doses above the LOAEL. • Accelerated preputial separation was not observed at 20 mg/kg/day. • Body weight in treated animals tended to be higher than in controls as animals aged (until age 60 wks). **• Maternal serum levels on GD 18 shown graphically**. • This study is also summarized in liver weight tables (Table 10).
Macon et al. (2011)	CD-1	0, 0.3, 1, or 3 Gavage	GD 1-17	NA	NA	NA	• Body weight PND 7-21 **• ↑ relative liver weight on PND 7** **• MG at PND 14 & 21[a]**	3 -- --	-- ***0.3[a]*** ***0.3***	• Body weight on PND 42-84 **• MG at PND 42 & 84[a]**	3 --	-- ***0.3***	• LOAELs for ↑ pup liver weight were higher at later pre-weaning and post-weaning time points. • LOAEL for MG was higher at PND 7. • MG was not significant at 3 mg/kg/day on PND 63 and 84, possibly due to small n (n=2)
		0, 0.01, 0.1, or 1 Gavage	GD 10-17	NA	NA	NA	• Body weight at birth (females) • Body weight on PND 1-21 **• ↑ relative liver weight on PND 4, 7, 14[a]** **• MG at PND 21**	1 1 **0.1** --	-- -- **1** ***0.01***	NA	NA	NA	**• Pup serum levels measured on PND 1 (end of dosing).** • Increased relative liver weight not significant on PND 1 and 21. • Delayed mammary gland development more sensitive endpoint than increased relative liver weight in offspring.

[a] See comments.

Citation and Study Design				Maternal/Reproductive Effects			Effects in Offspring through Weaning (including fetal body weight and malformations)			Effects in Offspring: Post-weaning			Comments
Citation	***Strain***	***Administered dose(s) and routes (mg/kg/day)***	***Duration***	***Effect***	***NOAEL***	***LOAEL***	***Effect***	***NOAEL***	***LOAEL***	***Effect***	***NOAEL***	***LOAEL***	
Suh et al. (2011)	CD-1	0, 2, 10 or 25 Gavage	GD 11-16	↓ Maternal body weight on GD 13-16 *Endpoints assessed on GD 16*. • ↓ placenta weight • Necrotic changes in placenta[a] • ↓ levels of gene expression for three placental lactogens (PRL family) and two pituitary-specific positive transcription factor 1 isoforms. • ↓ # of placental trophoblast cells • ↓ fetal weight • ↓ fetal/ placental weight ratio • # of implantations • ↑ # of resorptions/dead fetus • ↓ # of live fetus	10[a] -- 2 -- 10 2 -- 25 -- 2	25[a] ***2*** 10 ***2*** 25 10 ***2*** -- ***2*** 10	NA	NA	NA	NA	NA	NA	• Doses at which maternal body weight ↓ are not stated, although significance for trend is shown on various GDs. NOAEL & LOAEL in this table is based on graph of data. • Effects on placenta and production of placental factors may contribute to decreased fetal growth. • Fetal/placental ratio indicates efficiency of placenta. • Levels of placental lactogens significantly correlate with fetal weight on individual basis. • Statistical evaluation for necrotic changes in placenta is not provided; doses where this effect occurred are discussed in text. • NOAELs for potentially important placental effects not identified.

[a] See comments.

Citation and Study Design				Maternal/ Reproductive Effects			Effects in Offspring through Weaning (including fetal body weight and malformations)			Effects in Offspring: Post-weaning			Comments
Citation	***Strain***	***Administered dose(s) and routes (mg/kg/day)***	***Duration***	***Effect***	***NOAEL***	***LOAEL***	***Effect***	***NOAEL***	***LOAEL***	***Effect***	***NOAEL***	***LOAEL***	
Tucker et al. (2015)	CD-1	0, 0.01, 0.1, 0.3, 1 Gavage	GD 1-17	NA	NA	NA	• ↓ body weight on PND 21 • ↓ body weight minus liver weight on PND 21 • Estrogen & progesterone, PND 21 • **↑ relative liver weight, PND 21** • **MG at PND 21**	1 0.3 1 0.3 0.01	-- 1 -- 1 0.1	• Body weight & body weight minus liver weight on PND 35 & 56 • Day of vaginal opening • Day of first estrus • Estrogen & progesterone on PND 35 & 56 • **↑ relative liver weight, PND 35 & 56** • **MG at PND 35 & 56**	1 1 1 1 1 --	-- -- -- -- -- ***0.01***	PFOA serum data provided from PND 21 and later time points. Higher serum levels at same PFOA dose in CD-1 mice than C57Bl/6 mice may contribute to the greater sensitivity of CD-1.
	C57Bl/6						• ↓ body weight on PND 21 • ↓ body weight minus liver weight on PND 21 • Estrogen & progesterone on PND 21 • **↑ relative liver weight, PND 21** • **MG at PND 21**	1 1 1 1 0.1	-- -- -- -- ***0.3***	• Body weight & body weight minus liver weight on PND 61 • Day of vaginal opening • Day of first estrus • Estrogen & progesterone, PND 61 • **↑ relative liver weight on PND 61** • **MG at PND 61**	1 1 1 1 1 0.1	-- -- -- -- -- ***0.3***	

[a] See comments.

Citation and Study Design				Maternal/ Reproductive Effects			Effects in Offspring through Weaning (including fetal body weight and malformations)			Effects in Offspring: Post-weaning			Comments
Citation	***Strain***	***Administered dose(s) and routes (mg/kg/day)***	***Duration***	***Effect***	***NOAEL***	***LOAEL***	***Effect***	***NOAEL***	***LOAEL***	***Effect*** [a]	***NOAEL***	***LOAEL***	
van Esterik et al. (2016)	C57Bl/6J	0, 003, 0,01, 0.1, 0.3, 1, or 3 mg/kg/day Dietary	2 weeks before mating through weaning	• Maternal weight gain • ↓ litter size • Male/female ratio	3[a] 0.3[a] 3[a]	-- 1[a] --	• ↓ Body weight on PND 4 • Neonatal survival	-- 3[a]	0.003[a] --				• Exposure regimen differed from other studies. Exposure began before mating and continued through lactation. • Data presented as BMD and BMDL for each endpoint. NOAELs and LOAELs in table are based on interpretation of graphs and/or discussion in text; statistical significance is not presented. Personal communication with authors indicates that study was not intended to identify LOAELs and NOAELs. Data for some endpoints (e.g. relative liver weight) not presented in usable form. • The LOAEL for decreased neonatal body weight appears to be much lower than in other studies. As above, personal communication with authors indicates that study was not intended to identify LOAELs and NOAELs. • Text states that body weight decrements persisted through lactation and until wk. 21. Doses at which this occurred not stated. • Later in life studies relate to mode of action and effects with high fat versus control diet.

[a] See comments.

Citation and Study Design				Maternal/ Reproductive Effects			Effects in Offspring through Weaning (including fetal body weight and malformations)			Effects in Offspring: Post-weaning			Comments
Citation	***Strain***	***Administered dose(s) and routes (mg/kg/day)***	***Duration***	***Effect***	***NOAEL***	***LOAEL***	***Effect***	***NOAEL***	***LOAEL***	***Effect***	***NOAEL***	***LOAEL***	
White et al. (2007)	CD-1	0 or 5 Gavage	GD 1-17, GD 8-17, GD 12-17 *Restricted exposure study*****	• ↓ Maternal weight gain, GD 18 • # of implants • Live fetuses/litter • % prenatal loss/live litter	GD 1-17 GD 1-17 GD 1-17 GD 8-17	-- -- -- GD 1-17	• ↓ Body weight on PND 1, 5, 10, 20 • **MG on PND 21**	-- --	***GD 12-17*** ***GD 12-17***	NA	NA	NA	
White et al. (2009)	CD-1	0 or 5 Gavage	GD 8-17 *Cross-foster study******	• ↑ Maternal weight gain (GD 8-17) • Litter size • **↑ relative liver weight, PND 1, 3, 5, 10**	-- 5 0 dam, 5U pups	5 -- 5 dam, control pups	• ↓ Body weight on PND 1, 3 • ↓ Body weight on PND 10, 20 • **↑ relative liver weight, PND 1, 3** • **↑ relative liver weight, PND 5, 10** • **MG, PND 1, 3, 5, 10**	5L, 5U -- 5L -- --	5U+L ***5L, 5U*** 5U ***5L, 5U*** ***5L, 5U***	NA	NA	NA	
White et al. (2009); Wolf et al. (2007)	CD-1	0, 3, or 5 Gavage	GD 1-17 *Cross-foster study******	• ↑ Maternal wt. gain, GD 17 • FLR • # of implants • Live fetuses/litter • Prenatal loss/live litter • **↑ relative liver wt., PND 22** • **MG at PND 22**	-- 3 5 5 5 5 -- 3L	3 5 -- -- -- -- 3 3U	• ↓ Body weight at birth • ↓ Body weight on PND 22 (males) • ↓ Body weight on PND 22 (females) • Delayed eye opening • ↓ survival to PND 22 • **↑ relative liver weight, PND 22** • **MG at PND 22**	3 3U, 5L 3U, 3L 3U, 5L 3U+L, 5U, 5L -- 3L	5 3U+L, 5U 3U+L, 5U, 5L 3U+L, 5U 5U+L ***3L, 3U*** 3U	• ↓ Body weight until PND 36 (M) • ↓ Body weight until PND 85 (F) • **MG at PND 42, 63**	3U+L, 5U, 5L 3U+L, 5L --	5U+L 5U ***3L, 3U***	Body weight of male 3U group was increased from PND 85 (week 12) to week 35.

U = exposure *in utero* only; L = exposure through lactation only; U + L = exposure both *in utero* and through lactation.

Citation and Study Design				Maternal/ Reproductive Effects			Effects in Offspring: Fetal through Weaning (including fetal body weight and malformations)			Effects in Offspring: Post-weaning			Comments
Citation	***Strain***	***Administered dose(s) and routes (mg/kg/day)***	***Duration***	***Effect***	***NOAEL***	***LOAEL***	***Effect***	***NOAEL***	***LOAEL***	***Effect***	***NOAEL***	***LOAEL***	
Wolf et al. (2007) *(See White et al., 2009 for additional study from this public-ation)*	CD-1	0 5; GD 15-17, 13-17, 10-17, or 7-17. 20; GD 15-17 *(Post-natal endpoints were not assessed at this dose due to decreased post-natal survival.)* Gavage	GD 15-17, GD 13-17, GD 10-17, GD 7-17 *Restricted exposure study*****	• ↑ Maternal weight gain • # of implants • Pups/ litter • % prenatal loss	5, GD 10-17 5, GD 7-17 5, GD 7-17 5, GD 7-17	5, GD 13-17 -- -- --	• ↓ Body weight at birth (males) • ↓ Body weight at birth (females) • ↓ survival until PND 22 • Delayed eye opening • ↓ Body weight at PND 22	5, GD 13-17 20,GD 15-17; 5, GD 7-17 5, GD 7-17 5, GD 13-17 --	20, GD 15-17; 5, GD 10-17 -- 20,GD 15-17 5, GD 10-17 ***5, GD 15-17***	• ↓ Body weight until age 10-11 weeks (males)	5, GD 13-17	5, GD 10 -17	Body weight of 5 mg/kg/day females exposed on GD 13-17 was increased above controls after PND 161 (week 23).
White, et al. (2011b)	CD-1	0, 1 or 5 (gavage) 0 or 1 (gavage) plus 5 ppb (5000 ng/L) in drinking water .	GD 1-17 (P0) for gavage exposures. GD 1 (P0) – PND 63 (F2) for drinking water exposures *Multi-generation study. (See comments.)*	• ↓ Maternal weight gain • Implants/ litter • Live fetus/litter • Prenatal loss	5 5 1 1	-- -- 5 5	•↓ Body weight at PND 22 • ↓ Survival until PND 22 • **↑ relative liver weight, PND 22** • **MG at PND 22**	5 -- -- --	-- 5 1 (not significant at 1 + 5 ppb in drinking water) ***5 ppb in drinking water; 1 mg/kg/day***	• ↓ Body weight at PND 42 • ↓ Body weight at PND 63 • **↑ relative liver weight, PND 42** • **MG at PND 42, 63**	1 5 1 --	5 -- 5 ***5 ppb in drinking water; 1 mg/kg/ Day***	Only effects for P0 dams and F1 offspring presented here. See Table 14 and individual study table in Appendix 5 for additional information on this study.

Citation and Study Design				Maternal/ Reproductive Effects			Effects in Offspring: Fetal through Weaning (including fetal body weight and malformations)			Effects in Offspring: Post-weaning			Comments
Citation	***Strain***	***Administered dose(s) and routes (mg/kg/day)***	***Duration***	***Effect***	***NOAEL***	***LOAEL***	***Effect***	***NOAEL***	***LOAEL***	***Effect***	***NOAEL***	***LOAEL***	
Yahia et al. (2010)	ICR	0, 1, 5, 10 Gavage	GD 0-17 (assessed on GD 18) GD 0-18 (postnatal assessment)	• ↓ Maternal weight gain • ↑ Relative kidney weight • ↓ absolute brain weight • ↑ serum liver enzymes. • ↓ serum lipids • **↑ relative liver weight, PND 22**.	1 -- 5 5 5 --	5 1 10 10 10 ***1***	• ↓ fetal GD 18 body weight & birth weight • ↓ survival until PND 4 • ↑ cleft sternum, delayed ossification of phalanges, delayed incisor eruption	1 1 1 5	5 5 5 10	NA	NA	NA	NOAEL for delayed ossification is higher than LOAEL (1 mg/kg/day) for this effect in Lau et al. (2006). All pups died by PND 4 at LOAEL for delayed ossification (10 mg/kg/day).

* General notes:

NOAEL is defined as the highest dose that did not produce a statistically significant effect ($p \leq 0.05$) compared to control. LOAEL is defined as the lowest dose with statistically significant effects observed ($p \leq 0.05$) compared to control. For some studies, there were dose-related trends that included non-statistically significant changes at lower doses.

- In some studies, not all dose groups were evaluated for some endpoints. For some studies, not all endpoints evaluated are listed.
- Several studies that focused on other specific endpoints also evaluated reproductive and/or developmental endpoints but did not present data for these endpoints in usable form, or did not provide statistical significance. These studies are mentioned in the text but are not included in this table.
- Serum data are useful for dose-response modeling only when measured at end of dosing period. Serum data in many studies were from later time points; these data provide useful pharmacokinetic information but are not appropriate for dose-response modeling.

** See Table 14 and individual study tables in Appendix 5 for more details on studies in which mammary gland development was evaluated.
*** Effect occurred at low, but not high, doses.
**** In **restricted exposure studies**, gestational exposure was to the same dose for varying periods of time. In determining LOAELs and NOAELs, shorter time periods of dosing were considered to have been lower exposures than longer time periods of dosing.
***** In **cross foster studies**, pups were exposed *in utero* (U), through lactation (L), or both (U+L). In determining LOAELs and NOAELs, all groups to exposed lower doses (L, U, or U+L) were considered to have had lower exposure than those with higher doses. Within the same dosage level (mg/kg/day), L and U groups were considered to have had lower exposure than U+L groups.

FLR – full litter resorptions. MG – Delayed mammary gland development. NA – not assessed.

Table 13. Identification of most sensitive endpoints in mouse developmental studies of PFOA*

Citation and Study Design				Most Sensitive Reproductive/Developmental Endpoint(s) (other than increased relative liver weight or delayed mammary gland development)	Delayed Mammary Gland Development	Increased Relative Liver Weight
Citation	***Strain***	***Administered dose(s) and routes (mg/kg/day)***	***Duration***			
Abbott et al. (2007)	129S1/SvlmJ wildtype	0, 0.1, 0.3, 0.6, 1, 5, 10, or 20 Gavage	GD 1-17	% litter loss (includes full litter resorption); postnatal mortality NOAEL – 0.3 LOAEL – 0.6	NA	Pup, PND 22 ***NOAEL-ND*** ***LOAEL-0.1***
	129S1/SvlmJ PPAR-alpha null	0, 0.1, 0.3, 1, 3, 5, 10, or 20 Gavage	GD 1-17	% full litter resorptions; % litter loss (includes full litter resorption) NOAEL – 3 LOAEL – 5	NA	Maternal and pup, PND 22 ***NOAEL - 1*** ***LOAEL - 3***
Albrecht et al. (2013)	Sv/129 wild-type	0 or 3 Gavage	GD 1-17	All effects: NOAEL – 3 LOAEL - ND	NOAEL – 3 LOAEL - ND	Maternal and offspring, GD 18 and PND 20 ***NOAEL – ND*** ***LOAEL - 3***
	Sv/129 PPAR-alpha null			All effects NOAEL – 3 LOAEL - ND	NOAEL – 3 LOAEL - ND	Maternal, GD18, offspring GD 18 and PND 20 ***NOAEL – ND*** ***LOAEL - 3***
	Sv/129 humanized PPAR-alpha			Male/female offspring ratio ***NOAEL – ND*** ***LOAEL - 3***	NOAEL – 3 LOAEL - ND	Maternal andoffspring, GD 18 ***NOAEL – ND*** ***LOAEL - 3***
Hines et al. (2009)	CD-1	0, 0.01, 0.1, 0.3, 1, 3 or 5 Gavage	GD 1-17	Increased body weight, insulin & leptin in female offspring at weeks 20-29 ***NOAEL – ND*** ***LOAEL – 0.01*** *(No effects at some higher doses)*	NA	NA
Hu et al. (2010)	C57BL/6N	0, 0.5, 1 Drinking water	GD 6-17	Decreased offspring body weight at PND 2 ***NOAEL – ND*** ***LOAEL – 0.5***	NA	Offspring, PND 48 and 63 NOAEL – 1 LOAEL – ND *(not evaluated at earlier times)*
Hu et al. (2012)	C57BL/6N	0.02, 0.2 or 2 Gavage	Pre-mating – PND 21	↓ Body weight at PND 1 – 21; ↓ T cells in spleen at ~ 6 wks ***NOAEL – 0.2*** ***LOAEL – 2***	NA	NA

Citation and Study Design				Most Sensitive Reproductive/Developmental Endpoint(s) (other than increased relative liver weight or delayed mammary gland development)	Delayed Mammary Gland Development	Increased Relative Liver Weight
Citation	***Strain***	***Administered dose(s) and routes (mg/kg/day)***	***Duration***			
Lau et al. (2006)	CD-1	0, 1, 3, 5, 10, 20, or 40 Gavage	GD 1-17 or GD 1-18	Reduced ossification (not significant at some doses >LOAEL); accelerated preputial separation (did not occur at 20 mg/kg/day) ***NOAEL – ND*** ***LOAEL – 1***	NA	Maternal, GD 18 ***NOAEL – ND*** ***LOAEL – 1***
Macon et al. (2011)	CD-1	0, 0.3, 1, or 3 Gavage	GD 1-17	Offspring body weight, PND 7-84 NOAEL – 3 LOAEL - ND	***NOAEL – ND*** ***LOAEL – 0.3***	Offspring, PND 7 ***NOAEL – ND*** ***LOAEL – 0.3***
		0, 0.01, 0.1, or 1 Gavage	GD 10-17	Body weight, birth – PND 21 NOAEL – 1 LOAEL – ND	Offspring, PND 21 ***NOAEL – ND*** ***LOAEL – 0.01***	Offspring, PND 4,7, 14 NOAEL – 0.1 LOAEL – 1
Suh et al. (2011)	CD-1	0, 2, 10 or 25 Gavage	GD 11-16	Decreased placenta weight; decreased gene expression for placental factors; decreased fetal/ placental weight ratio; increased number of resorptions/dead fetuses NOAEL – ND LOAEL – 2	NA	NA
Tucker et al. (2015)	CD-1	0, 0.01, 0.1, 0.3, 1 Gavage	GD 1-17	Decreased body weight minus liver weight, PND 21 NOAEL – 0.3 LOAEL – 1	Offspring, PND 35 and 56 ***NOAEL – ND*** ***LOAEL – 0.01***	Offspring, PND 21 NOAEL – 0.3 LOAEL – 1
	C57Bl/6			All effects evaluated: NOAEL – 1 LOAEL – ND	Offspring, PND 61 ***NOAEL – 0.1*** ***LOAEL – 0.3***	Offspring, PND 21 and 61 NOAEL – 1 LOAEL – ND
van Esterik et al. (2016)	C57Bl/6 J	0, 003, 0,01, 0.1, 0.3, 1, or 3 mg/kg/day Dietary	2 weeks before mating through weaning	Decreased body weight on PND 4 ***NOAEL – ND*** ***LOAEL – 0.003***	NA	NA
White et al. (2007)	CD-1	0 or 5 Gavage	GD 1-17, GD 8-17, or GD 12-17 *Restricted exposure study***	% prenatal loss per live litter NOAEL – 5 mg/kg, GD 8-17 LOAEL – 5 mg/kg, GD 1 – 17	Offspring, PND 10, 20 ***NOAEL – ND*** ***LOAEL - 5 mg/kg, GD 12-17***	NA

Citation and Study Design				Most Sensitive Reproductive/Developmental Endpoint(s) (other than increased relative liver weight or delayed mammary gland development)	Delayed Mammary Gland Development	Increased Relative Liver Weight
Citation	***Strain***	***Administered dose(s) and routes (mg/kg/day)***	***Duration***			
White et al. (2009)	CD-1	0 or 5 Gavage	GD 8-17 *Cross-foster Study****	Decreased body weight on PND 10, 20 ***NOAEL – ND*** ***LOAEL – 5L, 5U***	Offspring, PND 1-10 ***NOAEL – ND*** ***LOAEL – 5L, 5U***	Offspring, PND 5-10 ***NOAEL – ND*** ***LOAEL – 5L, 5U***
White et al. (2009); Wolf et al. (2007)	CD-1	0, 3, or 5 Gavage	GD 1-17 *Cross-foster Study****	Decreased body weight on PND 22 NOAEL – 3U, 3L LOAEL – 3 U+L, 5L, 5U	Offspring, PND 42, 63 ***NOAEL – ND*** ***LOAEL – 3L, 3U***	Offspring, PND 22 ***NOAEL – ND*** ***LOAEL – 3L, 3U***
White, et al. (2011b) *Multi-generation study – onlyP0 dam and F1 offspring data shown here*	CD-1	0, 1 or 5 (Gavage) 0 or 1 (gavage) with 5 ppb in drinking water .	GD 1-17 (P0) for gavage exposures. GD 1 (P0) – PND 63 (F2) for drinking water exposures	Increased prenatal loss; decreased live fetus/litter; decreased body weight on PND 42 NOAEL – 1 LOAEL - 5	NA	Offspring, PND 22 ***NOAEL-ND*** ***LOAEL-1*** (Not significant at 1 mg/kg/day plus 5 ppb in drinking water)
Wolf et al. (2007) *(See White et al., 2009 for other study from this publication)*	CD-1	0 5; GD 15-17, 13-17, 10-17, or 7-17. 20; GD 15-17 Gavage	GD 15-17, GD 13-17, GD 10-17, or GD 7-17 *Restricted exposure study***	Increased maternal weight gain NOAEL – 5 mg/kg, GD 13-17 LOAEL – 5 mg/kg, GD 15–17	Offspring, PND 10,20 ***NOAEL – ND*** ***LOAEL – GD 15-17***	NA
Yahia et al. (2010)	ICR	0, 1, 5, 10 Gavage	GD 0-17 or GD 0-18	Increased maternal relative kidney weight, GD 18 ***NOAEL – ND*** ***LOAEL - 1***	NA	Increased maternal relative liver weight, GD 18 ***NOAEL – ND*** ***LOAEL - 1***

* General notes:

- NOAEL is defined as the highest dose that did not produce a statistically significant effect ($p \leq 0.05$) compared to control. LOAEL is defined as the lowest dose with statistically significant effects observed ($p \leq 0.05$) compared to control. For some studies, there were dose-related trends that included non-statistically significant changes at lower doses.
- In some studies, not all dose groups were evaluated for some endpoints. For some studies, not all endpoints evaluated are listed.

- Several studies that focused on other specific endpoints also evaluated reproductive and/or developmental endpoints but did not present data for these endpoints in usable form, or did not provide statistical significance. These studies are mentioned in the text but are not included in this table.

** In **restricted exposure studies**, gestational exposure is to the same dose for varying periods of time. In determining LOAELs and NOAELs, shorter time periods of dosing were considered to have been lower exposures than longer time periods of dosing.

*** In **cross foster studies**, pups were exposed *in utero* (U), through lactation (L), or both (U+L). In determining LOAELs and NOAELs, both all groups to exposed lower doses (L, U, U+L) were considered to have had lower exposure than those with higher doses. Within the same dosage level (mg/kg/day), L and U groups were considered to have had lower exposure than U+L groups.

FLR – full litter resorptions

NA – not assessed

ND – not determined

Table 14A. Summary of publications/studies evaluating effects of PFOA on mammary gland (MG) development in mice – Includes studies with exposure during pregnancy, gestation, and/or lactation (6 publications/10 studies). (Note: Other effects evaluated in these studies are discussed in other tables in main document and/or individual study tables in Appendix 5)

Citation	*Strain*	*Lifestage(s) for Dosing*	*Dose(s) (mg/kg/day)*	*Dosing Duration*	*Lifestage for MG Assessment*	*Timepoint for MG Assessment*	*Endpoint(s) Assessed*	*Effect*	*NOAEL* (mg/kg/day)*	*LOAEL* (mg/kg/day)*	*Comments/Other Mammary Gland Effects*
Albrecht et al. (2013)	Sv/129 Wild Type (KO), PPAR-alpha null (KO), and humanized PPAR-alpha (H)	Gestation	0, 3	GD 1-17	Female pups	PND 20	MG whole mounts. # terminal end buds/gland; ductal length	No effect	3	----	-This is the only study of MG gland development in this strain. -Other developmental effects seen at similar serum PFOA levels in this strain by Abbott et al. (2007) were not observed in this study. - Some control animals had elevated PFOA levels in serum and liver. - These issues create uncertainty about the conclusion that PFOA did not affect MG development in this study.
Macon et al. (2011)	CD-1	Gestation	0, 0.3, 1, 3	GD 1-17 "Late Gestation"	Female pups	PND 7, 14, 21, 28, 42, 63, 84	MG whole mounts, scored 1-4.	Delayed development	-----	0.3	- Delays occurred in absence of effects on body weight. - Delays persisted until end of study on PND 84 (12 weeks of age)
Macon et al. (2011)	CD-1	Gestation	0, 0.01, 0.1, 1	GD 10-17 "Full Gestation"	Female pups	PND 14, 21	MG whole mounts. PND 14-longitudinal growth, change in long. Growth PND 22 – Score 1-4, longitudinal and later growth, lateral growth, change in long. and lateral growth, # terminal endbuds and terminal ends.	Delayed development	----	0.01 (Developmental Score, $p<0.05$)	- Serum levels in offspring on PND 1 are provided and can be used for dose-response modeling. Serum levels are highest on PND 1 (end of dosing period) and decline thereafter. - The serum level at the LOAEL (0.01 mg/kg/day) in PND offspring was 285 ng/ml. - Liver weight and body weight in the pups were not affected at 0.01 mg/kg/day and higher doses, indicating that MG development is a more sensitive endpoint than decreased body weight or increased liver weight

* NOAELs are defined as the highest dose that did not produce a statistically significant ($p<0.05$) effect, and LOAELs are defined as the lowest doses with statistically significant ($p<0.05$) effects.

Table 14A. Summary of publications/studies evaluating effects of PFOA on mammary gland (MG) development in mice – Includes studies with exposure during pregnancy, gestation, and/or lactation (6 publications/10 studies). (Note: Other effects evaluated in these studies are discussed in other tables in main document and/or individual study tables in Appendix 5)

Citation	*Strain*	*Lifestage(s) for Dosing*	*Dose(s) (mg/kg/day)*	*Dosing Duration*	*Lifestage for MG Assessment*	*Timepoint for MG Assessment*	*Endpoint(s) Assessed*	*Effect*	*NOAEL* (mg/kg/day)*	*LOAEL* (mg/kg/day)*	*Comments/Other Mammary Gland Effects*
Tucker et al. (2015)	CD-1 C57Bl/6	Gestation	0, 0.01, 0.1, 0.3, 1	GD 1-17	Female pups	CD-1: PND 21, 35, 56 C57Bl/6: PND 21, 61	MG whole mounts. Scored 1-4.	Delayed development	--- (CD-1) 0.1 (C57Bl/6)	0.01 (CD-1) 0.3 (C57Bl/6)	- Delayed MG development occurred in the absence of effects on body wt, liver wt, day of vaginal opening, day of first estrus, or serum estradiol and progesterone in both strains. - Serum PFOA levels were first measured on PND 21 (3 weeks after dosing ended) and therefore cannot be used for dose-response modeling. - Higher serum levels from same dose in CD-1 mice than C57Bl/6 mice may contribute to the greater sensitivity of CD-1 to effects on MG development.
White et al. (2007)	CD-1	Gestation	0, 5	GD 1-17	Dams	GD 18	MG whole mounts, scored 1-4	Delayed development	-----	5, GD 1-17	
White et al. (2007)	CD-1	Gestation	0, 5	GD 1-17, 8-17, or 12-17 "Restricted Exposure"	Dams	PND 10 and 20	MG whole mounts, scored 1-4; H&E slides also evaluated.	Delayed development	5 GD 8-17 exposure (PND 10)	5 GD 12-17 exposure (PND 10). All treated groups on PND 20.	- No effect in non-pregnant adult females dosed for 17 days. - Expression of milk proteins changed on PND 10 and 20, consistent with delays on PND 20.
					Female Pups		MG whole mounts, scored 1-4	Delayed development	--------	5, GD 8-17 exposure	

* NOAELs are defined as the highest dose that did not produce a statistically significant ($p<0.05$) effect, and LOAELs are defined as the lowest doses with statistically significant ($p<0.05$) effects.

Table 14A. Summary of publications/studies evaluating effects of PFOA on mammary gland (MG) development in mice – Includes studies with exposure during pregnancy, gestation, and/or lactation (6 publications/10 studies). (Note: Other effects evaluated in these studies are discussed in other tables in main document and/or individual study tables in Appendix 5)

Citation	*Strain*	*Lifestage(s) for Dosing*	*Dose(s) (mg/kg/day)*	*Dosing Duration*	*Lifestage for MG Assessment*	*Timepoint for MG Assessment*	*Endpoint(s) Assessed*	*Effect*	*NOAEL* (mg/kg/day)*	*LOAEL* (mg/kg/day)*	*Comments/Other Mammary Gland Effects*
White et al. (2009)	CD-1	Gestation	0, 5	GD 8-17 (Cross-fostering: exposure during gestation, lactation, or both)	Dams	PND 1,3,5, 10	MG whole mounts, numerical data not shown.	Delayed development	-------	5 In both treated dams nursing treated or untreated pups beginning on PND 1, and untreated dams nursing treated pups beginning on PND 3.	
					Female pups		MG whole mounts, scored 1-4	Delayed development	-----	5 All treated groups beginning on PND 1, including lacation only exposure (serum level ~2000 ng/ml).	
White et al. (2009)	CD-1	Gestation	0, 3, 5	GD 1-17 (Cross-fostering: exposure during gestation and/or lactation)	Female pups	PND 22, 42, 63, and 18 months	MG whole mounts, scored 1-4	Delayed development	-----	3 All treated groups including gestation or lactation only exposure beginning PND 22 and persisting to PND 63.	At 18 months, changes in MG persisted in treated groups (whole mounts and H&E slides) including increased number of darkly staining foci per gland and reduced epithelial density. These effects could not be scored using the criteria that were used at earlier time points.
White et al. (2009)	CD-1	Gestation	0, 5	GD 15-17, 13-17, 10-17, 7-17 "Restricted Exposure"	Female pups	PND 29, 32 and 18 months	MG whole mounts, scored 1-4	Delayed development	------	5, GD 15-17 exposure.	At 18 months, changes in MG persisted in treated groups (whole mounts and H&E slides) including increased numbers of darkly staining foci per gland in all treated groups.

* NOAELs are defined as the highest dose that did not produce a statistically significant ($p<0.05$) effect, and LOAELs are defined as the lowest doses with statistically significant ($p<0.05$) effects.

Table 14A. Summary of publications/studies evaluating effects of PFOA on mammary gland (MG) development in mice – Includes studies with exposure during pregnancy, gestation, and/or lactation (6 publications/10 studies). (Note: Other effects evaluated in these studies are discussed in other tables in main document and/or individual study tables in Appendix 5)

Citation	*Strain*	*Lifestage(s) for Dosing*	*Dose(s) (mg/kg/day)*	*Dosing Duration*	*Lifestage for MG Assessment*	*Timepoint for MG Assessment*	*Endpoint(s) Assessed*	*Effect*	*NOAEL* (mg/kg/day)*	*LOAEL* (mg/kg/day)*	*Comments/Other Mammary Gland Effects*
											These effects could not be scored using the criteria used at earlier time points
White et al. (2011b)	CD-1	Gestation and/or postnatal (multi-generation study)	GD 1-17 (0, 1, 5 mg/kg/day) and/or 5 ppb in drinking water (through PND 22 in P0 and F1 dams; through PND 63 in F1 and F2 pups)		P0 Dams	PND 22	MG whole mounts, scored 1-4. H&E slides for some groups.	Delayed development	---- (P0 dams)	1 mg/kg/day (exposure P0 GD 1-17) *or* 5 ppb in drinking water (exposure P0 GD 7- F1 PND 22)	In lactational challenge test in F1 dams and F2 litters on PND 10, milk volume was decreased and time to initiate was increased in all treated groups. However, these changes were not statistically significant. High variability limited power to detect statistically significant differences.
					F1 Dams	PND 10, 22			---- (F1 dams on PND 10).	On PND 10: 1 mg/kg/day (exposure P0 GD 1-17) *or* 5 ppb in drinking water (exposure P0 GD 7- F2 PND 22)	
					F1 Female pups	PND 22,42, 63			----- (F1 pups)	1 mg/kg/day (exposure P0 GD 1-17) *or* 5 ppb in drinking water (exposure P0 GD 7- F1 PND 63). Serum PFOA level in 5 ppb group was 21.3 ng/ml	
					F2 Female pups	PND 10, 22, 42, 63			No significant effects at most doses and time points. Significant delays at 3 of 16 data points. (F2 pups)		

* NOAELs are defined as the highest dose that did not produce a statistically significant ($p<0.05$) effect, and LOAELs are defined as the lowest doses with statistically significant ($p<0.05$) effects.

Table 14B. Summary of publications/studies evaluating effects of PFOA on mammary gland (MG) development in mice – Studies with peripubertal exposure (3 publications)

Citation	*Strain*	*Lifestage(s) for Dosing*	*Dose(s) (mg/kg/day)*	*Dosing Duration*	*Lifestage for MG Assessment*	*Timepoint for MG Assessment*	*Endpoint(s) Assessed*	*Effect*	*NOAEL* (mg/kg/day)*	*LOAEL* (mg/kg/day)*	*Comments/Other Mammary Gland Effects*
Yang et al. (2009a)	Balb/C C57Bl/6	Peripubertal	0,1,5,10	4 weeks, starting at 3 wks of age.	Peripubertal females	7 weeks of age	MG whole mounts. Ductal length, # terminal end buds, # terminal ducts	*Balb/C* - Delayed development *C57Bl/6* - Stimulated development & totally inhibited development	*Balb/C* – 1 *C57Bl/6* - 1	*Balb/C* – 5 *C57Bl/6* - Stimulation at 5 *Total inhibition at* 10	MG cell proliferation measured by BrDu incorporation: - In Balb/C, proliferation of MG cells was inhibited at 1 and 5 mg/kg/day. - In C57Bl/6, proliferation of MG cells was stimulated at 1 and 5 mg/kg/day, and totally stopped at 10 mg/kg/day Serum PFOA data provided in Zhao et al. (2012)
Zhao et al. (2010)	C57Bl/6 Wild Type Ovariecto-mized (OVX)	Peripubertal. OVX at 3 weeks (before PFOA treatment)	0, 5	4 weeks starting at 4 weeks of age	Peripubertal OVX females	8 weeks of age	MG whole mounts scored based on longitudinal growth, # of terminal end buds, stimulated/ enlarged terminal ducts. Numerical data not shown.	Total inhibition of development in both control and PFOA-treated OVX mice	NA	NA	Groups of non-OVX mice were not included in these studies.
	C57Bl/6 PPAR-alpha null (KO)	Peripubertal. OVX at 7 weeks (after PFOA treatment)		4 weeks starting at 3 weeks of age	Peripubertal OVX Females, treated with saline, or progesterone and/or estradiol after OVX	9 weeks of age		In mice treated with hormones, stimulation of development by PFOA compared to controls (no PFOA).	NA	NA	
		Peripubertal		4 weeks, starting at 3 wks of age.	Peripubertal females	7 weeks of age		Stimulated development	----	5	C57Bl/6 WT were not included in this study. Serum PFOA data provided in Zhao et al. (2012)

* NOAELs are defined as the highest dose that did not produce a statistically significant ($p<0.05$) effect, and LOAELs are defined as the lowest doses with statistically significant ($p<0.05$) effects.

Table 14B. Summary of publications/studies evaluating effects of PFOA on mammary gland (MG) development in mice – Studies with peripubertal exposure (3 publications))

Citation	***Strain***	***Lifestage(s) for Dosing***	***Dose(s) (mg/kg/day)***	***Dosing Duration***	***Lifestage for MG Assessment***	***Timepoint for MG Assessment***	***Endpoint(s) Assessed***	***Effect***	***NOAEL* (mg/kg/day)***	***LOAEL* (mg/kg/day)***	***Comments/Other Mammary Gland Effects***
Zhao et al. (2012)	Balb/C C57Bl/6 (WT) C57Bl/6 PPAR-alpha null (KO)	Peripubertal	Balb/C: 0, 2.5 C57Bl/6 WT and KO: 0, 7.5	4 weeks, starting at 3 wks of age.	Peripubertal females	7 weeks of age	MG whole mounts. Ductal length, # terminal end buds, # terminal ducts	Balb/C – Delayed development C57Bl/6 WT – Delayed development KO – no effect	*Balb/C & C57Bl/6 WT* – ------ *C57Bl/6 KO* - 7.5	*Balb/C* – 2.5 *C57Bl/6 WT* – 7.5 *C57Bl/6 KO* – --------	- PFOA doses in all 3 strains of mice in this study are different than those used in earlier studies of these strains (Yang et al., 2009a; Zhao et al., 2010). Doses from the earlier study in each strain were not repeated in this study. Data from different studies may not be directly comparable. - Lack of inhibition of MG development in KO mice may be due to lower serum PFOA levels rather than PPAR status. - Serum PFOA levels for this study and the 2 earlier studies are presented in this study.

* NOAELs are defined as the highest dose that did not produce a statistically significant ($p<0.05$) effect, and LOAELs are defined as the lowest doses with statistically significant ($p<0.05$) effects.

MODE OF ACTION

Overview

The mode(s) of action of PFOA are not fully characterized. PFOA structurally resembles a free fatty acid, and it thus may act similarly to a free fatty acid in activating nuclear receptors, binding to transporters and carrier proteins, and interacting with membranes (Butenhoff, 2009). However, it is non-reactive and therefore is not a substrate for biochemical reactions involving fatty acids. It also has been shown to have estrogenic activity and to act through other mechanisms. The mode of action for some of the effects of PFOA is unknown. A summary of information on PFOA's mode of action, with emphasis on potential human relevance, is presented below.

Genotoxicity

Since PFOA is not chemically reactive, it is not metabolized to reactive intermediates and does not covalently bind to nucleic acids and proteins. Therefore, it is considered unlikely to be genotoxic. Information on the genotoxicity of PFOA was reviewed by Butenhoff et al., (2014). PFOA was not mutagenic or genotoxic in most of a series of *in vitro* assays in bacterial and mammalian cells, with or without metabolic activation. It did not cause chromosomal aberrations in Chinese hamster ovary cells at non-cytotoxic concentrations, although some positive results were reported at cytotoxic concentrations. It did not cause chromosomal aberrations in human lymphocytes in whole blood with or without metabolic activation, and it did not transform cells in culture. Although PFOA caused DNA strand breaks and increased the incidence of micronuclei in cultured human hepatoma cells in a dose-related manner (Yao and Zhong, 2005), high doses of PFOA did not induce micronuclei in mice *in vivo*. In male rats, PFOA increased the levels of 8-hydroxydeoxyguanosine in liver DNA, but not in kidney DNA (Takagi et al., 1991). These effects were accompanied by a significant increase in reactive oxygen species, which the investigators suggested caused the DNA damage. In contrast, PFOA increased the formation of reactive oxygen species but was not genotoxic in *in vitro* studies by other investigators.

PPAR-alpha and Other Nuclear Receptors

PFOA activates the nuclear receptor, peroxisome proliferator-activated receptor-alpha (PPAR-alpha) as well as other nuclear receptors including PPAR-gamma, CAR (constitutive activated receptor), PXR (pregnane X receptor) and estrogen receptor-alpha (reviewed by Peters and Gonzalez, 2011). When activated, nuclear receptors bind to DNA and alter the expression of genes that control many biological processes involved in development, homeostasis, and metabolism. PPAR-alpha and other nuclear receptors are found at varying levels in many tissues in rodents, humans, and other species. PPARs affect many biological processes beyond stimulation of peroxisome proliferation in rodents, the effect for which they were originally named. Levels of PPARs may vary during different stages of development, and their role in development is discussed further below.

Much attention has been focused on the role of PPAR-alpha activation in the toxicity of PFOA and on the potential human relevance of effects that occur through activation of this receptor. The

major role of PPAR-alpha is regulation of energy homeostasis. Levels of PPAR-alpha are highest in tissues with high rates of catabolism of fatty acids and peroxisomal activity, including liver, brown adipose tissue, heart, kidney, and intestine. Hepatic effects of PPAR-alpha include increased fatty acid oxidation, increased degradation of cholesterol, increased gluconeogenesis, synthesis of ketone bodies, and control of lipoprotein assembly. By increasing oxidation of fatty acids, activation of PPAR-alpha decreases serum triglycerides and serum cholesterol and prevents the accumulation of fat in the liver (Michalik et al., 2006). Consistent with PPAR-alpha's inhibition of hepatic lipid accumulation, PPAR-alpha null mice developed fatty livers, which were more severe with a high fat diet, while fatty liver did not occur in wild type mice on either a regular or high fat diet (Abdelmegeed et al., 2011). In addition to its effects on metabolism, activation of PPAR-alpha also decreases inflammatory response. PPAR-alpha activators such as fibrate drugs are used in humans to decrease serum lipids. The anti-inflammatory effects of these drugs may contribute to their actions in preventing atherosclerosis and reducing the incidence of cardiovascular events (Michalik et al., 2006).

In vitro activation of human and rodent PPAR-alpha by PFOA has been evaluated in several studies of cultured cells transfected with plasmids containing PPAR-alpha from these species. As noted by Vanden Heuvel et al. (2006), these *in vitro* assays measure only the first step in the series of complex steps involved in regulation of gene expression by PPAR-alpha. Therefore, interspecies comparisons based solely on these *in vitro* data may not necessarily be valid.

Relative sensitivities of human and rodent PPAR-alpha to PFOA differed among these studies, and the results do not clearly indicate that human PPAR-alpha is less sensitive than rodent PPAR-alpha in *in vitro* systems. Maloney and Waxman (1999) reported that somewhat higher concentrations of PFOA were needed to cause maximal activation of human PPAR-alpha as compared to mouse PPAR-alpha. In contrast, Vanden Heuvel et al. (2006) found that mouse and human PPAR-alpha were similarly responsive to PFOA, while rat PPAR-alpha was less responsive than mouse or human PPAR-alpha. Two studies from the same laboratory reported differing results on the responsiveness of mouse and human PPAR-alpha to PFOA. In the first study (Wolf et al., 2008), human PPAR-alpha was somewhat less responsive to PFOA than mouse PPAR-alpha. In the second study (Wolf et al., 2012), the dose-response curves for activation by PFOA were identical for mouse and human PPAR-alpha.

Many PPAR-alpha activators (e.g. phthalates, trichloroethylene, and perchloroethylene) cause liver tumors in rodents. The human relevance of these tumors is subject to debate because of the lower levels and/or differences in intrinsic activity of hepatic PPAR-alpha in humans as compared to rodents (NRC, 2006; Corton, 2010). However, the uncertainty about human relevance does not necessarily apply to PPAR-alpha mediated effects other than liver tumors, as illustrated by the use of fibrate drugs to decrease cholesterol and lipids in humans by activation of PPAR-alpha.

Furthermore, as discussed in detail below, effects of PFOA clearly occur through both PPAR-alpha independent and PPAR-alpha dependent processes. Therefore, conclusions from studies with pure PPAR-alpha activators such as Wyeth 14,643 (WY) cannot necessarily be extrapolated to PFOA.

Hepatic Toxicity

As discussed in the Epidemiology section, PFOA is associated with increased serum liver enzymes in human. The studies in non-human primates, standard strains of rats and mice, PPAR-alpha null mice, and humanized PPAR-alpha mice that are summarized below support the conclusion that hepatic effects of PFOA in experimental animals are relevant to humans for the purposes of risk assessment.

Data from non-human primates

In the 13-week study in male cynomolgus monkeys (Butenhoff et al., 2002), PFOA increased relative liver weight in a dose-related fashion. Several monkeys that did not complete the study due to overt toxicity exhibited notable increases in liver weight, highly elevated serum liver enzymes, and/or severe hepatic toxicity.

Subcellular biochemical markers were evaluated in the livers of the monkeys that completed the study; animals that had been removed due to toxicity were not included. Hepatic DNA content was decreased by PFOA (statistically significant at highest dose); a marker of peroxisome proliferation (palmitoyl CoA oxidation; PCO) was increased in a dose-related manner (2.6-fold and statistically significant at highest dose; Figure 12); and the mitochondrial enzyme, succinate dehydrogenase, was increased although not in a dose related manner (statistically significant at highest dose). Hepatic alkaline phosphatase (a lysosomal marker) and glucose-6-dehyrogenase (a marker for endoplasmic reticulum) were not affected by PFOA treatment.

Although no histopathological changes were reported in the livers of the treated monkeys that completed the study, the authors state that the dose-related decrease in hepatic DNA content is indicative of hepatocellular hypertrophy. The dose-related increase in peroxisome proliferating activity (PCO) was similar in magnitude to the increases reported in PFOA treated rats (below), demonstrating that substantial hepatic PPAR-alpha activity occurs in response to PFOA in non-human primates. The authors also state that the increased succinate dehydrogenase activity suggests mitochondrial proliferation, although this possibility was not evaluated by microscopic studies.

As discussed below, data from rats (Perkins et al., 2004) indicate that the increase in PCO activity in response to PFOA becomes smaller in magnitude with longer exposure duration, while the increase in relative liver weight remains stable over time. Since PCO data are available only from a single time point (13 weeks) in the monkeys, it is unknown whether they represent the maximum PCO activity in response to PFOA. The USEPA SAB (2006) stated: "Because the available data for PFOA in rats and monkeys indicate similar responses in the livers of rodents and primates (increased liver weight and induction of hepatic peroxisomal enzyme activity), about three quarters of the Panel members shared the view that human relevance for liver effects induced by PFOA by a PPARα agonism mode of action cannot be discounted."

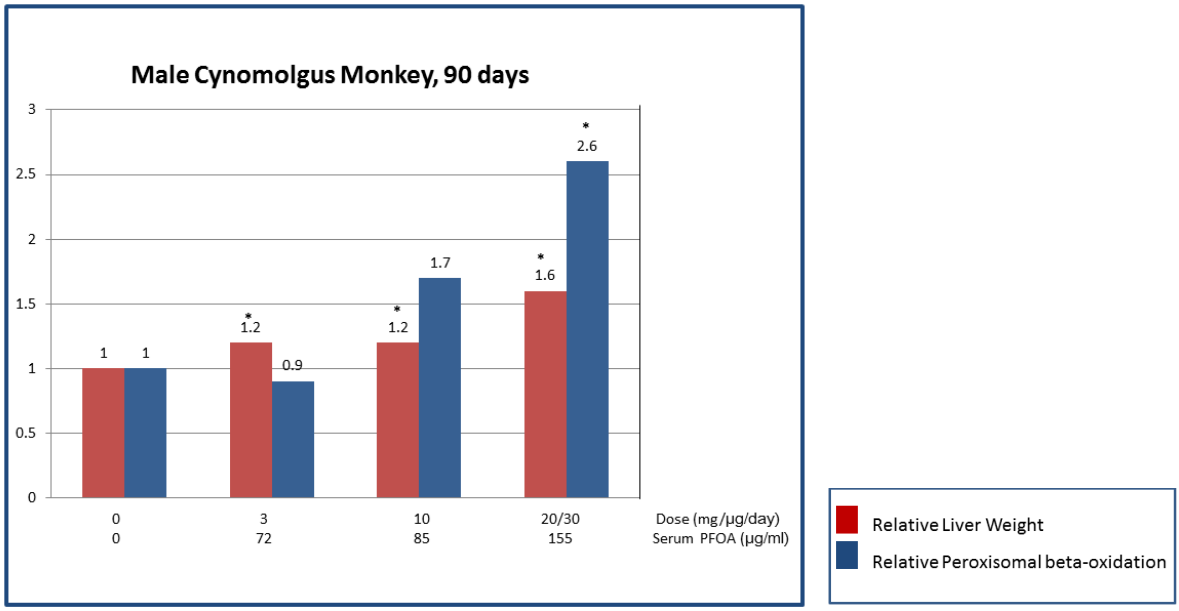


Figure 12. Relative liver weight and relative PCO activity (compared to controls) versus serum PFOA concentrations in male cynomolgus monkeys dosed with PFOA for 6 months (Butenhoff et al., 2002).

Data from a 90-day study of rhesus monkeys (Goldenthal, 1978) also suggest that PFOA caused increased liver weight, although this effect was not statistically significant likely due to the small number of animals.

<u>Data from standard strains of laboratory rodents</u>

In a standard strain of laboratory rats, PFOA activated PPAR-alpha, as indicated by increased PCO activity, and also activated other nuclear receptors, CAR and PXR, as indicated by induction of specific cytochrome P450 proteins associated with these receptors (Elcombe et al., 2010). Similarly, PFOA treatment increased gene expression for cytochrome P450 proteins associated with each of these three receptors in wild type mice (Cheng and Klaasen, 2008).

Loveless et al. (2006) evaluated the effects of three isomeric forms of PFOA (linear isomers, branched isomers, and mixed linear/branched isomers) on relative liver weight and hepatic PCO activity in standard strains of male rats and mice. As can be seen in Figure 13, increased relative liver weight did not correlate with hepatic peroxisome proliferation, as indicated by PCO activity. In mice, liver weight increased with administered dose and serum PFOA level, but PCO activity was lower at the highest administered doses and serum levels than at lower doses and serum levels. Additionally, in rats (Figure 14), branched isomers of PFOA were more potent in increasing relative liver weight than linear isomers, but were less potent in increasing PCO activity. These results illustrate the involvement of PPAR-alpha independent processes in the increased relative liver weight caused by PFOA even in standard strains of rodents with normal PPAR-alpha function.

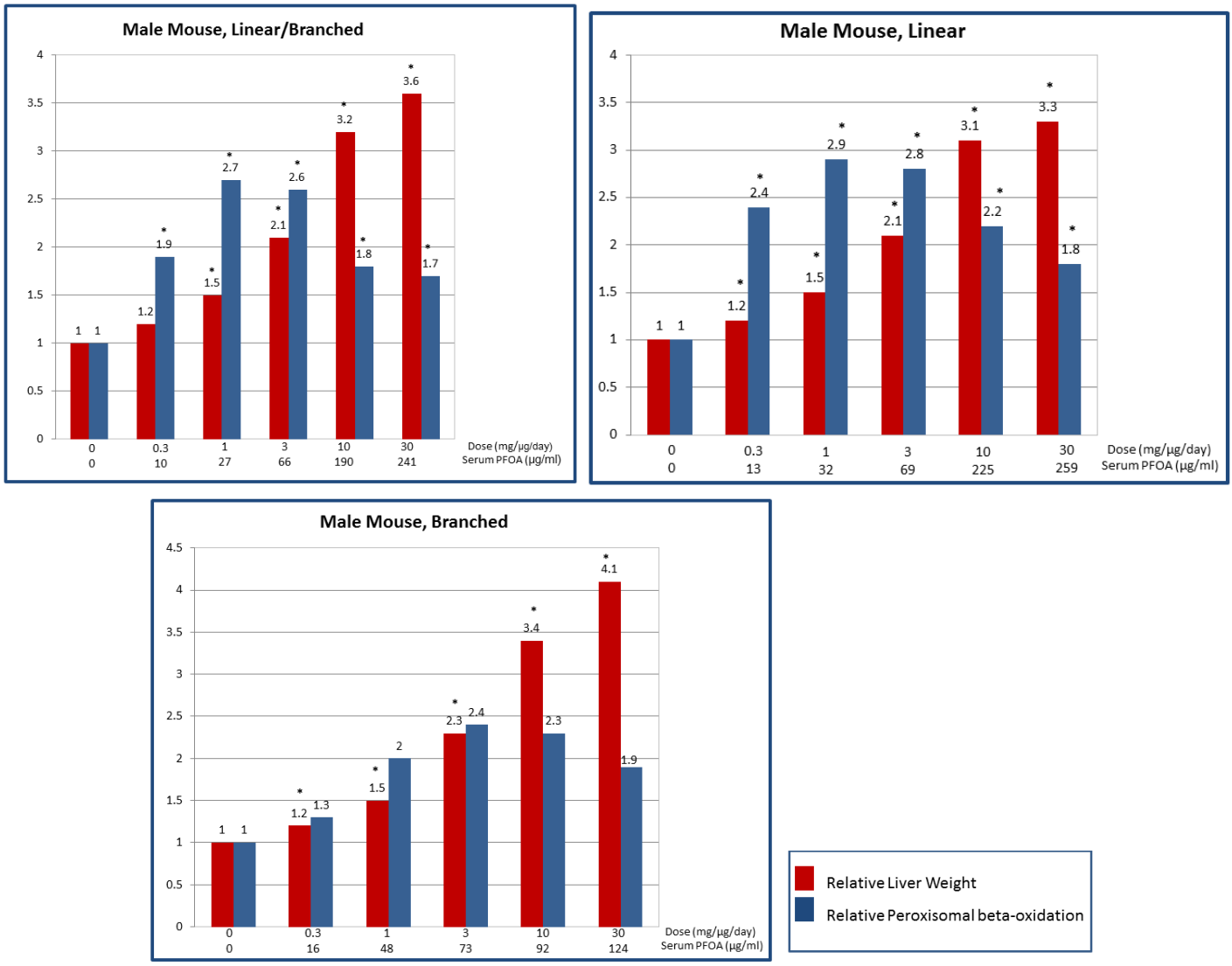


Figure 13. Relative liver weight and relative PCO activity (compared to controls) versus serum PFOA concentrations in male mice dosed with linear/branched, linear, or branched PFOA for 14 days (Loveless et al., 2006).
* $p < 0.05$.

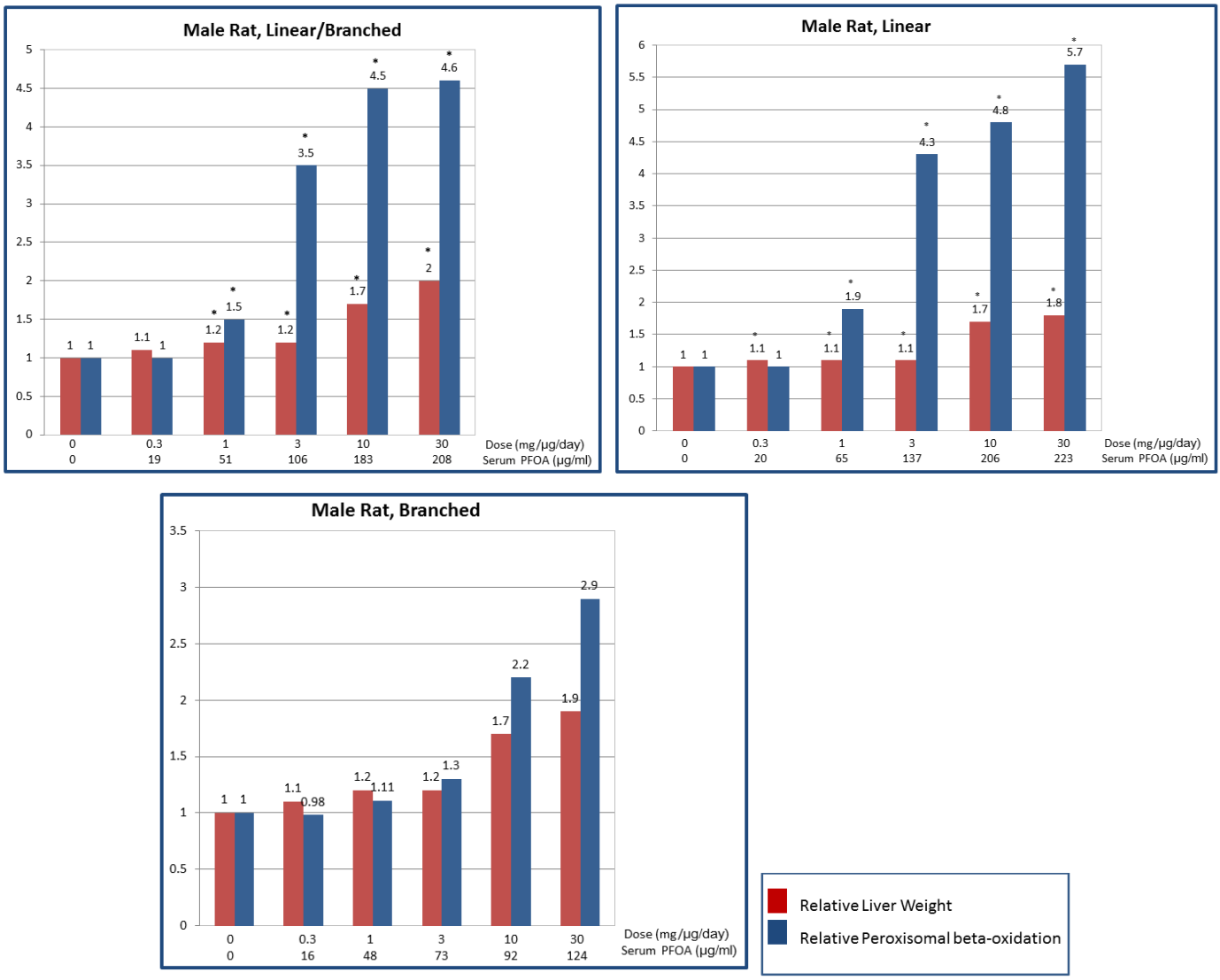


Figure 14. Relative liver weight and relative PCO activity (compared to controls) versus serum PFOA concentrations in male rats dosed with linear/branched, linear, or branched PFOA for 14 days (Loveless et al., 2006).
* *p < 0.05.*

Data from a standard strain of male rats exposed for 4, 7, or 13 weeks (Figure 15) also indicate that PCO activity does not necessarily correlate with the increased liver weight caused by PFOA (Perkins et al., 2004). Although relative PCO activity was much greater at 7 weeks than 4 weeks, and was lower at 13 weeks than at the two earlier time points, the dose-response curve for increased relative liver weight was similar at all three time points.

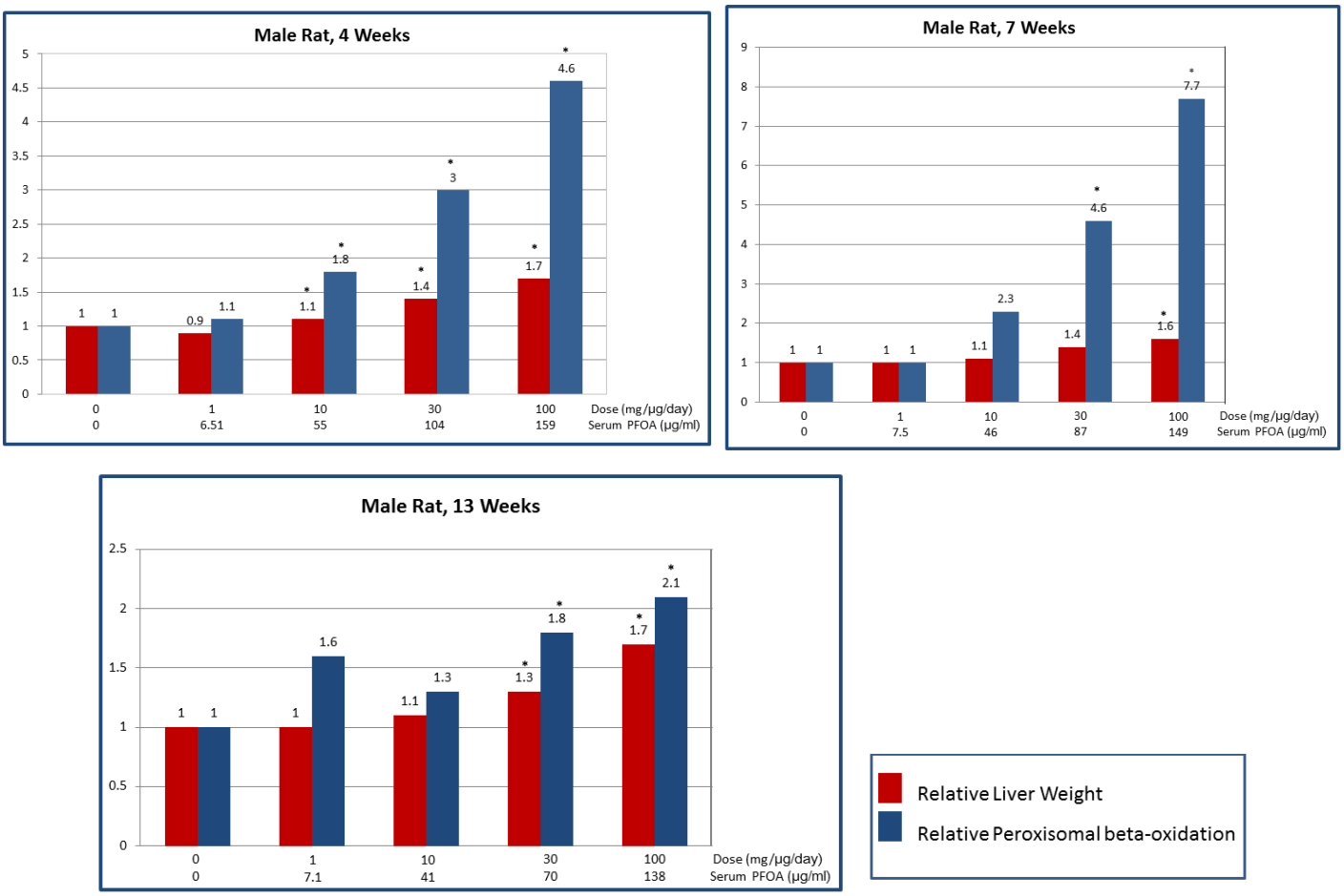


Figure 15. Relative liver weight and relative PCO activity (compared to controls) versus serum PFOA concentrations in male rats and mice dosed with PFOA for 4, 7, or 13 weeks (Perkins et al., 2004).

A recent study of histopathological change in livers of female CD-1 mouse offspring with developmental exposure to PFOA does not support the involvement of peroxisome proliferation in the observed hepatic toxicity (Quist et al, 2015; described in the Animal Toxicology section). Transmission electron microscope studies of the livers found no evidence that PFOA caused peroxisome proliferation, but rather indicated that it caused mitochondrial proliferation and abnormal mitochondrial morphology.

Activation of PPAR-alpha inhibits accumulation of fat in the liver. In contrast, PFOA increased the accumulation of hepatic triglycerides, cholesterol, and phospholipids in a standard strain of laboratory rats, and the authors noted that these effect are not consistent with the actions of other peroxisome proliferators such as clofibric acid and di(2-ethylhexyl) phthalate (Kawashima et al., 1995). Additionally, PFOA caused a dose-dependent increase in hepatic triglycerides in mice exposed to perilla oil and soybean oil, although there was no effect in mice exposed to fish oil (Kudo and Kawashima, 1997). In more recent studies of standard strains of mice given either afregular or high fat diet, PFOA caused increased triglyceride levels in the liver and decreased relative adipose tissue weight in animals on both diets (Tan et al., 2013; Wang et al., 2013).

Data from wild type mice, PPAR-alpha null mice, and humanized PPAR-alpha mice

Studies comparing wild type and PPAR-alpha null mice clearly demonstrate that PFOA causes PPAR-alpha independent and PPAR-alpha dependent hepatic effects. Yang et al. (2002) reported that liver weight was increased in both wild type and PPAR-alpha null mice treated with PFOA (0.02% in the diet) for 7 days, while peroxisomal acyl CoA oxidase (a marker of peroxisome proliferation) was increased only in wild type mice. In contrast, the model PPAR-alpha activator, WY, increased liver weight and peroxisome proliferating activity in wild type mice but had no effect in PPAR-alpha null mice. Based on this study, which was the only study of PFOA in wild type and PPAR- alpha null mice available at the time, the USEPA Science Advisory Board (2006) concluded that PPAR-alpha independent modes of action may be important in hepatic effects of PFOA in rodents. Numerous studies that have been published subsequent to the USEPA SAB (2006) review confirm this conclusion and provide additional relevant information.

Consistent with Yang et al. (2002), Wolf et al. (2008) also reported that PFOA (0, 1, 3, or 10 mg/kg/day for 7 days) increased liver weight similarly in both PPAR-alpha null mice and wild type mice, while WY increased liver weight only in wild type mice. Cell proliferation and hepatocyte hypertrophy were also increased by PFOA in a dose-related fashion in both wild type and PPAR-alpha null mice. However, the livers of wild type and PPAR-alpha null mice treated with PFOA differed histologically. Increased numbers of peroxisomes were seen in the livers of treated wild type mice, while the livers of PPAR-alpha null mice had no peroxisomes but had numerous vacuoles.

Gene profiling studies in wild type and PPAR-alpha null mice treated with PFOA and WY found that PFOA, but not WY, also activates CAR (constitutive activated/androstane receptor), a receptor that is activated by phenobarbital and other compounds, and possibly PXR (pregnane X receptor). Both compounds caused a similar profile of gene changes in wild type mice, including up-regulation of genes involved with PPAR-alpha activation. However, while there were few gene changes in WY treated PPAR-alpha null mice, gene expression was altered in PFOA-treated PPAR-alpha null mice, including some of the same genes affected by PFOA in the wild type mice (Rosen et al., 2008a). Further study (Rosen et al., 2008b) showed that 85% of the genes altered by PFOA were PPAR-alpha dependent. The PPAR-alpha independent genes included genes involved with lipid homeostasis and xenobiotic metabolism, and many were consistent with activation of CAR.

Additional studies suggest that PPAR-alpha may be protective against some types of hepatic toxicity caused by PFOA. Minata et al. (2010) showed that hepatic or biliary damage occurred in both wild type and PPAR-alpha null mice treated with PFOA, but the profile of toxicity differed greatly between the strains. They investigated the effects of PFOA in wild type and PPAR-alpha null mice given 4.4, 10.8, or 21.6 mg/kg/day for four weeks. Concentrations of PFOA in serum and liver were similar in wild type and PPAR-alpha null mice, but were much lower in bile of PPAR-alpha null mice than wild type mice, indicating a much lower capacity for transport of PFOA to bile in the null strain. Relative liver weights were similarly increased in both wild type

and PPAR-alpha null mice at all doses of PFOA, to about three times the control value. Hepatocyte and bile duct injury was assessed by light and electron microscopy, by levels of serum enzymes, bile acids, and bilirubin, and by biochemical markers. Hepatocyte hypertrophy and elevated liver enzymes occurred in both wild type and PPAR-alpha null mice, but bile duct injury was much more severe in PPAR-alpha null mice. PFOA increased the levels of two inflammatory cytokines associated with liver injury in PPAR-alpha null, but not wild type, mice. The authors concluded that PPAR-alpha protects against cholestasis and bile duct injury caused by PFOA in mice. They suggested that rodents may, in fact, be less sensitive to PFOA than humans at some doses.

Finally, the hepatic effects of developmental PFOA exposure in 18-month old offspring of gestationally exposed dams were studied in CD-1, wild type, and PPAR-alpha null mice (Filgo et al., 2015). At this time point long after PFOA has been eliminated from the body, the incidence and/or severity of hepatocellular hypertrophy was increased by prenatal and early life exposure to PFOA in all three strains of mice, and this effect was more pronounced in PPAR-alpha null than wild type mice. Additionally, consistent with Minata et al. (2010), PFOA caused a dose-dependent increase in incidence and severity of bile duct toxicity in PPAR-alpha null, but not wild type, mice. Additional findings from this study related to carcinogenicity of PFOA are discussed below.

Three additional studies evaluated hepatic effects of PFOA in wild type, PPAR-alpha null, and humanized PPAR-alpha (having hepatic expression of human PPAR-alpha) mice of a Sv/129 genetic background (Nakamura et al., 2010; Nagakawa et al., 2011; Albrecht et al., 2013). PFOA caused hepatic toxicity in all three of these mouse strains, including in fetal liver. It should be noted that differences in response in humanized mice are not necessarily indicative of a different response of human PPAR-alpha to PFOA, for reasonse that may include species differences in binding of PFOA to recognition sites on mouse DNA (cognate DNA). PFOA concentrations in liver and serum were measured in only one of these three studies (Albrecht et al., 2013).

Hepatic effects were studied in male wild type, PPAR-alpha null, and humanized PPAR-alpha mice treated with 0, 0.1, or 0.3 mg/kg/day PFOA for two weeks (Nakamura et al., 2009). Relative liver weight was not affected at 0.1 mg/kg/day in any strain and was increased at 0.3 mg/kg/day only in wild type mice. Expression (mRNA and protein levels) of five genes that are targets of PPAR-alpha was measured in the three strains. Expression of four of these genes was significantly increased only in the wild type mice, while expression of the fifth gene was significantly increased only in the humanized PPAR-alpha mice. None of the PPAR-alpha target genes were affected by PFOA in the PPAR-alpha null mice. Histopathological examination showed mild hepatocellular hypertrophy in both wild type and humanized PPAR-alpha mice. Cytoplasmic vacuoles indicating lipid accumulation were observed in PPAR-alpha null mice, consistent with those described in this strain by Wolf et al. (2008).

Interpretation of these results is complicated by the fact that PFOA levels in serum and liver were not measured in this study and may vary between strains. Relative liver weight was increased at 0.3 mg/kg/day in wild type mice, but was not increased at this dose in the other two strains. However, there was no effect in wild type mice at 0.1 mg/kg/day, indicating that 0.3 mg/kg/day is

relatively close to the NOAEL in this strain. In the absence of serum and liver PFOA data, it is not known whether the increased liver weight observed at 0.3 mg/kg/day in wild type, but not PPAR-alpha null or humanized PPAR-alpha, strains is due to an intrinsic difference in sensitivity or to toxicokinetic differences.

Hepatic effects were also evaluated in male wild type, PPAR-alpha null and humanized PPAR-alpha mice treated with 0, 1, or 5 mg/kg/day for six weeks (Nagakawa et al., 2011). The results of this study do not support the conclusion that rodent PPAR-alpha is required for the hepatic toxicity of PFOA. Hepatocyte hypertrophy occurred in both strains with PPAR-alpha activity (wild type and humanized), and activity of the liver enzyme ALT was increased in all three strains including PPAR-alpha null. Single cell hepatocyte necrosis occurred in all three strains, but hydropic degeneration of hepatocytes occurred only in PPAR-alpha null and humanized mice. Macrovesicular steatosis and inflammatory cells were found only in the PPAR-alpha null mice, while microvesicular steatosis occurred in the PPAR-alpha null and humanized strains but not the wild type strain. Liver triglyceride and cholesterol levels were increased several fold in the PPAR-alpha null mice, but not in the other strains, while plasma triglycerides were decreased only in the wild type mice. The authors concluded that PPAR-alpha may be important in protecting from hepatic damage caused by PFOA at the doses used in the study. They also concluded that, since hepatic PPAR-alpha may be weaker in its function and present in lower amounts in humans than in mice, humans may be susceptible to hepatic damage from similar doses of PFOA.

Finally, hepatic effects of PFOA were evaluated in pregnant wild type, PPAR-alpha null and humanized PPAR-alpha mice and their offspring after exposure to 3 mg/kg/day on GD 1-17 (Albrecht et al., 2013). Serum and liver PFOA levels were measured in dams and fetuses on GD 18, and in dams and offspring on PND 20. There were large differences in serum and/or liver PFOA concentrations among strains, and these differences were not consistent at the different life stages and time points. Therefore, differences in responses to PFOA among the strains may be due to toxicokinetic differences in addition to, or instead of, intrinsic strain differences in sensitivity to PFOA's effects.

In dams on GD 18, relative liver weight was significantly increased to a similar degree in all three strains. Expression of two target genes for PPAR-alpha was increased by PFOA in both wild type and humanized PPAR-alpha dams, but not PPAR-alpha null dams, although the increase in expression of one of the genes was not significant in the humanized mice. Expression of target genes for CAR and PXR was increased by PFOA in all three strains, with the biggest increase in the humanized PPAR-alpha dams. Minimal to mild hepatocellular hypertrophy was observed by histopathological examination in all three strains, although the morphological features differed among the strains.

On PND 20, relative liver weight remained increased in the wild type dams treated with PFOA, but not in PPAR-alpha null or humanized PPAR-alpha strains. Expression of a gene associated with PPAR-alpha was also increased by PFOA only in wild type dams. Expression of a gene associated with CAR was not affected in any strain, while expression of a gene associated with PXR

remained significantly increased in all three strains. Histopathological changes were observed in livers from PFOA-treated dams of all three strains, but were decreased in incidence and severity compared to on GD 18 in wild type and humanized PPAR-alpha dams.

In fetuses on GD 18, relative liver weight was significantly increased in wild type and humanized PPAR-alpha, but not PPAR-alpha null, mice. Expression of two genes associated with PPAR-alpha was significantly increased in wild type and humanized fetal livers, but not in PPAR-null. The increased expression compared to controls was greater for both genes in the humanized PPAR-alpha fetuses than in the wild type fetuses, and the increase for one of them was about 5-fold greater in humanized than wild type (humanized PPAR-alpha significantly different from wild type). Fetal expression of a gene associated with CAR was not affected by PFOA in any of the three strains, and expression of a gene associated with PXR was increased only in the humanized PPAR-alpha fetal liver. Histopathological changes consistent with peroxisome proliferation were seen in some wild type mice, while no definitive changes were seen in the other strains.

In offspring on GD 20, relative liver weight and expression of genes associated with PPAR-alpha were increased only in wild type mice. Expression of a gene associated with CAR was increased in all three strains, with the largest increase in the PPAR-null offspring, while expression of a gene associated with PXR was increased in wild type and humanized, with the larger increase in wild type. At this time point, histopathological changes in PFOA-treated wild type offspring were similar to those on GD 19, while changes were not clearly evident in livers from the other two strains.

These results indicate that hepatic effects of PFOA in adult, fetal, and neonatal mice are mediated by human PPAR-alpha and through PPAR-alpha independent pathways, as well as by mouse PPAR-alpha. As noted above, interpretation of these results is complicated by the fact that PFOA levels in serum and liver differed among the strains in dams, fetuses, and offspring. For example, serum and liver PFOA levels were lower in humanized dams and offspring than in wild type dams and offspring on PND 20. Therefore, it is unknown whether the greater hepatic effects in wild type than humanized PPAR-alpha mice at this time point are due to an intrinsic difference in sensitivity or to toxicokinetic factors.

Serum cholesterol and lipids

As discussed above, activation of PPAR-alpha causes decreased serum cholesterol and triglycerides in both humans and experimental animals, and this is the basis for the use of fibrates as cholesterol-reducing agents in humans. Consistent with other PPAR-alpha activators, PFOA decreased serum lipids levels in rodents in a number of studies (e.g. Loveless et al., 2006, and other studies summarized in Rebholz et al., 2016). In contrast, as discussed in the Epidemiology section, serum PFOA has been consistently associated with increased serum cholesterol, suggesting a human versus rodent difference in the effect of PFOA on this endpoint.

Two recent studies suggest the possibility that dietary factors, specifically differences in dietary fat content, may contribute to the observed differences in effects of PFOA on serum lipids in rodents

versus humans. Male Balb/C mice on a regular diet or a high fat diet were dosed with 0, 5, 10, or 20 mg/kg/day PFOA for 14 days (Tan et al., 2013). Consistent with earlier mouse studies, both serum cholesterol and serum triglycerides decreased in a dose-related fashion in mice on the regular diet. In contrast, serum cholesterol and triglycerides were unaffected by PFOA in mice on the high fat diet. Additionally, hepatic toxicity from PFOA was more severe in mice on the high fat diet (discussed in Animal Toxicity section).

In another study, male and female C57BL/6 and BALB/c mice were fed a high fat diet containing 32% of calories from fat (similar to the mean of 33% calories from fat in the U.S. diet; CDC, 2016) and 0.25% cholesterol for 6 weeks (Rebholz et al., 2016). In both sexes of C57BL/6 mice and male Balb/c mice, plasma cholesterol was significantly increased by PFOA treatment (3.5 ppm in the diet, resulting in a dose of about 0.5 mg/kg/day), while PFOA had no effect on plasma cholesterol in female Balb/C mice. These results contrast with the decreased serum cholesterol and triglycerides caused by PFOA reported in other studies in which rodents were fed a standard laboratory diet (e.g. containing 13% of calories from fat and 0.02 % cholesterol; LabDiet, 2016). Interestingly, Minata et al. (2010) also observed that cholesterol was increased at the highest PFOA dose, and triglycerides were increased at all doses, in PPAR-alpha null mice.

Immune System Toxicity

Effects of PFOA on the immune system in humans and experimental animals are discussed in the Epidemiology and Toxicology sections above. Additionally, immunotoxicity of PFOA and other PFCs, including mode of action studies, was reviewed by DeWitt et al. (2012). PFOA suppressed the immune system in studies of both non-human primates and mice. Effects include decreased absolute and relative spleen and thymus weights, decreased thymocyte and splenocyte counts, decreased immunoglobulin response, and changes in total numbers and/or specific populations of lymphocytes in the spleen, thymus, peripheral blood, and bone marrow. Based on the available data, rats are less sensitive than mice to immunotoxic effects of PFOA. Immune system effects were not observed in the two studies of rats; these studies included doses higher than those which generally caused immune effects in mice. In humans, exposures to PFOA within the general population range have been associated with decreased antibody levels in response to vaccination. Fletcher et al. (2009) also reported several statistically significant associations between several markers of immune function (decreased IgA, decreased IgE in females only, increased anti-nuclear antibody, decreased C-reactive protein) and serum PFOA levels in communities with drinking water exposure to PFOA in an unpublished C8 Science Panel report.

PPAR-alpha dependence of immune effects in rodents and humans

Two studies in wild type and PPAR-alpha null mice indicate that PFOA's effects on the immune system in mice occur through both PPAR-alpha dependent and PPAR-alpha independent mechanisms. Yang et al. (2002b) studied PFOA's effects on immune response in wild type and PPAR-alpha null mice given 0.02% PFOA in the diet (resulting in a dose of 30 mg/kg/day) for 7 days. Although food intake was similar in the treated wild type and PPAR-alpha null mice, body weight was significantly reduced by PFOA in the wild type mice, but not the null mice, suggesting that weight loss is related to activation of PPAR-alpha, possibly through PFOA's effects on

metabolism, rather than to non-specific toxicity. Consistent with studies discussed in the section on hepatic effects (above), liver weights were increased by PFOA similarly in wild type and null mice, but peroxisome proliferation (as measured by PCO oxidation) occurred only in wild type mice, indicating that increased liver weight was not PPAR-alpha dependent. PFOA caused some immune system effects in wild type but not PPAR-alpha null mice, including decreased spleen weight and splenocyte numbers, and reduced splenocyte proliferation in response to mitogens. However, PFOA also caused other effects (reduced thymus weight and thymocyte numbers, and alterations in the distribution of thymocyte subpopulations) in the null mice, although to a lesser degree than in the wild type mice. These findings led the authors to conclude that PFOA's effects on the immune system are due to mechanisms both dependent and independent of PPAR-alpha.

DeWitt et al. (2009a) studied C57 and Sv/129 wild type and PPAR-alpha null mice exposed to 30 mg/kg/day PFOA in drinking water for 15 days to determine the involvement of PPAR-alpha in the immunotoxic effects of PFOA. Sensitivity to PFOA toxicity appeared to differ in the C57 and the Sv/129 mice, regardless of their PPAR-alpha status, as PFOA treatment decreased body weight, spleen weight, thymus weight, or antibody titer in the C57 wild type mice but did not have these effects in the Sv/129 wild type mice. IgM titer was decreased in both C57 and Sv/129 PPAR-alpha null mice, although this effect did not occur in the wild type Sv/129 mice, suggesting that this effect is not PPAR-alpha dependent. Relative liver weight was similarly increased in all four types of mice given 30 mg/kg/day PFOA in drinking water for 15 days, indicating that this response was not dependent on strain or PPAR-alpha status.

A more recent study suggests that the T-cell dependent antibody response (TDAR) in mice is independent of PPAR-alpha. DeWitt et al. (2016) evaluated the IgM response to immunization with a T-cell dependent antigen (sheep red blood cells: SRBC) in wild type and PPAR-alpha C57Bl/6 null mice exposed to 0, 7.5, or 30 mg/kg/day PFOA for 15 days. PFOA at 7.5 mg/kg/day did not affect TDAR, and production of sheep red blood cell-specific antigens was inhibited equally in wild type and PPAR-alpha null mice at 30 mg/kg/day.

In the C8 Health Study population, serum PFOA was significantly associated with a strong downward trend with C-reactive protein, a liver protein that is a marker for inflammation (Fletcher et al., 2009). Genser et al. (2015) found consistent and significant associations of serum PFOA with decreased C-reactive protein, both within each of the six water districts included in the study and on an aggregated basis. They concluded that these within- and between-district associations strengthen the evidence of causality for this effect. Fibrate drugs act through activation of PPAR-alpha to decrease C-reactive protein in humans (Kleemann et al., 2003; Wagner et al., 2011). These drugs also cause decreases in other markers of inflammatory response *in vivo* in humans and animals, and *in vitro* through PPAR-alpha activation (Kleemann et al., 2003; Budd et al., 2007; Wagner et al., 2011). As it is well established that PFOA also activates PPAR-alpha, the decrease in C-reactive protein in populations exposed to PFOA through drinking water may have similarly occurred through PPAR-alpha activation.

Additionally, statins are a group of drugs that inhibit HMG-CoA reductase, a hepatic enzyme involved with cholesterol synthesis. Statins also activate PPARs including PPAR-alpha (Balakumar and Mahadevan, 2012). Two recent studies have reported that these drugs decrease the effectiveness of influenza vaccines in humans (Black et al., 2016; Omer et al., 2016). As above, it is well established that PFOA is an activator of PPAR-alpha, and HMG-CoA reductase activity was reduced by 50% in rats treated with 0.02% PFOA in the diet (22.7 mg/kg/day) for 7 days (Haughom and Spydevold, 1992). Taken together, these observations suggest that the decreased immune response associated with PFOA in humans may also be related to these effects.

Finally, an *in vitro* study in the human promyelocytic cell line THP-1 showed that PPAR-alpha plays a role in the immunotoxicity of PFOA in human cells (Corsini et al., 2011). Incubation of these cells with 100,000 ng/ml PFOA inhibited the lipopolysaccharide-stimulated release of two pro-inflammatory cytokines (TNF-alpha and IL-8) and matrix metallopeptidase-9, a molecule which plays a role in mobilization of inflammatory cells. This inhibition was reversed when PPAR-alpha was silenced by the addition of small interference RNA specific for PPAR-alpha.

<u>Role of corticosterone production secondary to stress in immune effects in mice</u>
Loveless et al. (2008) studied immune system and other effects in male CD rats and CD-1 mice given linear APFO by gavage at 0, 0.3, 1, 10, and 30 mg/kg/day for 29 days. This study evaluated the role of corticosterone in the immunotoxicity of PFOA. Hepatic effects from this study were described above.

Immunotoxicity caused by PFOA in the mice included decreased relative spleen weight at 1 mg/kg/day and above, and decreased numbers of spleen and thymus cells, decreased thymus weight, thymic depletion/atrophy, granulocytic bone marrow hyperplasia, and decreased anti-SRBC IgM titer at 10 and 30 mg/kg/day. The authors stated that the LOAEL for immunotoxic effects was 10 mg/kg/day, based on decreased IgM titers. However, the significant decrease in relative spleen weight at 1 mg/kg/day suggests that the LOAEL may actually have been 1 mg/kg/day.

Corticosterone levels were above the normal value of 400 ng/ml in 7 of 10 mice given 10 mg/kg/day PFOA and in 6 of 10 mice given 30 mg/kg/day PFOA, and decreased IgM correlated with increased corticosterone for individual mice ($p<0.002$). Based on this observation, the authors suggested that the IgM decreases from PFOA were secondary to stress-related increases in corticosterone.

In this study, rats were less sensitive to immunotoxic effects of PFOA than mice, and there were no effects of PFOA on spleen or thymus weight or on antibody production in the rats. Corticosterone levels were increased above the level considered normal (300 ng/ml) in 2 of 10 rats in the 10 mg/kg/day group and 4 of 10 rats in the 30 mg/kg/day group.

DeWitt et al. (2009b) further investigated the hypothesis that immune effects of PFOA are secondary to corticosterone production in response to stress by studying adrenalectomized and

sham-operated mice. Female C57BL/6N female mice (adrenalectomized or sham-operated) were dosed with 0, 7.5, or 15 mg/kg/day PFOA in drinking water for 10 days. Corticosterone levels were much higher in untreated sham-operated than adrenalectomized mice, and PFOA increased the levels of this hormone in sham-operated, but not adrenalectomized, animals. However, the immunotoxic effects of PFOA were not reduced by adrenalectomy, as SRBC-specific IgM levels were significantly reduced by 7.5 mg/kg/day PFOA in the adrenalectomized, but not sham-operated, mice, and in both groups of mice by 15 mg/kg/day PFOA. These authors concluded that the suppression of IgM synthesis by PFOA was not secondary to stress-related corticosterone production.

Developmental toxicity

As discussed in the Epidemiology section, a systematic review of relevant epidemiological evidence concluded that there is "sufficient" human evidence (the highest strength of evidence in the evaluation scheme) that developmental exposure to PFOA reduces fetal growth in humans (Johnson et al., 2014). The developmental effects of PFOA in mice have PPAR-alpha dependent and independent components (Dewitt et al., 2009; Abbott et al., 2007) and may involve toxicity to the placenta. Based on the information presented below, developmental effects of PFOA in laboratory animals are considered relevant to humans for the purposes of risk assessment.

The USEPA Science Advisory Board (2006) concluded that PPAR-alpha mediated effects of PFOA in human fetuses, neonates, and children are of potential concern because there was minimal information about human levels of PPAR-alpha during these life stages. Subsequent to the USEPA SAB (2006) review, additional relevant information on the role of PPARs in human development became available. It is now known that PPAR-alpha, -beta, and -gamma are expressed in many fetal and adult tissues in rodents and humans. Abbott et al. (2010, 2012) found that PPARs are present in nine human fetal tissues examined (liver, heart, lung, kidney, intestine, stomach, adrenal, spleen, and thymus) from embryonic days 54 to 125. They found that the levels may increase or decrease with age of the fetus, or between the fetus and the adult. In some fetal tissues, PPARs were expressed at levels equivalent to or higher than in adults. Although the role of PPAR-alpha and other PPARs in human and animal development is not well characterized, based on their physiological actions, they are expected to have important roles in reproduction and development (Abbott et al., 2010). For these reasons, it can be assumed that PPAR-alpha mediated effects on development are relevant to humans.

In 129S1/SvlmJ wild type and PPAR-alpha null mice dosed with PFOA on GD 1-17, full litter resorptions and increased pup liver weight caused by PFOA were independent of PPAR-alpha, while PFOA caused postnatal mortality, delayed eye opening, and decreased weight gain in wild type, but not PPAR-alpha null mice (Abbott et al., 2007). More recently, Albrecht et al. (2013) studied effects of gestational exposure of PFOA in wild-type, PPAR-alpha null, and humanized PPAR-alpha (expressing human PPAR-alpha) mice. They stated that PFOA decreased postnatal survival in wild type mice, but not PPAR-alpha null or humanized PPAR-alpha mice, suggesting that the humanized PPAR-alpha strain is less sensitive to this effect. However, a detailed review

of this study, presented in the Animal Toxicology section, suggests that a firm conclusion on this point cannot be made from the data presented.

In the lungs and the liver of fetal (GD 18) CD-1 mice exposed during gestation, PFOA primarily affected the expression of genes related to intermediary metabolism and inflammation, including genes both associated and not associated with PPAR-alpha activation (Rosen et al., 2007). The authors suggested that PFOA tends to shift metabolism in the direction of a fasted animal, consistent with the metabolic changes and obesity in adult mice exposed to PFOA during gestation (Hines et al., 2009).

Developmental exposure to PFOA was found to alter the expression pattern of PPAR-alpha, -beta, and –gamma in many tissues in fetal and neonatal CD-1 mice (Abbott et al., 2012). The expression of genes regulated by PPARs and other nuclear receptors (CAR and PXR), including genes involved with homeostatic control of lipid and glucose metabolism, was also altered as early as GD14. The authors suggested that these effects on metabolism could contribute to the neonatal mortality and decreased rate of growth caused by gestational exposure to PFOA.

Wolf et al. (2010) discussed the potential human relevance of developmental effects of PFNA that are mediated by PPAR-alpha in mice, and these comments are applicable to PFOA as well. They state: "Relevance of the PPAR-alpha mechanism to humans has been criticized primarily based on the lower number of these receptors in the liver of human versus mouse. However, PPAR-alpha is implicated here in the developmental effects of PFNA as well, and the etiology of PPAR-alpha in other tissues of the embryo, fetus and neonate of the human and the mouse that are involved in gross development has not been fully determined. Therefore, the possibility of relevance of PPAR-alpha to a human response to PFNA cannot be dismissed."

Although many of the developmental effects of PFOA appear to be PPAR-alpha dependent, PFOA's developmental effects are not shared by other potent PPAR-alpha activators. Palkar et al. (2010) evaluated the developmental effects of two well-studied PPAR-alpha activating compounds, WY and clofibrate, in wild type and PPAR-alpha null mice. Pregnant mice were given doses of WY and clofibrate known to cause the same increase in maternal liver weight that is produced by doses of PFOA that also cause developmental toxicity. As expected, both WY and clofibrate caused increased maternal relative liver weight on GD 18 in wild type, but not PPAR-alpha null, mice. However, WY did not increase fetal liver weight on GD 18, although PFOA caused this effect on GD 18 and/or at early postnatal time points (GD 1, GD 4) in other studies (Albrecht et al., 2013; Macon et al., 2011; White et al., 2009). Although WY did not increase fetal liver weight, both WY and clofibrate increased expression of two genes associated with PPAR-alpha in fetal and maternal liver of wild type mice on GD 18. Furthermore, unlike PFOA, which is considered a low affinity PPAR-alpha agonist, the two higher affinity PPAR-alpha activators, WY and clofibrate, had no effect on reproductive and developmental parameters such as litter loss, number of live pups, fetal growth, day of eye opening, or post-natal mortality in either wild type or PPAR-alpha null mice. Although the reasons for these differences between PFOA and the two higher affinity PPAR-alpha activators is not known, these results suggest that PFOA may cause

increased fetal liver weight, and reproductive and developmental effects, through a mechanism unrelated to PPAR-alpha activation.

Toxicity to the placenta may play a role in developmental effects of PFOA such as fetal growth retardation (Suh et al., 2011). In pregnant CD-1 mice treated with PFOA (2, 10, or 25 mg/kg/day) on GD 11-16, fetal and placental weights were decreased in a dose-dependent manner. PFOA also increased resorptions and dead fetuses, and decreased the number of live fetuses, resulting in significant increases in post-implantation loss. Placental efficiency (ratio of fetal weight to placental weight) showed a dose-dependent decrease. Necrotic changes occurred in the placenta at the two higher doses, and decreases in several types of placental trophoblast cells occurred at all doses. These placental trophoblast cells produce prolactin family hormones that are vital to placental and fetal growth, adaptation to physiological stressors, and maintenance of pregnancy. Placental mRNA for these hormones was significantly decreased in all dose groups. Additionally, mRNA for the pituitary-specific transcription factor (Pit-1) that activates the prolactin family genes was also decreased. The authors concluded that reduced placental efficiency due to effects on placental trophoblast cells and placental hormones may play a role in PFOA's reproductive and developmental effects.

Male reproductive effects

As discussed above, PFOA caused toxicity to the male reproductive system in several mouse studies. Additionally, PFCs have also been associated with adverse effects on sperm parameters and/or effects on male reproductive hormones in humans, although these associations are not necessarily specific to PFOA (Joensen et al., 2009; Toft et al., 2012). Additionally, prenatal exposure to PFOA was associated with decreased sperm count and concentration and increased LH and FSH in young men (Vested et al., 2013).

Li et al. (2011) suggest that PPAR-alpha plays a role in male reproductive effects of PFOA and that these effects occur through activation of either mouse or humanized PPAR-alpha. Decreased serum testosterone and an increased percentage of abnormal sperm were statistically significant effects in wild type and humanized PPAR-alpha mice, but statistically significant changes in these parameters did not occur in PPAR-alpha null mice. Additionally, abnormal seminiferous tubules with vacuoles or lack of germ cells were observed in the wild type and humanized PPAR-alpha mice, while no obvious structural changes occurred in the PPAR-alpha null mice.

Other studies suggest that PFOA may decrease levels of enzymes involved with testosterone synthesis (Zhang et al., 2014), disrupt the blood-testis barrier (Lu et al., 2015), and/or affect cellular pathways in the testes leading to increased oxidative stress (Liu et al, 2015). In regard to the latter effect, it was noted by Liu et al. (2015) that the testes are very sensitive to oxidative damage produced by reactive oxygen species, and that oxidative stress is known to be an important cause of male infertility in humans.

Effects on mammary gland development

Because the developmental patterns of the mammary gland are similar in humans and rodents, rodents are considered to be a good model for studying the effects of environmental contaminants on human mammary gland development (Rudel and Fenton, 2009; Fenton et al. 2012; Rudel et al., 2011; Fenton and Birnbaum, 2015; Osborne et al., 2015).

PPAR-alpha null mice are viable, healthy, and fertile (Lee et al., 1995), suggesting that PPAR-alpha is not required for mammary gland development (Yang et al., 2006). However, activation of PPAR-alpha was shown to disrupt maternal mammary gland development in mice in two different mouse models (Yang et al., 2006). First, mammary gland development during pregnancy was impaired in transgenic mice with constitutively activated PPAR-alpha. Additionally, mammary gland development was suppressed in wild type mice administered the PPAR-alpha activator WY during pregnancy, and this effect was decreased when dosing with WY was started later in pregnancy. In contrast, no significant effects on mammary gland development were observed in PPAR-alpha null mice similarly treated with WY during pregnancy.

The role of PPAR-alpha in PFOA's effects on mammary gland development in mice is not known. As discussed in detail above, prenatal and/or neonatal exposures to very low doses of PFOA cause persistent delays in mammary gland development in mice. However, the effects of gestational and/or lactational exposure to PFOA on mammary gland development have not been studied in wild type versus PPAR-alpha null mice. Limited data from two studies of effects of peripubertal exposure to PFOA on mammary gland in wild type and PPAR-alpha null mice (Zhang et al., 2010; Zhang et al., 2012) are insufficient to support a conclusion on this question.

In Zhao et al. (2010), PPAR-alpha null mice were treated with a single dose level of 5 mg/kg/day PFOA, 5 days per week for 4 weeks, starting at age 3 weeks. It was reported that PFOA caused stimulation of mammary gland development in the PPAR-alpha null mice, and, based on this single data point, the authors concluded that PFOA causes PPAR-alpha independent stimulation of mammary gland development. However, numerical data for this effect were not provided, and a similarly treated group of wild type mice was not included for comparison to the PPAR-alpha null mice in the study. A subsequent study (Zhao et al., 2012) reported no effect on mammary gland development in PPAR-alpha null mice from 7.5 mg/kg/day PFOA given 5 days per week for 4 weeks starting at age 3 weeks, while mammary gland development was inhibited in similarly treated wild type mice. However, the differences seen in the two strains may not have resulted from PPAR-alpha dependence of the effect, but rather from toxicokinetic differences, since the serum levels in the PPAR-alpha null mice (38,000 ng/ml) were much lower than in the wild type mice (93,000 ng/L). Additionally, the serum levels in PPAR-alpha null mice dosed with 5 mg/kg/day where stimulation was reported (28,000 ng/ml; Zhao et al., 2010) were close to the serum levels (38,000 ng/ml) in the same strain given 7.5 mg/kg/day at which no effects were reported. In summary, the stimulation of mammary gland development in PPAR-alpha null mice has not been clearly demonstrated. It was reported only in one study using a single dose level without a comparison group of wild type mice, and was not replicated in a second study of PPAR-alpha null mice with similar serum PFOA levels.

Estrogenic effects

Estrogenic activity and/or increased estrogen levels may be involved in the mode of action of PFOA. As discussed in the Toxicology section, serum estradiol was increased in male Sprague-Dawley rats exposed to 13.6 mg/kg/day APFO in the diet for 1 month to 12 months in a two-year dietary study (Biegel et al., 2001). PFOA was also shown to bind to and/or activate human estrogen receptor-alpha in several *in vitro* studies (Benninghoff et al., 2011; Kjeldsen and Bonefeld-Jorgensen, 2013; Buhrke et al., 2015).

Exposure to PFOA increased levels of the sensitive estrogen-dependent biomarker protein, vitellogenin, and the expression of estrogen receptor-beta in the livers of mature male and female rare minnows (Wei et al., 2007). Male fish exposed to PFOA developed oocytes in the testes, and the ovaries of exposed females underwent degeneration. PFOA (as well as PFNA and perfluorodecanoic acid [PFDA, C10]), induced vitellogenin in young rainbow trout, and these PFCs also activated rainbow trout estrogen receptor *in vitro* (Benninghoff et al, 2011).

Studies in rainbow trout, a species used as a model for human liver carcinogenesis because it is insensitive to peroxisome proliferation, suggest that PFOA promotes liver tumor development through an estrogenic mechanism (Tilton et al., 2008; Benninghoff et al., 2012). PFOA and two other peroxisome proliferating compounds (clofibrate and dehydroepiandrosterone, DHEA) were tested for tumor promoting activity in rainbow trout which had been initiated with aflatoxin. PFOA and DHEA (Tilton et al., 2008), as well as PFNA and PFDA (Benninghoff et al., 2012), increased the incidence and number of liver tumors and also induced a genomic signature similar to that induced by 17-beta-estradiol, while clofibrate did not promote liver tumors and did not regulate genes in common with 17-beta-estradiol (Tilton et al., 2008).

Carcinogenicity

As discussed in the Epidemiology section, PFOA has been associated with testicular and kidney tumors in communities with drinking water exposure after adjustment for smoking and other relevant factors. As discussed in the Toxicology section, PFOA caused hepatic, testicular Leydig cell, and pancreatic acinar cell tumors in chronically exposed male rats (Biegel et al., 2001; Butenhoff et al., 2012). These tumor types are also caused by several other PPAR-alpha activating compounds in male rats (Klaunig et al., 2003).

Hepatic tumors

Many PPAR-alpha activators cause liver tumors in rodents through a mode of action involving PPAR-alpha activation. These chemicals are not directly genotoxic, and thus cause tumors through a non-genotoxic mechanism. Activation of PPAR-alpha causes a number of effects in the liver, but all of these effects are not necessarily causal for carcinogenesis. As summarized by USEPA (2009c), peroxisome proliferation and increases in biochemical markers for peroxisome proliferation such as PCO are considered indicative of PPAR-alpha activation, but they are not considered causal events for carcinogenicity since their correlation with carcinogenic potency is poor. As discussed by the USEPA SAB (2006), the key causal events for PPAR-alpha induced liver carcinogenesis are believed to be PPAR-alpha activation, followed by increased cell

proliferation and decreased apoptosis mediated by gene expression changes, leading to clonal expansion of preneoplastic foci and tumor formation (Klaunig et al., 2003; NRC, 2006). However, aside from the initial event (PPAR-alpha activation), the other causal events are not specific to a PPAR-alpha mode of action but rather are also common to other modes of action for hepatic carcinogenesis (USEPA, 2006; Klaunig et al., 2012).

Much attention has been focused on the potential human relevance of rodent liver tumors induced by PPAR-alpha activators. There is wide variation among species in the ability of PPAR-alpha activators to cause hepatic peroxisome proliferation and liver tumors, with rats and mice the most sensitive, hamsters intermediate in sensitivity, and humans, monkeys, and guinea pigs least sensitive to these effects. These species differences may be due to lower levels of PPAR-alpha expression in humans than in rodents (NRC, 2006) or to differences in PPAR-alpha structure and function among species (Corton, 2010). Studies of humans exposed to peroxisome proliferating fibrate drugs have not shown increased risk of liver cancer (NRC, 2006), although these studies have limitations that are discussed by Guyton et al. (2009).

The majority of the USEPA SAB (2006) panel believed that the human relevance of the PPAR-alpah mode of action for liver tumors caused by PFOA could not be dismissed. This conclusion was based on data indicating similar responses to PFOA in the livers of rodents and primates (increased liver weight and induction of hepatic peroxisomal enzyme activity, discussed above). Although increased cell proliferation was not found in the monkeys (Butenhoff et al., 2002), the USEPA SAB (2006) noted that this endpoint was only measured after 6 months of exposure in the monkeys but not at an earlier time point during the first 1-2 weeks of exposure when it would have been more likely to occur, and apoptosis was not evaluated at any time point. The lack of data on cell proliferation at an appropriate time point and apoptosis at any time point in the monkeys treated with PFOA precluded analysis of dose-response concordance between these key events and tumor induction for PFOA as compared to other PPAR-alpha agonists. Subsequent to the USEPA SAB (2006) evaluation, hepatic effects of PFOA were observed in three studies of mice with humanized PPAR-alpha, although chronic studies to evaluate tumor incidence in humanized PPAR-alpha mice have not been conducted (Nakamura et al., 2009; Nagakawa et al., 2011; Albrecht et al., 2013; discussed above).

The USEPA SAB (2006) also noted that the role of hepatic PPAR-alpha in human fetuses, neonates, and children is not known, and that a PPAR-alpha mode of action for hepatic effects could not be ruled out in these age groups. As discussed above, it was subsequently demonstrated that PPARs are present in many human fetal tissues, including liver, and that levels of PPARs increase and decrease with age of the fetus, and between the fetus and the adult (Abbott et al., 2010, 2012). Additionally, PFOA caused increased liver weight and expression of genes associated with PPAR-alpha in fetal livers from mice with humanized PPAR-alpha (Albrecht et al., 2013).

The USEPA SAB (2006) further concluded that PPAR-alpha activation may not be the sole mode of action for liver tumors caused by PFOA. This conclusion was based on the single study of

PFOA in wild type and PPAR-alpha null mice that was available at the time (Yang et al., 2002b). In this study, PFOA increased liver weight in both wild type and PPAR-alpha null mice, while a model PPAR-alpha activator (WY) increased liver weight in wild type mice but had no effect in PPAR-alpha null mice. Numerous studies published subsequent to the USEPA SAB (2006) review confirm this conclusion and provide additional relevant information. These include several studies demonstrating PPAR-alpha independent hepatic effects of PFOA in standard strains of rodents and PPAR-alpha null mice, as well at the PPAR-alpha independent hepatic tumors, possibly related to estrogenic effects, in rainbow trout exposed to PFOA. The USEPA SAB (2006) also noted that some of the causal events in the PPAR-alpha mode of action for liver tumors had not been demonstrated for PFOA, including increased cell proliferation in the liver at early time points after dosing and/or decreased apoptosis in liver cells.

Newer information relevant to this issue shows that the hepatic effects of PFOA and WY differ, and that increased hepatic cell proliferation caused by PFOA is not totally dependent on PPAR-alpha activation. PFOA caused liver tumors in a two-year study of male rats (Biegel et al., 2001), but it did not increase hepatic cell proliferation at any of eight time points between one and 21 months after dosing began. In contrast, the PPAR-activating compound WY, which also caused liver tumors in this study, significantly increased hepatic cell proliferation at most of these time points.

Elcombe et al. (2010) suggested that the earliest time point evaluated by Biegel et al. (2001), one month after dosing began, may have been too late to observe hepatic cell proliferation in response to PFOA since cell proliferation occurs early in the sequence of events leading to liver tumors. They studied effects of PFOA and WY on hepatic endpoints including nuclear receptor activation, cell proliferation, and apoptosis in male rats at earlier time points (one day after the end of dosing for 1, 7, or 28 days). While PFOA acted as an activator of PPAR-alpha, CAR, and PXR, WY was a specific activator of PPAR-alpha only. WY increased hepatic cell proliferation and decreased apoptosis, and these effects were accompanied by increased hepatic DNA content. PFOA also increased hepatocellular proliferation, but, unlike WY, it did not cause decreased apoptosis or increased liver DNA content.

Results from wild type and PPAR-alpha null mice demonstrate that PFOA causes hepatic cell proliferation, one of the key causal events for PPAR-alpha dependent liver carcinogenesis, through PPAR-alpha independent pathway(s). PFOA caused a similar or greater dose-dependent increase in hepatic cell proliferation in mice lacking PPAR-alpha as compared to wild type mice, and relative liver weight was also increased to a similar degree in both strains (Wolf et al., 2008). In contrast to PFOA, WY increased cell proliferation and relative liver weight only in the wild type mice in this study.

A carcinogenicity bioassay of PFOA has not been conducted in a standard strain of mice, or in wild type and PPAR-alpha null mice. However, the incidence of liver tumors at age 18 months was evaluated in female CD-1, 129/Sv wild type, and 129/Sv PPAR-alpha knockout offspring after developmental exposures to PFOA (Filgo et al., 2015). The authors emphasize that the study was

not designed or intended to be a carcinogenicity bioassay, but that tumor incidence was assessed because of the unexpected finding of liver tumors in some animals that died before the scheduled end of the study.

In CD-1 mice, single or multiple hepatocellular adenomas occurred in one or more animals in each of the treated groups (n=21 to 37 per group; 0, 0.01, 0.1, 0.3, 1, and 5 mg/kg/day) except for at the lowest dose (0.01 mg/kg/day), but were not found in controls (n=29). In total, adenomas occurred in 4.9% (7 of 144) treated animals, compared to a historic control incidence of 0.4% in untreated female CD- mice. Additionally, hepatocellular carcinomas occurred in two treated mice (0.3 and 5 mg/kg/day) but not in controls.

In 129/Sv wild type mice, hepatocellular adenomas did not occur in control or treated groups (n=6 to 10 per group; 0, 0.1, 0.3, 1, and 3 mg/kg/day). In contrast, in PPAR-alpha null mice of this strain, there were no adenomas in the controls (n=6), one adenoma in each of the 0.1, 0.3, and 1 mg/kg/day PFOA-treated groups (n=9 or 10), and two adenomas in the 3 mg/kg/day group (n=9). These tumors occurred in 13.2% of all PFOA-treated PPAR-alpha null mice. The results of this study suggest the possibility that developmental exposure to low doses PFOA may cause hepatic tumors later in life through a PPAR-alpha independent mode of action, although more research is needed before any firm conclusions can be made.

Testicular Leydig cell and pancreatic acinar cell tumors

Modes of action that have been suggested for the non-hepatic tumors caused by PFOA are reviewed in USEPA (2005a) and Klaunig et al. (2012). Several other PPAR-alpha agonists have been found to induce the same three tumor types (hepatic adenomas, pancreatic acinar cell tumors, and testicular Leydig cell tumors) as PFOA in Sprague-Dawley rats. However, the modes of action for the latter two types of tumors have not been fully characterized, and it has not been shown that they occur through a PPAR-alpha mediated mode of action.

The mode of action for testicular Leydig cell tumor induction by PFOA is unknown. Proposed modes of action include 1) increased serum estradiol through PPAR-alpha mediated induction of hepatic aromatase activity, leading to estradiol-dependent increased production of growth factors inducing the tumors, and 2) inhibition of testosterone biosynthesis leading to an increase in luteinizing hormone (LH), which promotes Leydig cell tumor development (Klaunig et al., 2012). However, for each of these proposed modes of action, data from the chronic mechanistic rat study (Biegel et al., 2001) do not support one of the key events and the mode of action (Klaunig et al., 2012).

The key events in the first proposed mode of action above are:

> Activation of PPAR-alpha in the liver → increased hepatic aromatase → increased serum and testicular interstitial fluid estradiol levels → increased transforming growth factor-alpha → increased testicular Leydig cell proliferation → testicular Leydig cell tumors (Klaunig et al., 2012).

As discussed by Klaunig et al. (2012), testicular Leydig cell proliferation was not increased by PFOA at numerous time points during the chronic rat study of Biegel et al. (2001).

The key events in the second proposed mode of action above are:

Decreased testosterone levels → increased LH levels → testicular Leydig cell tumors.

As discussed by Klaunig et al. (2012), LH was not increased by PFOA at numerous time points during the chronic rat study (Biegel et al., 2001).

The mode of action for pancreatic acinar cell tumor induction by PFOA is also unknown. One possible hypothesis that has been suggested is that these tumors occur as a result of an increase in cholecystokinin secondary to hepatic effects of PFOA, but this has not been demonstrated experimentally (Klaunig et al., 2012).

The USEPA SAB (2006) concluded that insufficient data were available to determine the mode of action for the testicular Leydig cell tumors and the pancreatic acinar cell tumors found in PFOA treated male rats (Biegel et al., 2001). More recently, Klaunig et al. (2012) concluded that, based on information presented above, the available data do not definitively establish a mode of action for these tumors. In the absence of a defined mode of action for these tumor types, the USEPA SAB (2006) concluded that "they must be presumed to be relevant to humans, as suggested by EPA's Cancer Guidelines," and this conclusion remains valid at this time.

Other possible modes of action

A number of other modes of action for PFOA have been suggested. Although a complete review of this topic is beyond the scope of this document, information on several potential modes of action is summarized below.

PFOA and other perfluorinated carboxylates of carbon chain lengths C7-C10 inhibited intercellular gap junction communication *in vitro* in cultured rat liver cells, while non-fluorinated fatty acids and PFCs of chain length C2-C5 or C16 and C18 did not (Upham et al., 1998). In further *in vivo* studies, male F-344 rats fed diets containing 0.02% PFOA (resulting in a dose of 37.9 mg/kg/day) for 7 days had significantly increased liver weight and significantly decreased hepatic gap junction intercellular communication, as measured by the distribution of a fluorescent dye that travels through gap junction channels (Upham et al., 2009). Perfluoropentanoic acid (C5) did not cause either of these effects. The authors suggested that disruption of gap junction intercellular communication can lead to tumorigenesis and can be important in tumor promotion. However, Lau et al. (2007) stated that inhibition of gap junction intercellular communication is a widespread phenomenon, and that these effects of PFOA and other PFCs have not been shown to be species or tissue specific.

The mode of action for PFOA may also involve effects on mitochondria. As discussed above, developmental exposure to PFOA in female mice caused hepatic mitochondrial proliferation and

abnormal mitochondrial morphology later in life (Quist et al., 2015). Furthermore, hepatic activity of the mitochondrial enzyme, succinate dehydrogenase, was increased in cynomolgus monkeys treated with PFOA (Butenhoff et al., 2002). Hepatic mitochondrial DNA copy number was also significantly increased in male rats 3 days after a single dose of 100 mg/kg PFOA and after 28 days of exposure to 30 mg/kg/day (Berthiaume and Wallace, 2002; Walters et al., 2009).

Starkov and Wallace (2002) investigated effects of PFOA and other PFCs in isolated mitochondria. The involvement of mitochondria in the mode of action of PFCs was suggested by earlier reports of proliferation of hepatic mitochondrial membranes after PFC exposure and uncoupling of mitochondrial respiration in isolated mitochondria exposed to high concentrations of PFCs (reviewed in Starkov and Wallace, 2002). They found that PFOA and PFOS caused slight effects only at high concentrations (e.g. 100 μM for PFOA) which may have resulted from changes in membrane fluidity due to the surfactant properties of PFCs. In contrast, other PFCs were potent uncouplers of oxidative phosphorylation, such as perfluorooctane sulfonamide which had an IC_{50} of 1 μM for this effect.

MicroRNAs (miRNAs) are small non-coding RNAs which affect gene expression by binding to complementary mRNA to block translation or trigger degradation. Changes in miRNA may play a role in various diseases by affecting the expression of relevant genes (Wang et al., 2012; Yan et al., 2014). Recent studies suggest that PFOA may alter miRNA levels in both humans and rodents. Elevated levels of specific miRNAs in serum were associated with serum PFOA concentrations in a study of Chinese residents and workers with PFOA exposures from a fluoropolymer manufacturing facility (Wang et al., 2012). In this study, 63 of the 754 miRNAs that were evaluated were detected in all serum samples. Levels of 9 miRNAs were significantly higher in workers compared to less highly exposed residents. In a further analysis, levels of two of these (miR-26b and miR-199a-3p) were significantly increased in an exposure-related fashion within the worker group. A subsequent study found that levels of circulating miRNAs were increased in male BALB/cJ mice dosed with PFOA for 28 days, and that more miRNAs were affected as PFOA dose increased. Interestingly, the two miRNAs that were associated with PFOA in workers in a dose-related fashion were also increased by PFOA exposure in mice (Yan et al., 2014).

In addition, the mode of action of PFOA may involve effects related to organic anion transporters (OATs), organic anion transporting polypeptides (OATPs), and other multispecific transporter proteins such as multidrug resistance-associated proteins (MRPs/Mrps). These highly conserved transporters are present in the membranes of many tissues throughout the body in humans, other mammalian species, and lower vertebrates. They are responsible for the transport of numerous endogenous and xenobiotic compounds both into and out of cells. Substrates for transporter proteins include fatty acids, hormones, bile acids, drugs, environmental contaminants, and other exogenous and endogenous substances that are critical in homeostatic pathways (Roth et al., 2011).

It is well established that renal tubular secretion and reabsorption of PFOA occur through transport by various OATs and OATPs, and that the slow rate of excretion of PFOA in male (in contrast to female) rats and both genders of humans is due to reabsorption of PFOA by specific renal OATs

and/or OATPs (Han et al., 2012). Recent studies have shown that effects of PFOA in organs other than the kidney may involve these transporter proteins as well. PFOA can act as an inhibitor of specific OATs and OATPs (Yang et al., 2009b; 2010), and it induced MRP in cultured human cells (Rusiecka and Skladanowski, 2008) and Mrp in mouse liver (Maher et al., 2008), potentially affecting transport of endogenous substances, drugs, and other transporter substrates. The role of OAT4 in placental transfer of PFOA in humans was recently studied (Kummu et al., 2015). OAT4 is found primarily in human kidney and placenta and has been identified as one of the specific transporters responsible for renal reabsorption of PFOA in humans (Han et al., 2012). The level of placental OAT4 was inversely correlated with the rate of transfer of PFOA from the maternal circulation to fetal circulation in studies of perfused human placenta, suggesting that higher levels of placental OAT4 may protect the fetus from PFOA after maternal exposure (Kummu et al., 2015).

Summary of conclusions about human relevance of toxicological effects of PFOA

Based on the information presented above, the toxicological effects of PFOA are generally considered relevant to humans for the purposes of risk assessment.

Hepatic effects

Increased serum levels of liver enzymes are associated with exposure to PFOA in humans. Data from non-human primates, standard strains of rats and mice, PPAR-alpha null mice, and humanized PPAR-alpha mice support the conclusion that hepatic effects of PFOA in experimental animals are relevant to humans for the purposes of risk assessment.

PFOA caused increased relative liver weight in non-human primates, species for which the human relevance of hepatic effects is not in question. In a subchronic study in cynomolgus monkeys, several animals that did not complete the study due to overt toxicity exhibited notably increased liver weight, highly elevated serum liver enzymes and/or severe hepatic toxicity. In this study, hepatic peroxisome proliferating activity was increased in a dose-related fashion. As was noted by the USEPA SAB (2006), the effects of PFOA on both liver weight and peroxisome proliferation were similar in cynomolgus monkeys and rats. In contrast to rodents, the human relevance of hepatic effects mediated by PPAR-alpha in non-human primates is not subject to debate. Potential PPAR-alpha independent pathways for hepatic effects of PFOA have not been thoroughly investigated in non-human primates, although increased activity of a mitochondrial enzyme (succinate dehydrogenase) was reported in the subchronic cynomolgus monkey study**.**

Data from standard strains of mice and rats clearly show that PPAR-alpha independent mechanisms contribute to the hepatic effects of PFOA. Studies which evaluated both liver weight and hepatic peroxisome proliferating activity provide data relevant to this question. At the doses and time points evaluated in these studies, the dose-response curves for increased liver weight and hepatic peroxisome proliferating activity did not directly correspond. These data indicate that hepatic effects of PFOA involve PPAR-alpha independent mechanisms even in standard strains of rodents with normal PPAR-alpha status. Furthermore, PFOA caused fatty liver in standard strains of mice and rats, although PPAR-alpha activation causes decreased hepatic lipid accumulation.

Additionally, recent studies suggest that the differences in the effects of PFOA on serum lipids in rodents (decreased cholesterol and triglycerides) versus humans (increased cholesterol and triglycerides) may be related to the low fat diet generally used in laboratory rodent studies versus the higher fat content of a typical Westernized human diet, rather than solely to interspecies differences. Finally, developmental exposure to PFOA caused abnormal mitochondria in livers of a standard strain of laboratory mice, with no evidence of peroxisome proliferation.

Data from studies comparing mice with normal PPAR-alpha status (wild type) and mice lacking PPAR-alpha (PPAR-alpha null) provide further evidence that hepatic effects occur through both PPAR-alpha dependent and PPAR-alpha independent pathways. PFOA causes similar increases in liver weight in wild type and PPAR-alpha null mice. PFOA also causes histopathological changes and increased liver enzymes in PPAR-alpha null mice; the histopathological changes differ from those seen in wild type mice, particularly as related to damage to the bile duct. Additionally, developmental exposures to PPAR-alpha null mice caused persistent histopathological changes in the liver.

Data from mice expressing human PPAR-alpha in the liver indicate that activation of human PPAR-alpha by PFOA causes hepatic effects. PFOA caused similar increases in liver weight in humanized PPAR-alpha mice and wild type mice. Other hepatic effects of PFOA in humanized PPAR-alpha mice include activation of genes associated with PPAR-alpha, and histopathological changes including hepatocellular hypertrophy and single cell necrosis.

PPAR-alpha is known to be active in human fetal tissues, including liver. Fetal liver weight was increased similarly in wild type and PPAR-alpha humanized mice after *in utero* exposure. Additionally, PFOA increased the expression of genes associated with PPAR-alpha in fetal liver to a greater degree in PPAR-alpha humanized mice than in wild type mice.

<u>Immune system effects</u>

Data from epidemiological studies suggest that the immune system is a sensitive target for PFOA in humans. PFOA suppresses the immune system in both non-human primates and mice. Data from mouse studies indicate that these effects on the immune system occur through both PPAR-alpha dependent and PPAR-alpha independent modes of action. Both PPAR-alpha dependent and independent effects on the immune system are considered relevant to humans for the purposes of risk assessment. Potentially relevant to this conclusion, lipid-lowering drugs that activate PPAR-alpha have recently been associated with decreased effectiveness of the influenza vaccine in humans, consistent with inhibition of human immune response by agents that activate PPAR-alpha such as PFOA.

<u>Developmental and reproductive effects</u>

PFOA is associated with decreased fetal growth in humans. PPAR-alpha and other PPARs are present in human fetal tissues and are expected to have important roles in reproduction and development. Therefore, PPAR-alpha mediated effects of PFOA on development are considered relevant to humans for the purposes of risk assessment. Developmental effects of PFOA in rodents

appear to occur primarily through PPAR-alpha dependent mechanisms, while some reproductive effects such as full litter resorptions appear to be PPAR-alpha independent. However, high affinity "pure" PPAR-alpha activators (WY and clofibrate) do not cause the developmental effects in mice that were caused by PFOA.

Delayed mammary gland development after developmental exposure is a sensitive endpoint for PFOA toxicity in mice. The rodent is considered a good model for human mammary gland development, and there is no mode of action evidence suggesting that the effects of PFOA on this endpoint are not relevant to humans.

PFOA also causes reproductive toxicity in male mice, and there is no mode of action information to suggest that these effects are not relevant to humans.

Carcinogenicity

PFOA is associated with testicular and kidney cancer in communities with drinking water exposure after adjustment for smoking and other relevant factors. PFOA caused tumors of the liver, testicular Leydig cells, and pancreatic acinar cells in male rats. USEPA (2006) SAB did not dismiss the potential human relevance of the liver tumors in rats, based on similarities in hepatic effects of PFOA in monkeys and rodents and the limited evidence available at the time for hepatic effects of PFOA in PPAR-alpha null mice. Subsequent studies have provided substantial additional information showing hepatic effects of PFOA in PPAR-alpha null mice. Importantly, hepatic cell proliferation, a causal event for tumor formation, is increased similarly by PFOA in wild type and PPAR-alpha null mice. Although a carcinogenicity bioassay for PFOA has not been conducted in PPAR-alpha null mice, a recent study suggests that developmental exposures to PFOA may cause hepatic tumors in adulthood in this strain. Finally, studies in rainbow trout, a species used as a model for human liver cancer because it lacks PPAR-alpha, suggest that PFOA causes liver tumors through an estrogenic mode of action.

Because the modes of action for the testicular and pancreatic tumors caused by PFOA have not been established, these tumors are considered relevant to humans for the purposes of risk assessment.

DEVELOPMENT OF RECOMMENDED HEALTH-BASED MCL

Health-based MCLs developed by the DWQI are intended to be protective for chronic (lifetime) exposure through drinking water. The 1984 Amendments to the New Jersey Safe Drinking Water Act (N.J.S.A. 58:12A-20) stipulate that Health-based MCLs are to be based on a one in one million lifetime cancer risk level for carcinogens and no adverse effects from lifetime ingestion for non-carcinogens.

Consideration of human epidemiological data

Both the human epidemiology data and the animal toxicology data were considered as part of the overall weight of evidence for the potential human health effects of PFOA. As reviewed in the

Epidemiology section, associations of PFOA with numerous health endpoints have been found in human populations, with evidence supporting multiple criteria for causality for some effects. These health endpoints include non-carcinogenic effects in the general population, and both non-carcinogenic effects and cancer in communities with drinking water exposure. The epidemiologic data for PFOA are notable because of the consistency between results among human epidemiologic studies in different populations, the concordance with toxicological findings in experimental animals, the use of serum concentrations as a measure of internal exposure, the potential clinical importance of the endpoints for which associations are observed, and the observation of associations within the exposure range of the general population and communities with contaminated drinking water. These features of the epidemiologic data distinguish PFOA from most other organic drinking water contaminants and justify concerns about exposures to PFOA through drinking water.

Although the data for some endpoints support multiple criteria for causality, the human epidemiology data have limitations and are therefore not used as the quantitative basis for the Health-based MCL. Instead, the potential Health-based MCLs developed below are based on sensitive and well established animal toxicology endpoints that are considered relevant to humans based on mode of action data. Notwithstanding, continued exposure to even relatively low levels of PFOA in drinking water are known to cause substantial increases in PFOA in blood serum, to levels several fold higher than those found in the general population. The considerable evidence for increased risks of health effects from the low-level PFOA exposures prevalent in the general population (e.g. <10 ng/ml, NHANES 2007-2010; CDC 2015) and in communities with contaminated drinking water suggests a need for caution about drinking water exposures that will result in such elevations in serum PFOA level. The human epidemiological data thus support the use of a public health-protective approach in developing a Health-based MCL recommendation based on animal toxicology data.

Weight of Evidence for Carcinogenicity

PFOA caused tumors in male rats in two chronic studies (Sibinski et al., 1987, Butenhoff et al., 2012; and Biegel et al., 2001). It caused a statistically significant increase in testicular Leydig cell tumors in both studies, as well as a statistically significant increase in liver tumors and pancreatic tumors in Biegel et al. (2001). Human exposure to PFOA has also been associated with increased risk of cancer, including increased risk of kidney and testicular cancer in communities with contaminated drinking water after adjustment for smoking and other relevant factors (Barry et al, 2013; Vieira et al., 2013).

Based on the chronic animal data and mode of action studies available at the time, PFOA was described as “likely to be carcinogenic to humans” by the USEPA Science Advisory Board (USEPA, 2006). PFOA was classified as “possibly carcinogenic to humans” by IARC based on review of data from human epidemiology, animal toxicology, and mechanism of action studies (Benbrahim-Tallaa et al., 2014). More recently, PFOA was described by the USEPA Office of Water (2016a) as having “suggestive evidence of carcinogenic potential”, a descriptor similar to the classification of “possibly carcinogenic to humans” used by IARC (2015).

The USEPA Guidelines for Carcinogen Risk Assessment (2005b) recommend dose-response modeling and low dose extrapolation for chemicals described as likely to be carcinogenic. For chemicals with suggestive evidence of carcinogenicity, dose-response modeling and low dose extrapolation may be performed when data to support it are available. The guidelines recommend that the risk-based estimates for suggestive carcinogens be used to obtain "a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities." Risk-based drinking water concentrations based on both non-carcinogenic and carcinogenic endpoints are presented below.

Potential Health-based MCLs based on non-carcinogenic endpoints

Selection of toxicological endpoints for consideration as basis for potential Health-based MCLs
As reviewed in the Toxicology section above, PFOA causes numerous systemic effects in experimental animals. These include liver toxicity, immune system toxicity, adverse developmental effects, and other adverse effects. The Health Effects Subcommittee concluded that delayed mammary gland development and increased relative liver weight are the most sensitive systemic endpoints with data appropriate for dose-response modeling.

Delayed mammary gland development from perinatal exposure is the most sensitive systemic endpoint for PFOA with data appropriate for dose-response modeling. It is a well-established toxicological effect of PFOA that is considered to be adverse and relevant to humans for the purposes of risk assessment. An RfD based on this endpoint is presented below.

To the knowledge of the Health Effects Subcommittee, an RfD for delayed mammary gland development has not previously been used as the primary basis for health-based drinking water concentrations or other human health criteria for environmental contaminants. Because the use of this endpoint as the basis for human health criteria is a currently developing topic, the Health Effects Subcommittee decided not to recommend a Health-based MCL with the RfD for delayed mammary gland development as its primary basis. However, the occurrence of this and other effects at doses far below those that cause increased relative liver weight (the endpoint used as the primary basis for the recommended Health-based MCL) clearly requires application of an uncertainty factor to protect for these more sensitive effects.

Increased relative liver weight is a well-established effect of PFOA that is more sensitive than most other toxicological effects such as immune system toxicity and most reproductive/developmental effects (Table 12 of Animal Toxicology section). A recommended Health-based MCL with increased liver weight as its primary basis is presented below.

Selection of studies and data for dose-response modeling
Only those studies that provide serum PFOA data were considered for dose-response modeling of non-carcinogenic effects. A risk assessment approach based on measured serum PFOA levels is less uncertain than one based on pharmacokinetic modeling of estimated serum PFOA levels or an

approach in which interspecies extrapolations is based on interspecies half-life differences. For example, as discussed above, serum PFOA concentrations from the same administered dose vary among strains and even between animals of the same strain obtained from different sources. Available approaches based on pharmacokinetic modeling or interspecies half-life conversions do not account for these intra-strain differences.

In some studies, serum PFOA was measured only at time points well after dosing had ended. Serum data from these later time points are not appropriate for dose-response modeling because PFOA body burdens would have decreased, and the serum levels thus do not represent the maximum exposures during the study. Therefore, only studies which provide serum PFOA levels close to the end of the dosing period were considered for dose-response modeling. Use of the maximum serum levels that occurred during the study is a non-conservative approach (i.e. would result in a less stringent, rather than more stringent, risk assessment than other potential approaches such as area under the curve modeling), since the observed toxicological effects could have resulted from the lower average exposures experienced over the entire dosing and post-dosing time periods.

Benchmark dose (BMD) modeling is a quantitative approach commonly used to estimate the lower 95% confidence limit (the BMDL) on the dose corresponding to a minimal response (the benchmark response, BMR) that is consistent with the observed data. The BMDL is considered to be an estimate of the NOAEL, but it is based on the entire dose-response curve for the endpoint of interest rather than just the fixed doses administered in the study. When the necessary data are available, BMD modeling can be performed using serum concentrations instead of administered doses. Serum concentrations are preferable to administered doses as the basis for BMD modeling because they are a better representation of the shape of the internal dose response curve. BMD modeling based on serum PFOA data was used to determine BMDLs for serum PFOA concentrations used as the points of departure (PODs) to develop RfDs for both increased relative liver weight and delayed mammary gland development.

Reference Dose based on delayed mammary gland development

Delayed mammary gland development in mice from developmental exposures is a sensitive endpoint for PFOA's toxicity. This effect has been reported in nine separate studies presented in five publications (Table 14 of Toxicology section). Only one study (Albrecht et al., 2013), which has several general problematic issues (discussed in Toxicology section) did not find this effect. Gestational and/or lactational exposures to PFOA caused delayed mammary gland development in pregnant dams and/or female offspring in two strains of mice. In one study, this effect was statistically significant in mouse pups exposed to concentrations of 5000 ng/L (5 µg/L) in drinking water *in utero* and after birth. The serum PFOA levels (80 ng/ml in the dams and 20-70 ng/ml in the pups) that resulted in delayed mammary gland development in these mice are relevant to human serum PFOA levels from contaminated drinking water. Histological changes in the mammary glands of exposed offspring persisted until adulthood and were considered permanent. Delayed mammary gland development occurs in a dose-related fashion, and there is no information indicating it is not relevant to humans. For these reasons, delayed mammary gland

development from developmental exposures to PFOA is considered a sensitive and relevant endpoint for dose-response modeling.

Selection of study and data for dose-response modeling of delayed mammary gland development
The late gestational exposure study conducted by Macon et al. (2011) is the only developmental exposure study of mammary gland development that provides serum PFOA data from the end of the dosing period (PND 1) that can be used for dose-response modeling. In this study, pregnant dams were dosed with PFOA (0.01 to 1 mg/kg/day) on GD 10-17. Mammary gland development was assessed on PND 1, 4, 7, 14, and 21, and delays in development were most evident on PND 21. Of the several endpoints related to mammary gland development reported by Macon et al. (2011), two endpoints (decreased mammary gland developmental score and decreased number of terminal end buds) showed a statistically significant dose-related decrease at PND 21 over the dose range studied. These endpoints were selected for dose-response modeling (Table 15).

Table 15. Mammary gland development parameters selected for dose-response modeling from PND 21 female offspring after exposure on GD 10-17 (Macon et al., 2011)

Dose (mg/kg/day)	**Pup Serum PFOA, PND 1 (ng/ml)**	**Developmental score (1-4)**	**Number of Terminal End Buds**
Control	22.6 ± 5.5	3.3 ± 0.3	40 ± 4
0.01	284.5 ± 21.0	2.2 ± 0.2	33 ± 4
0.1	2303.5 ± 114.1	1.8 ± 0.3	24 ± 4
1.0	16,305.5 ± 873.5	1.6 ± 0.1	15 ± 2

Determination of Point of Departure for delayed mammary gland development
USEPA Benchmark Dose Modeling Software 2.1.2 was used to perform BMD modeling of the data for mammary gland developmental score and number of terminal endbuds at PND 21 from Macon et al. (2011), using serum PFOA data from PND 1 as the dose. Continuous response models were used to obtain the BMD and the BMDL for a 10% change from the mean (the percent change typically used as the BMR) for the two endpoints. Modeling was based on serum levels at PND 1, since they were higher at this time than at later time points. In this study, the serum level in the control group was 22.6 ng/ml, indicating that some PFOA exposure occurred in these non-dosed animals. The BMD and BMDL values presented in Table 16 were derived using this value from the control group (22.6 ng/ml) as the baseline. The serum level BMDs and BMDLs may have been lower if the baseline serum level had been lower (Post et al., 2012).

Compared to controls, the developmental score at PND 21 significantly decreased at all three doses; the number of terminal end buds also decreased at all three doses, with significance at the two higher doses. The BMDLs for the two endpoints, 24.9 and 22.9 ng/ml (Table 16), are close to the NOAEL of 28.5 ng/ml that is estimated by applying a standard uncertainty factor of 10 for LOAEL-to-NOAEL extrapolation to the serum level of 285 ng/ml at the LOAEL, 0.01 mg/kg/day. Outputs of the BMD modeling are provided in Appendix 6.

Table 16. Benchmark Dose modeling of serum PFOA data (PND1) for mammary gland developmental effects (PND 21) in CD-1 mouse pups (Macon et al., 2011)[a]				
Model	**Chi-square p-value[b]**	**Akaike Information Criterion (AIC)[c]**	**BMD (Serum PFOA, ng/ml)**	**BMDL (Serum PFOA, ng/ml)**
Decreased Mammary Gland Developmental Score				
Hill (non-logged concs.)	0.70	-4.28	57.4	28.1
Polynomial - 2nd deg. (logged concs.)	0.83	-4.39	25.9	24.0
Exponential -model 2 (logged concs.)	**0.87**	**-36.86**	**25.7[d]**	**24.7[d]**
Linear (logged concs.)	0.83	-6.05	28.0	26.5
Decreased Number of Terminal End Buds				
Hill (non-logged concs.)	0.47	88.43	235.1	78.5
Exponential - model 4 (non-logged concs.)	0.30	88.97	399.8	110.5
Power (non-logged concs.)	0.62	88.15	64.8	23.4
Power (logged concs.)	0.81	87.96	87.2	29.0
Polynomial – 2nd deg. (logged concs.)	0.84	87.94	96.6	28.1
Linear (logged concs.)	0.12	89.47	27.0	25.7
Exponential – model 3 (logged concs.)	**0.98**	**87.90**	**25.1[d]**	**22.9[d]**

[a] Results are shown for all models that gave an acceptable visual fit.
[b] A larger Chi-square p-value indicates a better fit to the data.
[c] AIC: A measure of information loss from a dose-response model that can be used to compare a specified set of models. The AIC is defined as -2 × (LL - p), where LL is the log-likelihood of the model given the data, and p is the number of parameters estimated in the model. When comparing models, a lower AIC is preferable to a higher one (USEPA, 2012a).
[d] BMDs and BMDLs from the models with the lowest AIC statistic for each endpoint.

Application of uncertainty factors for delayed mammary gland development

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

The BMDL for decreased number of terminal endbuds of 22.9 ng/ml in serum (derived above) was used as the POD for RfD development. Uncertainty factors (UFs) were applied to the POD to obtain the Target Human Serum Level. The Target Human Serum Level (ng/ml in serum) is analogous to an RfD but is expressed in terms of internal dose rather than administered dose.

The total of the uncertainty factors (UFs) applied to the POD serum level was 30, and included the following factors:

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

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

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

1 – UF for LOAEL to NOAEL.

The point of departure is a BMDL, not a LOAEL. Therefore, this uncertainty factor is not applied.

1 – UF for duration of exposure.

Delayed mammary gland development occurs from exposures during development. Therefore, an UF to account for effects from longer term exposures is not used.

1 – UF for more sensitive effects that are not considered (e.g. incomplete database).

Because delayed mammary gland development is a sensitive endpoint for PFOA toxicity, the UF is not applied.

The target human serum level is: $\frac{22.9 \text{ ng/ml}}{30} = 0.8$ ng/ml (800 ng/L).

As discussed above, the most recent median and 95th percentile NHANES values for serum PFOA in the general population are 2.1 and 5.7 ng/ml (CDC, 2015). Therefore, the target human serum level for delayed mammary gland development, 0.8 ng/ml, is below the median serum PFOA level in the U.S. general population.

Development of Reference Dose based on delayed mammary gland development

USEPA (2016a) used a pharmacokinetic modeling approach to develop a species-independent clearance factor, 1.4×10^{-4} L/kg/day that relates serum PFOA level (µg/L) to human PFOA dose (µg/kg/day). As discussed in the Toxicokinetics section, this clearance factor predicts a serum:drinking water ration of 114:1 with average drinking water consumption, consistent with the empirically observed average serum:drinking water ratio of greater than 100:1 in human populations exposed to PFOA through drinking water. The clearance factor can be used to calculate the RfD, as follows:

$$800 \text{ ng/L} \times 1.4 \times 10^{-4} \text{ L/kg/day} = 0.11 \text{ ng/kg/day}$$

Where: 800 ng/L = Target Human Serum Level
1.4×10^{-4} L/kg/day = Clearance
0.11 ng/kg/day = RfD

Potential Health-based MCL based on increased relative liver weight

Increased relative liver weight is a well-established toxicological effect of PFOA in both non-human primates and rodents and is more sensitive than most other toxicological effects. Increased liver weight occurs in newborn animals after *in utero* exposure, during early life from lactational exposure, and from exposures during adulthood. As discussed in the Mode of Action section, PFOA may cause increased relative liver weight through multiple biochemical and cellular pathways. Increased relative liver weight can co-occur with and/or progress to other types of hepatic toxicity and is considered relevant to humans for the purposes of risk assessment.

According to USEPA IRIS guidance (USEPA, 2012c), endpoints that are "adverse, considered to be adverse, or a precursor to an adverse effect" are appropriate as the basis for non-cancer risk assessment. The increased relative liver weight caused by PFOA is usually accompanied by hepatocellular hypertrophy, and it can co-occur with and/or progress to more severe hepatic effects including hepatocellular necrosis, fatty liver, increased serum liver enzymes, and hyperplastic nodules. Additionally, PFOA caused hepatocellular adenomas in chronically exposed male rats in the study conducted by Biegel et al. (2001). Although these tumors were not reported to be increased in males rats in the earlier chronic study (Sibinski, 1987), Butenhoff et al. (2012) noted that these lesions represent a regenerative process and that diagnostic criteria for hepatic hyperplastic nodules have changed since the livers from the study were evaluated in 1986.

Increased relative liver weight in mice can result either from *in utero* exposure during the prenatal period or from lactational exposure during the neonatal period (Wolf et al., 2007; White et al., 2009). In other studies, ultrastructural and/or histopathological changes indicative of liver toxicity persisted until adulthood (age 3 months, Quist et al., 2015; age 18 months, Filgo et al., 2015) in offspring of dams dosed with PFOA during gestation. Hepatocellular hypertrophy and periportal inflammation occurred at doses below those that caused increased liver weight (Quist et al., 2015). It is not known whether these sensitive hepatic effects resulted from *in utero* exposure, lactational exposure, or both. Additionally, results from offspring at age 18 months suggest the possibility of an increased incidence of liver tumors from developmental exposures to PFOA, although the study was not designed as a carcinogenicity bioassay (Filgo et al., 2015). Although data from these studies are not amenable dose-response modeling, they support the conclusions that liver toxicity is a sensitive endpoint for PFOA, that the developmental period is a sensitive lifestage for PFOA's hepatic effects, and that increased relative liver weight is a relevant and appropriate endpoint for PFOA's toxicity.

Selection of study and data for dose-response modeling of increased liver weight

Increased relative liver weight has been observed in many studies of PFOA in both rodents and non-human primates. The five publications reporting studies of relative liver weight that were considered for dose-response modeling are summarized in the first part of Table 10 of the Animal Toxicology section. Studies were included if they provide serum PFOA data from the end of the dosing period, and, for rodent studies, include relatively low doses (1 mg/kg/day or less). Rodent studies that meet these criteria were reported in four publications. The 90-day cynomolgus monkey study in which the lowest dose was 3 mg/kg/day is also included in Table 10 of the

Animal Toxicology section for comparison purposes, since it used a non-human primate species and has been the focus of risk assessments by other groups.

The 90 day cynomolgus monkey study (Thomford et al., 2001b; Butenhoff et al., 2002) was not considered appropriate for dose-response modeling for several reasons (discussed in detail in Appendix 3). The study does not provide serum PFOA data that can be used for dose-response modeling because serum PFOA levels did not differ at the two lower doses (3 and 10 mg/kg/day); the high dose (30/20 mg/kg/day) group is excluded for use in dose-response modeling due to overt toxicity. Additionally, the death of one of four animals in the low dose group may have been due to PFOA toxicity. Aside from its lack of utility for dose-response modeling, this study provides no indication of the NOAEL for PFOA toxicity in this species because of the lack of a relationship between administered or internal dose and response, and because of the possibility of overt toxicity at the lowest dose.

Two of the four rodent studies (Loveless et al., 2006; Perkins et al., 2004) used adult male rats, and one of these (Loveless et al., 2006), also used adult male mice. Loveless et al. (2006) administered three different isomeric mixtures of PFOA (linear/branched, linear, and branched) to adult male mice and rats for 2 weeks, while Perkins et al. administered PFOA to adult male rats for 4, 7, or 13 weeks.

As discussed in the Toxicology section, increased relative liver weight associated with hepatocellular hypertrophy is an early manifestation of PFOA's hepatic toxicity. This effect does not appear to increase in magnitude over time, but rather it appears to progress over time to other more severe hepatic effects (Butenhoff et al., 2012). Relative liver weight data from male CD-1 mice after 14 day exposures (Loveless et al., 2006) and 29 day exposures (Loveless et al., 2008) were compared based on administered dose, as Loveless et al. (2008) does not provide serum PFOA levels. This comparison shows that the dose-response curves for increased relative liver weight are similar for the 14 day and 29 day exposure periods. Furthermore, dose-response curves for relative liver weight in male rats were similar after 4, 7, and 13 week exposures (Perkins et al., 2004).

Two additional developmental studies in mice (Lau et al., 2006; Macon et al., 2011) also met the criteria for inclusion in Table 10 of the Toxicology section. Lau et al. (2006) evaluated increased liver weight on GD 18 in pregnant mice dosed with PFOA on GD 1-18. The data for liver weight and serum PFOA levels in pregnant mice in this publication are not presented in a form that is appropriate for dose-response modeling of increased relative liver weight. Data on absolute liver weight and serum PFOA levels are presented in graphical form in the publication; numerical data for absolute liver weight, and liver weight relative to body weight minus weight of gravid uterus, were obtained from the investigator.

Macon et al. (2011) evaluated relative liver weight on PND 1 in female offspring exposed *in utero* on GD 10-17. Comparison of serum PFOA level LOAELs for increased relative liver weight in

neonatal female mice in Macon et al. (2011) and in adult male mice (Loveless et al., 2006) suggest similar sensitivity to this effect at both life stages.

The relative liver weight data from male mice exposed to branched/linear PFOA for 14 days (Loveless et al., 2006) were selected for dose-response modeling. These data are shown in Table 17. The branched/linear isomeric mixture is relevant to environmental contamination and human exposure, and almost all toxicological studies of PFOA used the branched/linear isomeric mixture. An increasing response with dose was observed in mice for increased relative liver weight from branched/linear PFOA over the range of doses used in this study. Data from both the standard strain and PPAR-alpha null strains of mice demonstrate that increased liver weight and other types of hepatic toxicity occur through both PPAR-alpha dependent and independent modes of action in mice, and these effects are considered relevant to humans. As shown in Figure 13 in the Mode of Action section, increased liver weight was not correlated with PPAR-alpha activity in mice in Loveless et al. (2006). As discussed above, relative liver weight does not appear to increase in magnitude with longer exposure durations. Therefore, 14 days is considered to be of sufficient duration, particularly since dose-response modeling is based on serum PFOA level, rather than administered dose, thus avoiding uncertainties about whether internal dose increases with exposures longer than 14 days.

Table 17: Serum PFOA and relative liver weight in Male CD-1 mice dosed with branched/linear PFOA for 14 days (Loveless et al., 2006)

Dose (mg/kg/day)	**Serum PFOA (µg/ml)**	**Relative Liver Weight (g/100 g)**
0	0.04±0.02	5.14±0.27
0.3	10±1.4	6.12±0.25
1	27±5.0	7.92±0.49
3	66±8.6	10.72±0.63
10	190±29	16.27±1.05
30	241±28	18.28±1.57

Determination of Point of Departure (POD) for increased relative liver weight

USEPA Benchmark Dose Modeling Software 2.6.0.88 was used to perform BMD modeling of the data on increased relative liver weight in male mice exposed to linear/branched PFOA from Loveless et al. (2006). BMD and BMDL serum levels were determined for a BMR of a 10% increase in mean relative liver weight from the control values. All models for continuous data included in the software were run.

Results of the BMD modeling are shown in Table 18, and a more detailed explanation and the complete output of the BMDS software for each model are presented in Appendix 7. Both of the exponential models (models 4 and 5) gave identical fits. These exponential models and the 3rd

degree polynomial model gave acceptable fits to these data. The 3rd degree polynomial model over-fits the data at the high dose, forcing a fit and resulting in a biologically unlikely fit in this area of the dose-response curve. However, the fit of the 3rd degree polynomial model at the lower doses (i.e., in the range of the BMD) is regular and biologically appropriate. It is unlikely that the forced fit at the high dose has any significant influence on the fit of the model at the BMD. Although the 3rd degree polynomial model gave a slightly better fit than the exponential models and also yielded a slightly lower BMDL, the exponential models produced a highly comparable fit and a similar BMDL. As neither model appears to have a claim to greater biological significance, it was recommended that the point-of-departure be derived as the average of the BMDLs for both of these models. **This yielded an average BMDL of 4,351 ng/ml.**

Table 18. Benchmark Dose analysis for a 10% increase in relative liver weight from linear/branched PFOA in male mice (Loveless et al., 2006)[a]

Model	Chi-square p-value[b]	AIC[c]	BMD (Serum PFOA, ng/ml)	BMDL (Serum PFOA, ng/ml)
Exponential (Models 4 and 5)	**0.2636**	**2.12782**	**4,904[d]**	**4,466[d]**
Hill	-	-	-	-
Linear	-	-	-	-
Polynomial (2nd degree)	0.03245 [c]	6.92134	5,317	4,896
Polynomial (3rd degree)	**0.4678**	**1.66669**	**4,682[d]**	**4,236[d]**
Average of Exponential (Models 4 and 5) and Polynomial (3rd degree)			***4,793***	***4,351***

[a] Results are shown for all models that gave an acceptable visual fit.
[b] A larger Chi-square p-value indicates a better fit to the data.
[c] AIC: A measure of information loss from a dose-response model that can be used to compare a specified set of models. The AIC is defined as -2 × (LL - p), where LL is the log-likelihood of the model given the data, and p is the number of parameters estimated in the model. When comparing models, a lower AIC is preferable to a higher one (USEPA, 2012a).
[d] BMDs and BMDLs from the models used to derive the point of departure, as discussed in text.

Application of uncertainty factors for increased relative liver weight

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

The BMDL of 4,351 ng/ml was used as the POD for RfD development. UFs were applied to the POD serum level of 4,351 ng/ml to obtain the Target Human Serum Level. The Target Human Serum level (ng/ml serum) is analogous to the RfD but is expressed in terms of internal, rather than administered, dose.

The total of the UFs applied to the POD serum level was 300, and included the following factors:

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

3 - UF for animal-to-human extrapolation, to account for toxicodynamic differences between humans and mice.

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

1 - UF for LOAEL to NOAEL.

The point of departure is a BMDL, not a LOAEL. Therefore, an adjustment for use of a LOAEL is not necessary.

1 - UF for duration of exposure.

The POD is based on increased liver weight resulting from exposure for 2 weeks, while the Health-based MCL is intended to protect for chronic exposure. However, increased liver weight, usually associated with hepatocellular hypertrophy, is an early manifestation of PFOA's hepatic toxicity. Data from the relevant studies (reviewed above) indicate that the dose-response for this effect, on an internal dose (serum PFOA level) basis, is similar after 2 weeks of exposure and from longer exposures, and that this effect does not appear to occur at lower internal doses (serum PFOA levels) or increase in magnitude with chronic exposures. Rather, the initial effect (increased liver weight accompanied by hepatocellular hypertrophy) appears to progress over time to other more severe hepatic types of effects. Therefore, an adjustment based on duration of exposure is not necessary.

10 - UF for more sensitive effects that are not otherwise considered (e.g. incomplete database).

USEPA IRIS guidance (USEPA, 2012c) states that: "If an incomplete database raises concern that further studies might identify a more sensitive effect, organ system, or lifestage, the assessment may apply a database uncertainty factor." Adverse effects on mammary gland development occur at doses much more than 10-fold lower than those that cause increased relative liver weight. Additionally, hepatic toxicity not associated with increased liver weight occurs at similarly low doses after developmental exposures. Therefore, a UF of 10 to account for more sensitive effects was applied.

The target human serum level is: $\frac{4351 \text{ ng/ml}}{300} = 14.5 \text{ ng/ml}$ (14,500 ng/L)

Development of Reference Dose for increased relative liver weight

As above, the clearance factor (1.4×10^{-4} L/kg/day; USEPA, 2016a) was used to derive the RfD from the Target Human Serum Level. This factor was used to develop the RfD that is the basis for the recommended Health-based MCL. As discussed in the Toxicokinetics section, the clearance factor is consistent with empirical data on the serum:drinking water ratio from communities with contaminated drinking water. It should be noted that health-based drinking water values may also be developed from target human serum levels for PFOA and other PFCs using an approach based on this ratio.

$14{,}500 \text{ ng/L} \times 1.4 \times 10^{-4} \text{ L/kg/day} = 2 \text{ ng/kg/day}$

Where: 14,500 ng/L = Target Human Serum Concentration
1.4×10^{-4} L/kg/day = Clearance Factor
2 ng/kg/day = RfD

Relative Source Contribution factor

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

The Health Effects Subcommittee concluded that there are insufficient data to develop a chemical-specific RSC for PFOA. There are no New Jersey-specific biomonitoring data for PFOA, and its frequent occurrence in NJ PWS suggests that New Jersey residents may also have higher exposure from non-drinking sources than the U.S. general population (e.g. NHANES). Elevated levels of PFOA were detected in PWS located throughout NJ in USEPA UCMR3 and other monitoring studies; PFOA was detected much more frequently at > 20 ng/L in NJ PWS (10.5%) than nationwide (1.9%) in UCMR3 (discussed in the Drinking Water Occurrence section). Potential sources of this contamination have been identified in some instances, while sources are unknown in other locations. Environmental contamination with PFOA that results in its presence in drinking water can arise from a number of different types of sources (reviewed in Fate and Transport Relevant to Drinking Water Contamination). These include releases to air, soil, and water from fluoropolymer telomer manufacturing facilities, on-site and off-site disposal from smaller industrial facilities that make products from fluoropolymer dispersions containing PFOA, releases of aqueous firefighting foams, and land application of biosolids from wastewater treatment plants treating waste containing PFOA, among others. These various sources may potentially result in

human exposures through contamination of nearby soils, house dust, or other environmental media. In communities with drinking water contamination, consumption of produce from home gardens or grown locally was associated with higher serum levels of PFOA (Emmett et al., 2006a; Holzer et al., 2008; Steenland et al., 2009a).

The exposure factors used to develop the Health-based MCL (below) are based on an adult drinking water consumption rate and body weight. The default RSC of 20%, while not explicitly intended for this purpose, also partially accounts for the higher PFOA exposures in infants. Exposures to infants, both breastfed and consuming formula prepared with contaminated drinking water, are much higher than in than older individuals. Infants consume much more fluid (breast milk or formula) than older individuals on a body weight basis; about 10-fold more from birth to 1 month of age, and 4 -6 fold more between ages 6-12 months. Additionally, PFOA concentrations in breast milk are similar or higher than in the mother's drinking water source (Post et al., 2012).

For these reasons, although serum levels in infants are similar to their mother's at birth (Post et al., 2012), they increase rapidly by several-fold shortly after birth for a period of at least several months. As shown in Figure 16, this increase was five-fold or greater in a considerable portion of infants evaluated in two studies (Fromme et al., 2010; Mogensen et al., 2015). Additionally, Monte Carlo simulations of results of a pharmacokinetic model predict median, 95th percentile, and maximum infant:mother plasma PFOA ratios of 4.5-fold, 7.8-fold, and 15.3-fold, respectively, during the period of greatest infant exposure (Verner et al., 2016a; Figure 17).

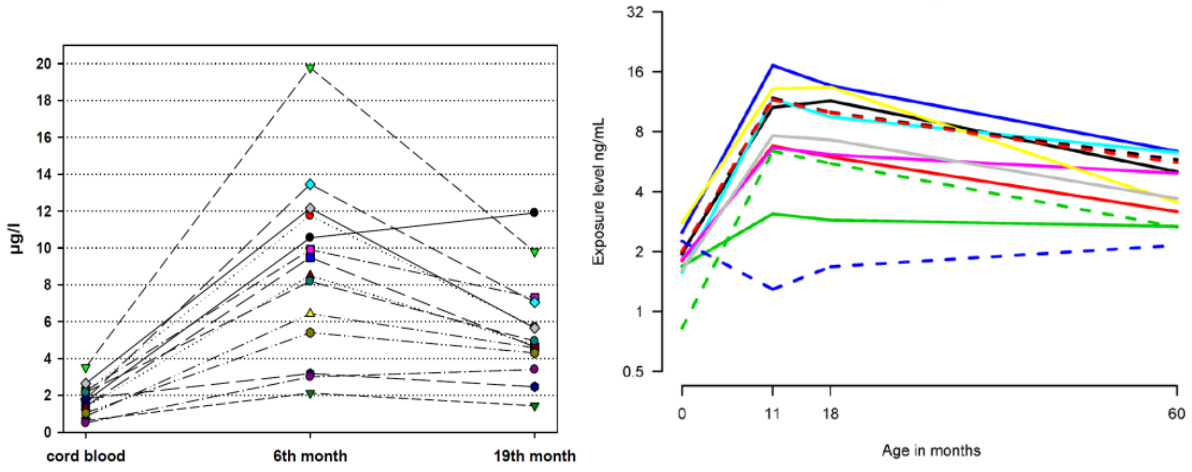


Figure 16. Changes in PFOA levels in breast-fed infants from birth to later timepoints (Fromme et al., 2010; Mogensen et al., 2015)

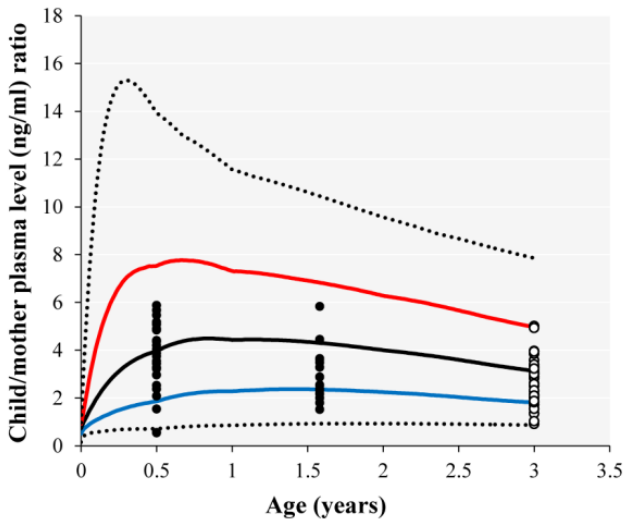


Figure 17. Monte Carlo simulations of child/mother ratios of plasma PFOA levels (ng/ml) a breastfeeding period of 30 months. Black line - 50th percentile; blue line - 5th percentile; red line - 95th percentile; dotted lines - minimum and maximum values (Verner et al., 2016).

These higher infant exposures must be considered because the toxicological effects of concern (delayed mammary gland development and increased relative liver weight) occur from short term exposures relevant to elevated exposures in infancy. Cross-fostering studies (discussed in Toxicology section) show that lactational exposure causes increased relative liver weight and delayed mammary gland development (White et al., 2007; White et al., 2009) in animals with no *in utero* exposure. Additionally, hepatic toxicity that persists until adulthood occurs in offspring of dams exposed to low doses of PFOA during gestation (Filgo et al., 2015; Quist et al., 2015). These effects could result from prenatal or lactational exposure, or both.

For the reasons discussed above, the default RSC of 20% is used to develop the Health-based MCL.

Development of potential Health-based MCL based on hepatic effects

$$\frac{2 \text{ ng/kg/day} \times 70 \text{ kg} \times 0.2}{2 \text{ L/day}} = 14 \text{ ng/L } (0.014\ \mu\text{g/L})$$

Where:

2 ng/kg/day = Reference Dose
70 kg = assumed adult body weight
0.2 = Relative Source Contribution from drinking water
2 L/day = assumed adult daily drinking water intake

Potential Health-based MCL based on carcinogenicity

PFOA caused tumors in male rats in two chronic studies (Sibinski et al., 1987, Butenhoff et al., 2012; and Biegel et al., 2001). It caused a statistically significant increase in testicular Leydig cell tumors in both studies, as well as a statistically significant increase in liver tumors and pancreatic tumors in Biegel et al. (2001). The testicular tumor data from the chronic dietary exposure rat

study reported by Sibinski et al. (1987) and Butenhoff et al. (2012) are appropriate for BMD modeling and can be used as the basis for development of a cancer potency factor. This study used two PFOA dose levels, and the incidence of testicular tumors increased in a dose-related fashion. Because Biegel et al. (2001) used only one dose, data from this study are not appropriate for dose-response modeling. As discussed above, the mode of action for rat testicular tumors has not been established and they are considered relevant to humans for the purposes of risk assessment. USEPA Guidelines for Carcinogen Risk Assessment (USEPA, 2005b) state that linear low-dose extrapolation should be used for dose-response modeling if the mode of action has not been established. Therefore, the linear low-dose extrapolation approach is appropriate for dose-response modeling of these testicular tumors. The linear low-dose extrapolation approach is based on the assumption that exposure to any dose of a carcinogen results in some risk of cancer.

The incidence of testicular tumors (Sibinski et al., 1987: Butenhoff et al., 2012) was 0/49, 2/50, and 7/50 in the control, 30 ppm (1.3 mg/kg/day), and 300 ppm (14.2 mg/kg/day) groups. Modeling was performed using EPA BMD software (version 2.6.0.86). Because serum PFOA levels were not measured in this study, the BMDL and slope factor were modeled in terms of dose administered to rats. The value based on administered dose to rats was then converted to the equivalent human dose, based on pharmacokinetic differences (ratio of half-lives) between rats and humans. A benchmark response (BMR) of 0.05 (5%) tumor incidence was selected for consistency with the recommendations for selection of the POD for cancer potency slope derivation in the USEPA (2005b) Guidance for Carcinogen Risk Assessment. This value is close to the 4% response at the lowest dose in this data set. Results of the modeling are shown in Table 21, and the complete output from the modeling is found in Appendix 8.

Table 19. BMD modeling (0.05 BMR; 5% response) of rat testicular tumor data (Butenhoff et al., 2012)[a]

Model	*Chi-square p-value* [b]	*AIC* [c]	*BMD (mg/kg/day)*	*BMDL (mg/kg/day)*	*Cancer Potency Slope (mg/kg/day)$^{-1}$*	*Rat Dose at 1 x 10^{-6} risk (mg/kg/day)*
Gamma (power restricted to ≥ 1) **Multistage (betas ≥ 0)** **Weibull (power restricted to ≥ 1)** **Quantal linear**	**0.2292**	**62.6851**	**4.42913**[d]	**2.50664**[d]	**0.020**	**5.0 x 10^{-5}**
Gamma (power unrestricted)	1.00	61.2908	4.42913	1.36483e-6		
Log-logistic (power unrestricted)	1.00	61.2908	1.95859	2.00091e-6		
Logisitic	0.1905	63.6843	8.85708	6.49805		
Log-logistic (slope restricted to ≤ 1)	**0.2338**	**62.5526**	**4.02707**[d]	**2.2101**[d]	**0.023**	**4.3 x 10^{-5}**
Probit	0.1948	63.625	8.32341	5.95965		
Weibull (power unrestricted)	1.00	61.2908	1.97407	1.65976e-6		
Average of Gamma (and other identical models) and Log-logistic (slope restricted to ≤ 1)				***2.36***	***0.021***	***4.8 x 10-5***

[a] Results are shown for all models that gave an acceptable visual fit.
[b] A larger Chi-square p-value indicates a better fit to the data.
[c] AIC: A measure of information loss from a dose-response model that can be used to compare a specified set of models. The AIC is defined as -2 × (LL - p), where LL is the log-likelihood of the model given the data, and p is the number of parameters estimated in the model. When comparing models, a lower AIC is preferable to a higher one (USEPA, 2012a).
[d] BMDs and BMDLs from the models used to derive the slope factor, as discussed in the text.

The Gamma model with power restricted to ≥ 1 (and the other models shown in the same cell in Table 19) and the Log-logistic model with slope restricted to ≤ 1 fit the data very similarly and yielded very similar BMDLs. Because neither of these models has a form that is obviously more biologically accurate, it is appropriate to average their BMDLs. The average BMDL for these models is **2.36 mg/kg/day**. For a 5% BMR, the corresponding cancer potency slope is **0.021 (mg/kg/day)$^{-1}$**, and the dose in rats corresponding to a 1 x 10^{-6} risk is estimated as **4.8 x 10^{-5} mg/kg/day**. These values are shown in the last row of Table 19.

As above, the dose-response modeling was based on administered PFOA dose to rats (mg/kg/day) instead of internal dose (serum PFOA level) since serum PFOA levels were not measured in the study. Thus, the rat doses derived through the modeling must be converted to equivalent human doses. As per USEPA (2005b) guidelines for carcinogen risk assessment, this adjustment is made based on pharmacokinetic differences between species instead of through the default adjustment

based on body weight$^{3/4}$. To make the interspecies adjustment, the dose in male rats corresponding to a 1 x 10^{-6} cancer risk is converted to the human equivalent dose based on the ratio of half-lives in the two species. This approach accounts for the much longer half-life of PFOA in humans than male rats, although it is associated with more uncertainty than an approach using measured serum PFOA levels.

The half-lives used for this adjustment were 7 days for male rats and 2.3 years (840 days) for humans. The half-life in male Sprague-Dawley rats after a single gavage dose of 0.1 to 25 mg/kg PFOA was about 7 days and was independent of dose (Kemper et al., 2003). Bartell et al. (2010a) estimated a human half-life of 2.3 years for a one-year period after exposure to contaminated drinking water ceased; elimination rate was not affected by age or gender. The ratio of these human and rat half-lives (840 days/7 days) is 120.

Therefore, the human dose corresponding to a 1 x 10^{-6} lifetime cancer risk is estimated as:

$$(4.8 \times 10^{-5} \text{ mg/kg/day}) / 120 = 4 \times 10^{-7} \text{ mg/kg/day } (4 \times 10^{-4} \ \mu\text{g/kg/day; } 0.4 \text{ ng/kg/day})$$

Using default drinking water assumptions (2 L/day water consumption; 70 kg body weight), the potential Health-based MCL at the 1 x 10^{-6} lifetime cancer risk level is:

$$\frac{0.4 \text{ ng/kg/day} \times 70 \text{ kg}}{2 \text{ L}} = \mathbf{14\ ng/L}\ (0.014\ \mu\text{g/L})$$

This value is identical to the Health-based MCL based on non-cancer endpoints developed above.

Recommended Health-based MCL

A potential Health-based MCL based on the RfD for delayed mammary gland development was not developed, for reasons discussed above. The potential Health-based MCL based on the RfD for increased relative liver weight is 14 ng/L (0.014 µg/L). The potential Health-based MCL based on a lifetime carcinogenic risk one in one million (1 x 10^{-6}), the cancer risk goal for New Jersey MCLs, is also 14 ng/L.

Therefore, the recommended Health-based MCL is 14 ng/L (0.014 µg/L).

DISCUSSION OF UNCERTAINTIES

● PFOA is associated with multiple human health effects in epidemiology studies of the general population and communities with drinking water exposure. There is evidence to support multiple criteria for causality for some of these endpoints. Although causality cannot be definitively proven for these associations of PFOA with human health effects, these numerous findings indicate the need for caution about drinking water exposures that will increase serum PFOA to levels substantially higher than in the general population. This is particularly true because elevated serum PFOA levels persist for many years after exposure ends, due to its long human half-life (several years).

Ongoing exposure to the recommended Health-based MCL of 14 ng/L is expected to increase serum PFOA levels, on average, by about 1.6 ng/ml (ppb) with average daily water consumption and 2.8 ng/ml (ppb) with upper percentile daily water consumption in adults. Increases in serum PFOA levels are predicted to be several-fold higher than in infants than in adults, including both breastfed infants whose mothers ingest PFOA in drinking water or from formula prepared with water contaminated with PFOA.

● The potential for prenatal and early life exposures to environmental contaminants to cause adverse health effects later in life is currently a focus of high interest in both epidemiology and toxicology (Boekelheide et al., 2012; Heindel and Vandenberg, 2015). Developmental exposures to PFOA caused effects in mice, with no threshold (NOAEL) identified, at doses far below those that caused effects in older animals. These effects include persistent changes in the mammary gland, persistent damage to hepatic cells, persistent neurobehavioral effects from a single relatively low dose to the pregnant dam, and obesity and metabolic changes in adulthood. Some of these effects were not evident until later in life and/or adulthood, long after the administered PFOA has been eliminated from the body. Some of these effects (obesity/overweight later in life after prenatal exposure; neurobehavioral effects) have been evaluated in humans, with both positive and negative findings among the studies. As discussed in the Development of Health-Based MCL section, the Target Human Serum Level based on delayed mammary gland development in mice is below the serum PFOA levels prevalent in the general population. The proposed Health-based MCL includes an uncertainty factor to protect for more sensitive developmental effects. However, there is still uncertainty about whether it is sufficiently protective for subtle effects later in life that may result from very low exposures during the developmental period.

● Chronic toxicity and carcinogenicity of PFOA have been studied only in the rat, a species in which PFOA is rapidly excreted by females. There is uncertainty about chronic effects including carcinogenicity in other species such as mice in which PFOA is persistent in both sexes. Furthermore, the chronic studies did not assess effects including carcinogenicity which might result from exposures during the critical developmental stages now known to be sensitive periods for PFOA toxicity.

● Uncertainties about the human relevance of effects seen in animals are inherent to all risk assessments based on animal data. As reviewed in detail in this document, the available information indicates that the effects of PFOA observed in experimental animals are relevant to humans for the purposes of risk assessment.

● Available information indicates that the target organs and modes of action are generally similar for PFOA and some other PFCs, including PFNA (DWQI, 2015c). Therefore, the toxicity of PFOA and other PFCs may be additive. Although PFOA and other PFCs, including PFNA, are known to co-occur in some NJ public water supplies, the potential for additive toxicity of PFOA and other PFCs was not considered in development of the Health-based MCL.

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PUBLIC REVIEW DRAFT

APPENDICES TO

HEALTH-BASED MAXIMUM CONTAMINANT LEVEL SUPPORT DOCUMENT: PERFLUOROOCTANOIC ACID (PFOA)

New Jersey Drinking Water Quality Institute
Health Effects Subcommittee
June 27, 2016

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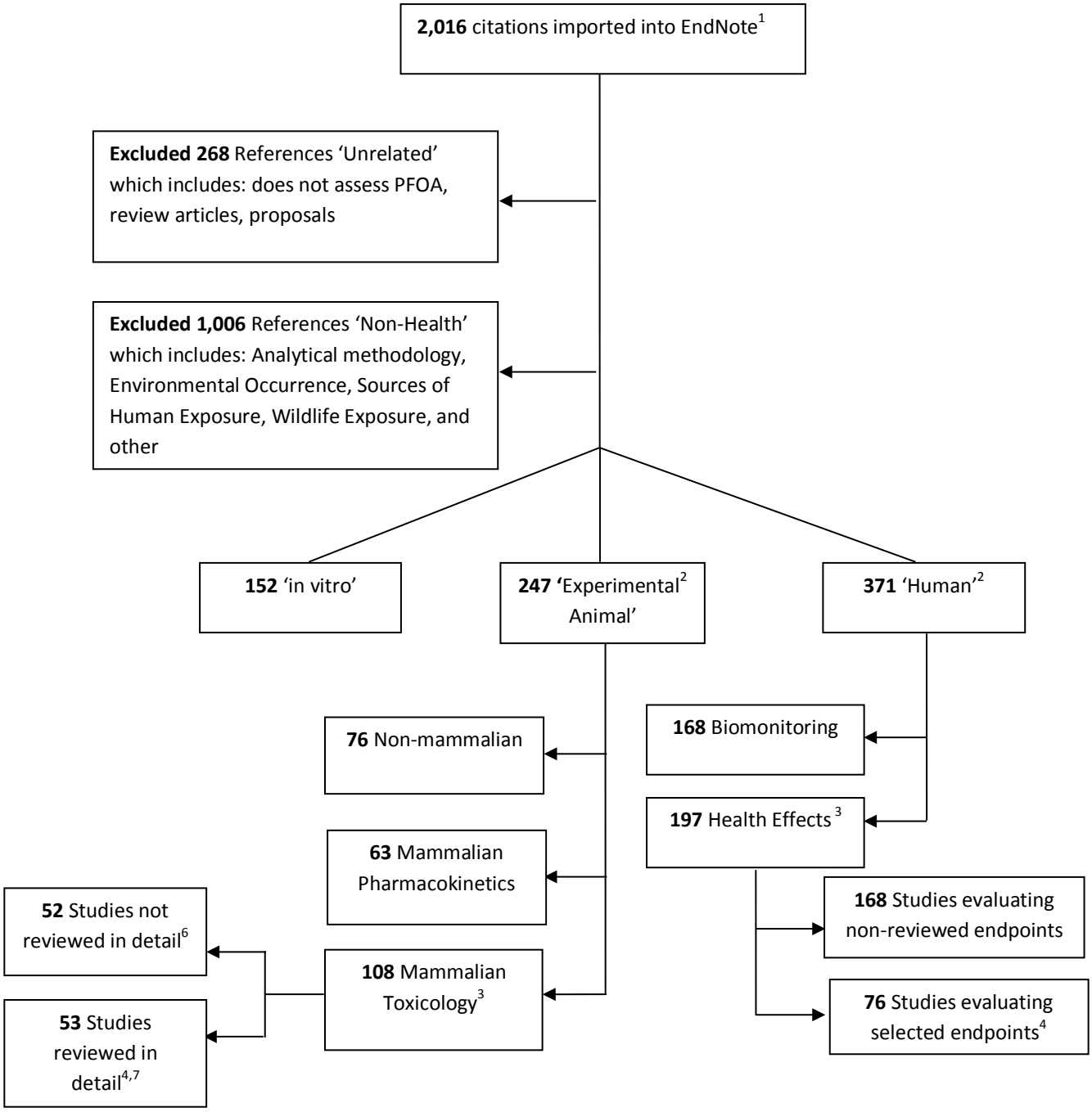
APPENDIX 1. Literature Search Criteria and Documentation - PFOA

1159 citations (2010 to present) and 857 citations (pre-2010) from PubMed (April 13, 2015): search "(pfoa OR (PFO AND (perfluoroalk* OR perfluorocarb* OR perfluorinat* OR perfluorooc*)) OR (C8 AND (perfluoroalk* OR perfluorocarb* OR perfluorinat* OR perfluorooc*)) OR apfo OR (perfluorooctanoic AND acid) OR (pentadecafluorooctanoic AND acid) OR (**perfluoroheptanecarboxylic** AND acid) OR (ammonium AND perfluorooctanoate) OR (ammonium AND pentadecafluorooctanoate) OR (**fluorad**) OR (ammonium AND perfluorocaprilate) OR (pentadecafluorooctanoic AND acid AND ammonium AND salt) OR (sodium AND perfluorooctanoate) OR (sodium AND pentadecafluorooctanoate) OR (sodium AND **perfluorocaprylate**) OR (**perfluorooctanoic AND** acid AND sodium AND salt) OR (potassium AND perfluorooctanoate) OR (potassium AND **perfluoroacrylate**) OR (perfluorooctanoic AND acid AND potassium AND salt) OR FC-143 OR (335-67-1[EC/RN Number]) OR (3825-26-1[EC/RN Number]) OR (335-95-5[EC/RN Number]) OR (2395-00-8[EC/RN Number])"; Limits: none



[1]Totals exceed number of imported files if articles are placed into more than one category. Additional studies were added via backward searching, relevant citations from earlier review by the Health Effects Subcommittee, and article title review of monthly PubMed key word searches.
[2]Title and/or abstract review
[3]Abstracts and/or text reviewed to be sorted into selected endpoints.
[4]Authors evaluated full text of each article to determine whether mammalian toxicology or human health effects were investigated in studies.
[5]This number does not represent unique studies.
[6]Some studies in this category are discussed in the text and others are not.
[7]Studies included in summary tables and/or individual study tables.

APPENDIX 2: Comparison of USEPA Office of Water Health Advisory and DWQI recommended Health-based MCL for PFOA

The basis for the USEPA (2016a) Health Advisory and the recommended DWQI Health-based MCL, and other relevant information about these two drinking water values, are compared in the table below. Additional information is provided in the text that follows the table.

Parameter	USEPA Office of Water Health Advisory	DWQI Recommended Health-based MCL
Value	70 ng/L	14 ng/L
General statement	"Protects the most sensitive populations, with a margin of protection from a lifetime of exposure."	"Developed using a risk assessment approach intended to be protective for chronic (lifetime) exposure."
	As discussed in this document, PFOA is associated with several human health effects, some with evidence supporting multiple criteria for causality, within the general population exposure range even without additional exposure from drinking water. Furthermore, the Reference Dose (RfD) for mammary gland effects in mice is below the average exposure level in the general population, and other toxicological effects occurred at similarly low doses in animal studies. Therefore, any additional exposure from drinking water may potentially pose some risk of health effects. For this reason, it cannot be concluded that lifetime exposure to a certain drinking water concentration, no matter how low, is protective of sensitive subpopulations with a margin of exposure. USEPA recognizes that human studies provide evidence of associations of several health effects with PFOA. However, USEPA concludes that the human studies do not provide quantitative information on the exposure levels or serum levels associated with these health effects. Therefore, USEPA did not consider the possibility that health effects may result from exposures within the general population range, even with no additional exposure from drinking water. USEPA also dismissed the low dose toxicological effects in animal studies from consideration in risk assessment. See discussion of these points below.	

Parameter (cont.)	USEPA (cont.)	DWQI (cont.)
Reference Dose (RfD)	20 ng/kg/day	2 ng/kg/day
	• Based on delayed ossification of phalanges and accelerated male puberty after developmental exposures to mice (Lau et al., 2006). • Data do not follow typical dose-response of greater response as dose increases. (See figures below.) An uncertainty factor of 10 was applied for LOAEL-to-NOAEL extrapolation. The basis and precedent for this approach with dose-response curves of this type is uncertain and may be subject to debate.	Based on Benchmark Dose modeling of increased liver weight in mice, with additional uncertainty factor for more sensitive developmental effects at much lower doses (delayed mammary gland development, persistent liver toxicity, and others).
	Note: USEPA and DWQI RfDs are **identical numerically**, with the exception of application of an additional uncertainty factor of 10 in the DWQI RfD to account for more sensitive developmental endpoints. These more sensitive endpoints were not considered in development of the USEPA RfD. Issues related to endpoints selected as the basis for RfDs by DWQI and USEPA are further discussed below.	
Interspecies conversion in RfD development	Pharmacokinetic modeling was used to predict average serum PFOA concentrations at the NOAELs and/or LOAELs in toxicological studies. A clearance factor that relates serum PFOA level to human administered dose (applicable to serum PFOA data from any species) was used to obtain the Human Equivalent Doses (HEDs) at the modeled average serum levels. Uncertainty factors were applied to the HEDs to obtain potential RfDs.	Benchmark Dose modeling was performed on measured serum PFOA concentrations at each dose from the end of the dosing period to derive a serum level Benchmark Dose (BMD). This approach has less uncertainty than use of modeled serum PFOA data. Uncertainty factors were applied to the BMD serum level to obtain the Target Human Serum Level (i.e. the RfD in terms of serum PFOA level). The same clearance factor used by USEPA was applied to the Target Human Serum Level to obtain the RfD.

Parameter (cont.)	USEPA (cont.)	DWQI (cont.)
Drinking water concentration estimated to result in one in one million (10^{-6}) lifetime cancer risk	500 ng/L. Predicted risk at this concentration based on the DWQI evaluation is about 4×10^{-5} (i.e. 40-fold higher than 1 in 1 million).	14 ng/L (Identical to the value based on non-cancer effects)
	USEPA and DWQI cancer assessments are both based on increased incidence of testicular tumors in a chronic rat study (Butenhoff et al., 2012), and the Benchmark Doses developed by USEPA and DWQI for these tumors are almost identical. The two assessments differ in the approach used for animal-to-human conversion. DWQI used the ratio of half-lives to account for interspecies pharmacokinetic differences, since serum PFOA data are not provided in this study. Although USEPA considered interspecies pharmacokinetic differences in animal-to-human conversion for non-cancer effects, the default animal-to-human extrapolation (ratio of body weights to the ¾ power) was used by USEPA for cancer risk assessment. This default approach does not account for interspecies pharmacokinetic differences. USEPA's use of an approach that does not account for pharmacokinetic differences for cancer risk assessment, although pharmacokinetic differences were considered for non-cancer risk assessment, does not appear to be logical or consistent.	
Relative Source Contribution Factor	20%. To account for non-drinking water exposures.	20%. To account for non-drinking water exposures. Also implicitly accounts for greater exposures to breastfed and formula fed infants than older individuals.
Assumed Drinking Water Consumption	0.054 L/kg/day. Based on 90th percentile for lactating woman. USEPA also developed an alternative Health Advisory of 100 ng/L that applies to adults in the general population based on standard adult exposure assumptions (2.5 L/day water consumption; 80 kg body weight). More information is provided below under <u>Sensitive Subpopulations.</u>	0.029 L/kg/day. Based on default upper percentile adult exposure assumptions (2 L/day water consumption, 70 kg body weight).

Parameter (cont.)	USEPA (cont.)	DWQI (cont.)
*Increase in serum PFOA concentration predicted from ongoing exposure to USEPA Health Advisory and DWQI recommended Health-based MCL (see **bar graph** below)*	• With average water consumption: 8.0 ng/ml; 4.8-fold increase from U.S general population median of 2.1 ng/ml (CDC, 2015). • With upper percentile water consumption: 14 ng/ml; 7.7-fold increase from U.S. general population median.	• With average water consumption: 1.6 ng/ml; 1.8-fold increase from U.S. general population median. • With upper percentile water consumption: 2.8 ng/ml; 2.3-fold increase from U.S. general population median.
	Note: A clearance factor that relates human PFOA exposures to human PFOA serum levels was developed by USEPA scientists (Lorber and Egeghy, 2011). This factor can be used to predict increases in serum PFOA from drinking water exposures. Although this factor was used in another part of the USEPA (2016a) assessment (to convert serum levels from laboratory animals to human doses, discussed above), USEPA does not acknowledge that it is possible to predict the increase in serum PFOA that will result from ongoing exposure to a given concentration of PFOA in drinking water (ASDWA, 2016).	
Sensitive Subpopulations	Pregnant and lactating women; bottle fed infants. USEPA does not include women who plan to become pregnant in sensitive subpopulations. This exclusion does not appear to be supportable because the body burden of PFOA remains elevated for many years after exposure ceases. However, USEPA (ASDWA, 2016) stated that states may choose to expand the sensitive subgroups to include women of child-bearing age. USEPA (2016a) also developed a lifetime Health Advisory for the general population (adults ages 21 and older) of 100 ng/L based on standard adult exposure assumptions. USEPA states that this value is protective for effects other than developmental toxicity, such as testicular and kidney cancer, liver damage, and immune effects. However, it has been reported in the news media that some states have interpreted the USEPA designation of sensitive subgroups to mean that those not in these sensitive subpopulations may continue to drink water exceeding the USEPA Health Advisory (e.g. WAAYTV, 2016).	As is the case for all Health-based MCLs developed by the DWQI, the Health-based MCL recommended for PFOA is intended to be protective of all individuals, including sensitive subpopulations. Sensitive subpopulations for health effects of PFOA include women who plan to become pregnant, pregnant women, lactating women, and breast-fed and bottle-fed infants.

Endpoints used as basis of DWQI recommended Health-based MCL

The primary basis for the recommended Health-based MCL is an RfD based on increased relative liver weight in mice (Loveless et al., 2006). As discussed elsewhere in this document, the Health Effects Subcommittee conducted an extensive and detailed review of the hepatic effects of PFOA. It was concluded that increased relative liver weight in animal studies is a sensitive and well established effect of PFOA which is accompanied by and/or can progress to more severe forms of liver toxicity, and that it should be considered relevant to humans. Additionally, human exposure to PFOA in the general population and in communities with contaminated drinking water is associated with increased serum levels of liver enzymes, strongly suggesting that low exposures to PFOA affect liver function in humans.

The Health Effects Subcommittee also conducted an extensive review of delayed mammary gland development in mice from developmental exposure to PFOA. This effect was observed in 9 separate studies from 5 publications and follows a typical (monotonic) dose-response curve, with greater effects at higher doses. The Health Effects Subcommittee concluded that this effect is adverse, results in persistent structural changes that are considered to be permanent, and should be considered relevant to humans. Additionally, recent human studies (Fei et al., 2010; Romano et al., 2016) suggest an association of PFOA exposure with decreased duration of breast feeding, an effect which may potentially be relevant to effects on mammary gland development.

The Health Effects Subcommittee developed an RfD for delayed mammary gland development (Macon et al., 2011) based on the Benchmark Dose presented in Post et al. (2012). A Health-based MCL based on this RfD would be 1 ng/L or less. The Health Effects Subcommittee chose not to use this RfD as the basis for a recommended Health-based MCL, not because of uncertainty about the scientific validity of doing so, but rather because of lack of precedent for use of this endpoint as the primary basis for health-based criteria for environmental contaminants. However, the Health Effects Subcommittee concluded that an additional uncertainty factor to protect for developmental effects (delayed mammary gland development; persistent liver toxicity) at much lower doses than those that caused increased liver weight should be incorporated into the RfD.

USEPA (2016a) provides a detailed summary of studies of mammary gland development in mice but chose not to consider this effect in risk assessment. The reasons provided by USEPA for not considering this endpoint (mode of action not known, effects occurred only at higher doses in a second strain of mice, functional significance is unclear) do not appear to be scientifically valid and/or are also equally or more applicable to the endpoints selected by USEPA as the basis for its Health Advisory (discussed further below in the section on Endpoints used as basis of EPA Health Advisory).

In regard to the issue of strain difference in susceptibility, it should be noted that almost all of the studies of developmental exposures to PFOA and mammary gland development were conducted in CD-1 mice. A second strain of mice (C57/Bl6) was included in only one study in which a very small number of mice of this strain were used in each dose group (Tucker et al., 2015). USEPA (2016a) does not discuss that the higher LOAEL for effects on the mammary gland in C57Bl/6 mice as compared to CD-1 mice may potentially be explained by pharmacokinetic

differences (lower serum PFOA concentrations from the same dose of PFOA in the C57/Bl6 strain) and/or the very small number of C57/Bl6 mice per dose group, limiting the ability to achieve statistical significance (Tucker et al., 2015).

The Health Effects Subcommittee also considered persistent liver toxicity from developmental exposures to very low doses of PFOA (Filgo et al., 2015; Quist et al., 2015). Although these effects cannot be used as the basis for an RfD because the serum PFOA data needed for dose-response modeling are not provided, they give further indication of effects at much lower doses than those that cause increased liver weight, the primary basis for the recommended RfD. The low doses at which these persistent hepatic effects from developmental exposures occurred were not considered by USEPA in developing its Health Advisory.

Endpoints used as basis of USEPA Health Advisory

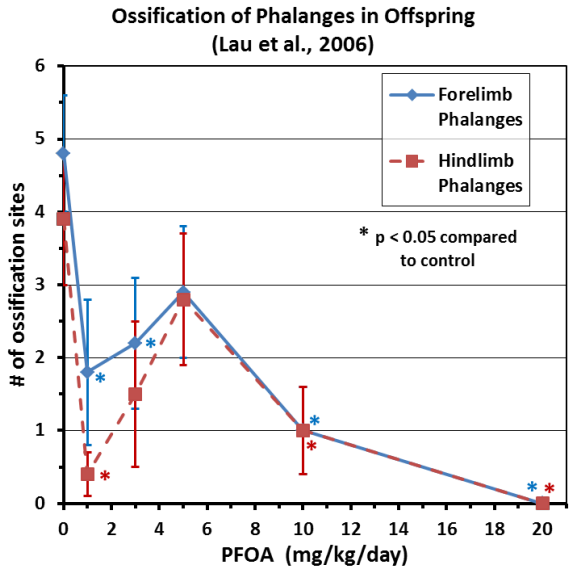
The USEPA Health Advisory is based on an RfD for delayed ossification (conversion of cartilage to bone during development) of proximal phalanges and accelerated male puberty in offspring in a developmental study of CD-1 mice (Lau et al., 2006).

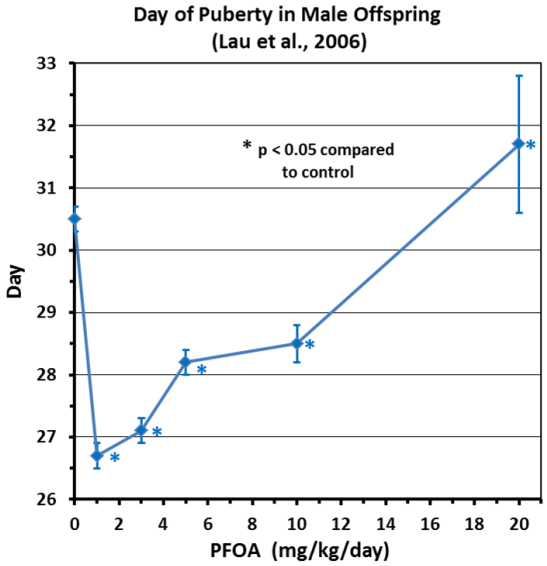
USEPA states that these effects have lifelong consequences. However, delayed ossification in Lau et al. (2006) represents a delay in timing of development rather than a permanent structural change, because the phalanges developed normally in mice treated with PFOA that were not sacrificed prior to delivery in this study (personal communication with C. Lau). Therefore, the long term consequences and functional significance of this effect are unclear. Similarly, the long term consequences and functional significance of accelerated puberty are unclear. This is particularly noteworthy because one of the reasons provided by USEPA for not considering delayed mammary gland development in risk assessment is that its functional significance is unclear, although developmental exposures to PFOA cause structural and/or histopathological changes that persist until adulthood in the mammary gland.

In regard to differences in strain sensitivity, there is a major difference in sensitivity to effects of gestational PFOA exposure on delayed ossification in ICR mice (Yahia et al., 2010) as compared to the CD-1 mice used by Lau et al. (2006). The LOAEL (10 mg/kg/day) and NOAEL (5 mg/kg/day) for delayed ossification in the ICR mice were much higher than the LOAEL (1 mg/kg/day) in CD-1 mice. In ICR mice, delayed ossification occurred only at a dose at or above the doses that caused maternal toxicity, decreased fetal weight, and decreased pup survival; no pups survived until 4 days of age at 10 mg/kg/day, the LOAEL for delayed ossification. This large difference in strain sensitivity is especially noteworthy because one of USEPA's reasons for dismissing effects on mammary gland development for consideration in risk assessment is the strain difference observed by Tucker et al. (2015), which may be explained by factors other than differences in intrinsic sensitivity (discussed above).

Accelerated puberty in male animals has not been reported in studies of PFOA other than Lau et al. (2006). To the knowledge of the Health Effects Subcommittee, this effect was not assessed in developmental studies of mice other than Lau et al. (2006). Timing of puberty in offspring was assessed in a two generation rat study (Butenhoff et al., 2004), and accelerated puberty was not observed in male offspring in this study.

The endpoints selected by USEPA (2016a) as the basis for the Health Advisory do not follow a typical (monotonic) dose-response in which greater effects occur with increasing dose (see graphs of data from Lau et al., 2006, below). In Lau et al. (2006), delayed ossification occurred at the lowest dose (1 mg/kg/day) but was not significantly increased at some of the higher doses. Acceleration of puberty in males in Lau et al. (2006) was greatest at the lowest dose (1 mg/kg/day), with a lesser effect with each increasing dose, and puberty was delayed rather than accelerated at the highest dose. USEPA (2016a) did not mention the shapes of these dose-response curves in its discussions of these endpoints. They considered the lowest dose to be the LOAEL for both of these effects and applied an uncertainty factor of 10 for extrapolation from LOAEL-to-NOAEL. However, the Health Effects Subcommittee is not aware of any precedent for use of an uncertainty factor for extrapolation from LOAEL-to-NOAEL for these types of dose response curves. The basis for application of this uncertainty factor, based on the assumption that the NOAEL is 10-fold lower than the lowest dose, is uncertain and may be subject to debate for dose-response curves of this type. In contrast, effects considered by the Health Effects Subcommittee for dose-response modeling (increased relative liver weight and delayed mammary gland development) follow typical monotonic dose-response curves and provide data appropriate for Benchmark Dose modeling.





Finally, the modes of action for delayed ossification and accelerated puberty in males are not known. This is noteworthy because one of the reasons provided by USEPA for not considering effects on mammary gland development in risk assessment is that the mode of action is not known.

Consideration of data from human epidemiological studies

As discussed in detail in the Epidemiology section of this document, PFOA is associated with several human health effects, some with evidence supporting multiple criteria for causality, in the general population exposure and in communities with exposure from contaminated drinking water. USEPA (2016a) acknowledges that associations of PFOA and numerous health endpoints

are observed in these human populations and that associations with some effects have consistently been found in multiple human studies.

However, USEPA states that, while these human studies are useful for hazard identification, they cannot be used quantitatively because the PFOA exposures at which the associations were observed are unknown or highly uncertain. Although the Health Effects Subcommittee agrees that the human data have limitations that preclude their use as the primary basis for risk assessment, it does not agree with USEPA that the serum PFOA concentrations and PFOA exposures associated with human health effects are highly uncertain or unknown.

USEPA (2016a) provides the following reasons for its conclusions:

- Serum levels may have decreased (in both the general population and in communities with drinking water exposure) prior to when the blood sample was taken. Therefore, the effects may have been due to earlier exposures that were higher than indicated by the measured serum PFOA levels.

  It is unlikely that this is a major source of uncertainty in evaluation of exposure since PFOA serum levels decrease slowly (half-life of several years) and are generally stable over time. In the C8 Health Study, blood samples were taken before or soon after the installation of treatment to remove PFOA from drinking water. Participants' blood was drawn between August 2005 and August 2006, with over 80% of samples taken in March 2006 or earlier, and treatment removal began between March 2006 and September 2008 (WVU School of Medicine, 2008; Frisbee et al., 2009; Fitz-Simons et al, 2013). Additionally, if effects were actually due to previous exposures that were higher than those at the time of blood sampling, it would mean that the detrimental effects of PFOA are persistent and do not resolve when exposures decrease, which would increase the level of concern about the effects.

- PFOA measured in serum may result from metabolism of precursors to PFOA rather than direct exposure to PFOA itself.

  This does not appear to be a valid reason to dismiss consideration of serum PFOA levels as a measure of PFOA exposure. Effects of PFOA would be the same regardless of whether the source of exposure is PFOA itself or metabolism of precursors to PFOA.

- Co-exposure to other PFCs, even if accounted for as a potential confounding factor in the statistical analysis, increase uncertainty about observed associations of health endpoints with PFOA.

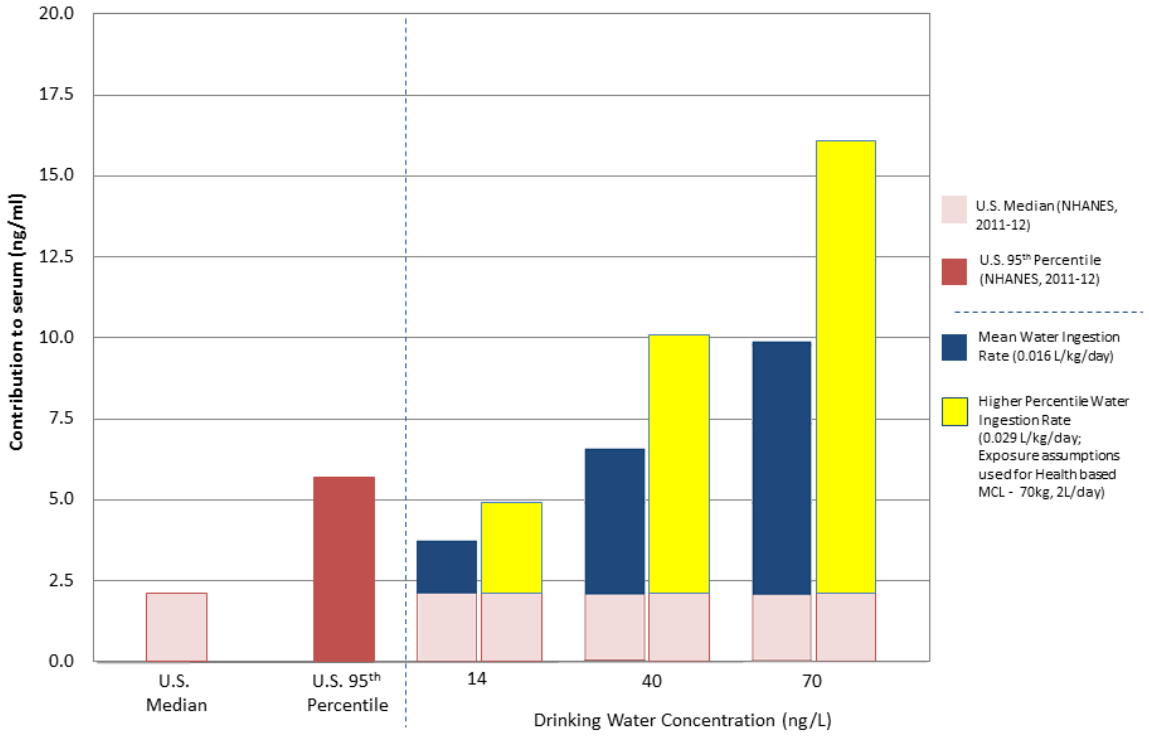
  However, co-exposure to other chemicals is a general issue for all human studies of exposure to environmental contaminants and does not preclude evaluation of the levels of PFOA exposure associated with health endpoints.

Consideration of increases in serum PFOA levels from exposure to PFOA in drinking water

As noted in the table at the beginning of this Appendix, a clearance factor developed by USEPA scientists (Lorber and Egeghy, 2011) relates human PFOA exposures to human PFOA serum levels. This factor can be used to predict increases in serum PFOA from ongoing drinking water exposures. The bar graph below shows the predicted increases in serum PFOA levels from ongoing exposure in drinking water at the USEPA (2016a) Health Advisory (70 ng/L), the NJDEP (2007) guidance (40 ng/L), and the DWQI recommended Health-based MCL (14 ng/L). Predictions based on both average and upper percentile drinking water ingestion rates are shown.

USEPA (2016a) used the clearance factor to convert NOAEL and LOAEL serum levels from laboratory animals to human equivalent doses (p. 4-13 of USEPA, 2016a) and discusses that it relates human PFOA dose to human PFOA serum level, including from drinking water exposure (p. 2-51 of USEPA, 2016b). However, USEPA does not acknowledge that it is possible to predict increases in serum PFOA levels from exposures to PFOA at 70 ng/L, the Health Advisory concentration (ASDWA, 2016). The Health Effects Subcommittee has evaluated this question and concludes that the use of the clearance factor to predict increases in serum PFOA levels from drinking water exposures is technically sound and is not subject to debate.

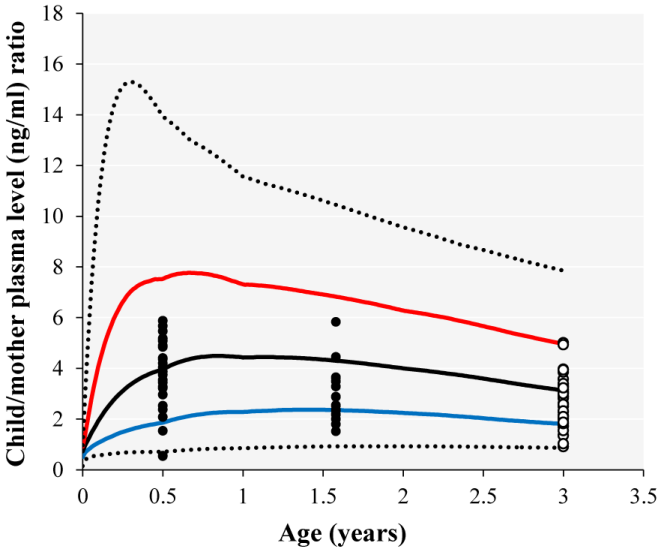
Several health effects, some with evidence supporting multiple criteria for causality, are associated with PFOA exposures at serum levels well below those that would result from exposure to 70 ng/L in drinking water. The Health Effects Subcommittee therefore concludes that elevations in serum PFOA levels of the magnitude expected from ongoing exposure to 70 ng/L (the USEPA Health Advisory) in drinking water are not desirable and may not be protective of public health.



14 ng/L – Health-based MCL recommended by DWQI Health Effects Subcommittee
40 ng/L – NJDEP drinking water guidance (2007) based on older toxicology data
70 ng/L – USEPA Lifetime Health Advisory

Finally, as discussed elsewhere in this document, several studies have shown that serum PFOA concentrations in breastfed infants increase several fold from levels at birth (which are similar to maternal levels) within the first few months of life (see figure below). Exposures to infants who consume formula prepared with contaminated water are also highest during this time period, and serum PFOA levels remain elevated for the first several years of life (see figure below). Therefore, increases in blood serum levels in infants and children from exposure to PFOA in drinking water are expected to be several-fold higher than those shown in the bar graph above.

USEPA recognizes that lactating women and bottle-fed infants are sensitive subpopulations for exposure to PFOA in drinking water. The Health Effects Subcommittee also concludes that the elevated exposures during infancy and early childhood are of particular concern because early life effects are sensitive endpoints for the toxicity of PFOA. Additionally, the Health Effects Subcommittee concludes women who may become pregnant should also be included in the sensitive subpopulations, because the body burden of PFOA remains elevated for many years after exposure ceases.



From Verner et al. (2016). Modeling simulation of the ratio of PFOA in blood plasma in breast fed infants/children to plasma concentration in mother.
Black line - 50th percentile. Blue line - 5th percentile. Red line - 95th percentile. Dotted lines - minimum and maximum values.

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APPENDIX 3: Risk Assessment Considerations for Butenhoff et al. (2002) Subchronic Cynomolgus Monkey Study

The Butenhoff et al. (2002) six month study in male cynomolgus monkeys is described in detail in the Toxicology section of this document. As discussed in the Toxicology section, only 3 of 4 of the low dose monkeys and 2 of 6 of the high dose monkeys tolerated the administered dose well enough to complete the study, while all 6 animals in the mid-dose group (10 mg/kg/day) completed the study. The LOAEL in this study was 3 mg/kg/day based on mortality (25%) possibly attributed to treatment and increased liver weight, and the NOAEL was not identified. Furthermore, it is important to note that 6 months represents less than 2% of the lifespan of this species of monkey, which is about 30 years. It is not known whether additional or more severe effects would have occurred with continued dosing of the monkeys that were able to tolerate dosing for the full 6 months of the study. Therefore, if this study were to be used as the basis for risk assessment, an uncertainty factor (UF) for less than chronic exposure duration should be included.

In the study, serum PFOA levels were analyzed every 2 weeks, and it appeared that steady state was reached after 2 to 4 weeks of dosing. Importantly, the serum levels were highly variable between animals and over time in the same animal, and they did not increase proportionally with dose. The average serum levels in the 0, 3, 10, 30/20 mg/kg/day groups, respectively, were: 134 $\pm$113, 81,000$\pm$40,000, 99,000$\pm$50,000 ug/ml, and 156,000 $\pm$103,000 ng/ml (Butenhoff et al., 2004a). The PFOA concentrations in liver also did not appear to increase with dose or time, and the highest concentration in the liver was in a high dose animal sacrificed at week 5.

Butenhoff et al. (2004b) reported benchmark dose modeling of the liver-to-brain weight data from this study. A $BMIC_{10}$ (maximum likelihood estimate of PFOA serum concentration at 10% effect level) of 40,000 ng/ml and a $LBMIC_{10}$ (lower bound estimate of serum concentration at 10% effect level) of 23,000 ng/ml were derived. The data on liver-to-brain weight ratio versus serum PFOA concentration are shown in Figure A-1 (below).

As can be seen from the graph in Figure A-1, Benchmark Dose (BMD) modeling of these data is not appropriate for the following reasons: The dose-response relationship needed to support BMD modeling does not exist, since (as stated by Butenhoff et al. (2002) the liver-to-brain weight ratio did not increase with dose. Additionally, there are an insufficient number of data points for BMD modeling. In the modeling conducted by Butenhoff et al. (2004b), the highest dose group was dropped, leaving only two data points for dosed groups plus the control group. It should be noted that the reported $LBMIC_{10}$ of 23,000 ng/L is within the same range as the serum PFOA concentration in the low dose monkey that was sacrificed in moribund condition during the weeks prior to its sacrifice.

Based on the above considerations, this study does not appear to be appropriate as the basis for a health-based drinking water concentration. In addition to the lack of dose response, a very small number of animals completed the study, and there was a wide variation in the responses of the individual animals.

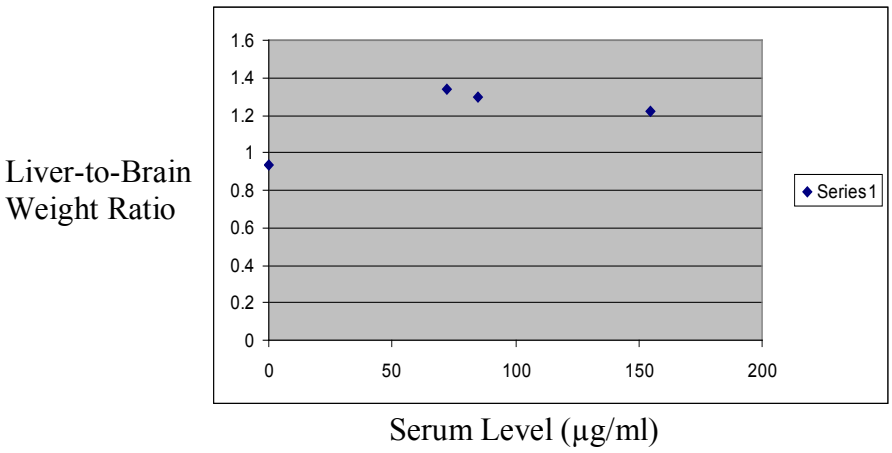
If a risk assessment were to be developed based on this study, in spite of its numerous limitations, the following point of departure (POD) and UFs appear to be appropriate:

- The serum level at the low dose, 3 mg/kg/day should be used as the POD. This dose was the LOAEL for both increased liver weight and increased mortality.

- In addition to the standard UFs for intraspecies (10) and interspecies toxicodynamic (3) variability used in risk assessments based on serum levels, a UF of 10 for extrapolation of a LOAEL to NOAEL and a UF of 10 for a study of less than chronic study duration, should be applied. These UFs combine to give a total UF of 3000.

- An additional UF for database limitations appears to be warranted due to the very small number of animals that completed the study, because mortality possibly related to treatment occurred in the low dose group, and because potentially more sensitive developmental endpoints were not evaluated.

Figure A-1: Cynomolgus Monkey
PFOA Serum Levels vs. Liver/Brain Weight Ratio



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APPENDIX 4. Individual tables for epidemiology studies

Reference and Study Design	Exposure Measures	Results	Comment
(Bloom, Kannan et al. 2010) **Study Design:** Cross-sectional **Location:** New York, USA **Population:** Licensed anglers in NYS, n=31. Participants are as subgroup of NYSACS participants (licensed anglers in New York state and their partners) **Outcome Definition:** Serum samples	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Geometric mean = 1.33 95% CI 1.15-1.53 ng/mL	**Stat Method:** Linear regression, covariates and confounders considered included age, gender, BMI, smoking, goiter or thyroid condition, race/ethnicity, use of thyroid medication, and self-reported consumption of sportfish caught from NY waters PFCs and TSH log transformed. **Outcome:** ln-TSH **Major Findings:** NS β = -0.06 (95% CI -0.78, 0.67) **Outcome:** FT4 **Major Findings:** NS β= -0.01 (95% CI -0.16, 0.14)	**Major Limitations:** Power analyses = would require 408-fold increase in sample size to see statistically significant associations with 80% power at observed effect sizes. Small sample size limited ability to control for potential covariates and confounders simultaneously, or other potential environmental compounds of interest or other PFCs. Cross-sectional design prevents causal inference. High proportion of values measured below the LODs for these compounds. **Funding Source**: Agency for Toxic Substances and Disease Registry (ATSDR), Grant H75-ATH 298338, the Great Lakes Protection Fund, Grant RM 791-3021, and the Centers for Disease Control and Prevention (CDC) National Center for Environmental Health (NCEH), Grant U59CCU22339202

Reference and Study Design	Exposure Measures	Results	Comment
(Chan, Burstyn et al. 2011) **Study Design:** Matched case-control **Location:** Edmonton, Alberta, Canada **Population:** Hypothyroxemic pregnant women at 15-20 weeks gestation, N=96 + 175 nonhypothyroxemic pregnant controls, matched on age and referring physician (Total N = 271), years 2005-2006 **Outcome Definition:** Hypothyroxinemic cases defined as (normal TSH, the lowest 10th percentile of ft4 – measured in serum)	**Exposure Assessment:** Serum PFOA **Population-Level Exposure:** Geometric mean (SD) nmol/mL Cases - 3.10 (3.10) Controls - 3.32 (2.72) Median nmol/L Cases – 3.94 Controls – 3.62	**Stat Method:** Student's t-test and chi-square were used to calculate differences in means between cases and controls by maternal age, maternal weight, gestation age at blood draw, and maternal race. Conditional logistic regression, covariates and confounders considered include maternal age, maternal weight, and gestational age at blood draw on mother. Each PFA was modelled separately and with three in a model (PFOA, PFOS, PFHxS). Adjusted analyses shown below. **Outcome:** maternal hypothyroxemia **Major Findings:** PFOA only OR = 0.94 (95% CI 0.74, 1.18) All PFCs in model OR=0.87 (95% CI 0.63,1.19)	**Major Limitations:** Small sample size. There are no known confounders to be accounted for in the study design. Possibility of confounding by socioeconomic or geographic factors Potential outcome misclassification due to timing of the blood draw. **Funding Source:** University Hospital Foundation (U of Alberta) and Alberta Health and Wellness.

Reference and Study Design	Exposure Measures	Results	Comment
(Costa, Sartori et al. 2009) **Study Design:** Case-control, cross-sectional **Location:** Miteni, Trissino, Italy **Population:** Cases (exposed): Workers (all male aged 20-63 years) engaged in the PFOA production department (n= 53). As of 2007,	**Exposure Assessment:** Serum PFOA (monitoring 2000-2007) **Population-Level Exposure:** Year 2007: Geometric mean (SD) µg/mL Currently Exposed – 4.02 (14.43) Formerly Exposed – 3.76 (6.06) Median (SD) µg/mL Currently Exposed – 5.71 (14.43) Formerly Exposed – 4.43 (6.06)	**Stat Method:** ANOVA, t test, multiple linear regression models, multivariate GEE models controlling form within-subject correlations, covariates and confounders considered include age, years of exposure, year of PFOA sampling, BMI, smoking, and alcohol consumption. People under treatment of primary hyperlidiemias and with a history of chronic hepatitis were excluded. Natural log transformation was performed where appropriate. The following chemical chemistry was compared among exposed and non-exposed: BMI, glucose, urea nitrogen,	**Major Limitations:** Small sample size Healthy worker effect Other environmental contaminants were not controlled for. Cross-sectional design prevents causal inference. **Funding Source:** not specified. Authors are

37 were active workers and 16 unexposed. Controls (unexposed): Other male workers never exposed to PFOA (n= 107) (12 executive clerks and 95 blue collar workers from other departments) Matched on age, time of shift, area of residence and job seniority **Outcome Definition:** Physical examination including blood chemical chemistry tests over 30 years (1978-2007)	Geometric Mean in Production Workers <u>Year</u> 2000 = 11.7 2001 = 10.2 2002 = 9.3 2003 = 6.9 2004 = 6.5 2006 = 5.8 2007 = 5.4 *renovation happened in the plant in 2002 partially automating the process	creatinine, uric acid, total cholesterol, HDL, triglycerides, total bilirubin, AST, ALT, GGT, ALP, proteins, albumin, α1 globulins (large proteins found in the blood), α2 globulins, β globulins, γ globulins, WBC, RBC, Haemoglobin, Haematocrit, platelets. **Outcome:** Chemical chemistry **Major Findings:** All the biochemical parameters turned out to be on average within the laboratory reference ranges. As compared with the control group some differences, both in terms of mean values and number of persons above the upper reference limited, were noted for some parameters such as uric acid, cholesterol, triglycerides, and liver enzymes. <u>*Analysis#1:* T-test (Nonexposed (34 controls) v. 34 exposed)</u> **Uric acid – p-value= 0.039** **Total cholesterol – p-value=0.003** All other findings are not significant. <u>*Analysis#2:* Multiple regression analysis (34 Exposed v. 107 controls)</u> **Uric acid – p-value= 0.027** **Total cholesterol – p-value=0.005** All other findings are not significant. <u>*Analysis#3:* GEE Model (subjects regardless of exposure status with concurrent outcome and PFOA in the last 6 years)</u> **Uric acid – p-value <0.05** **Total cholesterol – p-value <0.05** **Total bilirubin - p-value <0.01 (inversely)** **ALT - p-value <0.01** **GGT - p-value <0.01** **ALP - p-value <0.05** **α2 globulins – p-value <0.05** All other findings are not significant.	employed by Department of Occupational and Environmental Health (Costa, Sartori), University of Milano, Italy; Unit of Epidemiology (Consonni), IRCCS Maggiore Hospital, Mangiagalli and Regina Elena Foundation, Milano, Italy; and Institute of Medical Statistics (Sartori)

Reference and Study Design	Exposure Measures	Results	Comment
Darrow et al., 2016 **Study Design:** Cross-sectional; and a subanalysis with prospective analysis among those reporting liver disease in 2005/2006. **Location:** United States – West Virginia and Ohio **Population:** Adults (> 20 years); liver biomarkers (n=30,726, including 1,892 workers) and liver disease (n=32,254, including 3,713 workers) **Outcome Definition:** Alanine aminotransferase (ALT), γ-glutamyltransferase (GGT) and direct bilirubin, markers of liver toxicity (measures in 2005-2006) and self-reported liver disease (from surveys administered 2008 and 2011) which were medically reviewed. Liver disease was categorized as any medically-validated liver disease and liver disease restricted to medically-validated enlarged liver, fatty liver, or cirrhosis.	**Exposure Assessment:** Historically modeled yearly PFOA exposure estimated using environmental fate and transport models and participant residential histories, a measure of cumulative serum PFOA exposure was calculated by summing all previous yearly estimates **Population-Level Exposure:** Median estimated PFOA serum concentration in 2005-2006, 16.5 ng/mL (range 2.6 – 3559 ng/mL).	**Stat Method:** In linear regression models, natural log-transformed liver function markers were analyzed in relation to estimated cumulative PFOA serum concentrations through 2005 or 2006 and estimated year-specific PFOA serum concentration in 2005 or 2006. Logistic regression was used to model dichotomized measures of the liver function measures. PFOA was analyzed continuously (log-transformed) and by quintiles. Covariates and confounders considered include age, sex, BMI, alcohol consumption, race, regular exercise, smoking status, education, household income, fasting status, history of working at DuPont plant, and insulin resistance (HOMA-IR). Stratified Cox proportional hazard models with age as the time scale, time-varying cumulative serum PFOA as a predictor and stratified by birth year to control for any birth cohort trends used to analyzed liver disease. Covariates and confounders considered include sex, years of schooling, race, smoking, regular alcohol consumption, and BMI. A 10-year lag was also investigated. Stratified by history of working in PFOA plant, gender, and age. **Outcome:** Liver biomarkers **Major Findings:** *Cumulative PFOA (these findings are reflective of 2005/2006 PFOA results)* <u>ALT:</u> **Contin.: β=0.012 (95% CI 0.008. 0.016)** **Q2 v. Q1: β=0.023 (95% CI 0.006, 0.040)** **Q3 v. Q1: β=0.035 (95% CI 0.018, 0.052)** **Q4 v. Q1: β=0.039 (95% CI 0.022, 0.056)** **Q5 v. Q1: β=0.058 (95% CI 0.040, 0.076)** **Trend: p-value <0.001** <u>GGT:</u> Contin.: β=0.003 (95% CI -0.003, 0.008) Q2 v. Q1: β=0.009 (95% CI -0.014, 0.031) Q3 v. Q1: β=0.025 (95% CI 0.003, 0.047) Q4 v. Q1: β=0.011 (95% CI -0.011, 0.033) Q5 v. Q1: β=0.020 (95% CI -0.004, 0.044) Trend: p-value 0.1021 <u>Direct Bilirubin:</u> **Contin.: β=-0.005 (95% CI -0.008, -0.002)** Q2 v. Q1: β=0.012 (95% CI -0.002, 0.026)	**Major Limitations:** Cross-sectional design prevents causal inference. Other environmental contaminants were not controlled for. **Funding Sources:** C8 Class Action Settlement Agreement between DuPont and Plaintiffs

		Q3 v. Q1: β=-0.003 (95% CI -0.017, 0.011) Q4 v. Q1: β=-0.007 (95% CI -0.021, 0.007) **Q5 v. Q1: β=-0.017 (95% CI -0.032, -0.001)** **Trend: p-value 0.0029** **Outcome:** Liver disease **Major Findings:** Any liver disease (no lag): Contin.: HR=0.97 (95% CI 0.92, 1.03) Q2 v. Q1: HR=1.19 (95% CI 0.88, 1.59) Q3 v. Q1: HR=1.08 (95% CI 0.81, 1.45) Q4 v. Q1: HR=1.04 (95% CI 0.78, 1.40) Q5 v. Q1: HR=0.95 (95% CI 0.70, 1.27) Enlarged liver, fatty liver, cirrhosis Contin.: HR=0.97 (95% CI 0.91, 1.04) Q2 v. Q1: HR=0.90 (95% CI 0.65, 1.25) Q3 v. Q1: HR=0.83 (95% CI 0.60, 1.15) Q4 v. Q1: HR=0.75 (95% CI 0.54, 1.03) Q5 v. Q1: HR=0.83 (95% CI 0.60, 1.16)	

Reference and Study Design	Exposure Measures	Results	Comment
(de Cock, de Boer et al. 2014) **Study Design:** Cross-sectional **Location:** Six midwifery clinics in Netherlands **Population:** Mother-child cohort through neonatal screening programs, n=83 pairs **Outcome Definition:** Blood levels of thyroxine (T4) in cord plasma (immediately after birth) screening and heel prick blood spots (between day 4 and day 7 after birth)	**Exposure Assessment:** Cord plasma concentration **Population-Level Exposure:** Cord plasma: Median = 885 ng/L = 0.89 ng/mL Range 200-2700 ng/L = 0.2-2.7 ng/mL	**Stat Method:** Linear regression models (separate for each exposure). Models were stratified for gender. Covariates and confounders considered included health problems related to the thyroid gland, use of thyroid medication, birth weight, C-section, gestational weight gain, gestational age, parity, smoking, alcohol, maternal BMI, and maternal age at birth. Chemical compounds were analyzed as quartiles (after no linear associations were noted) Results reported for most adjusted model **Outcome:** T4 **Major Findings:** *In boys:* Q2 v. Q1, β=7.9 (95% CI -18.0, 33.9) Q3 v. Q1, β=-2.1 (95% CI -20.9, 19.8) Q4 v. Q1, β=6.2 (95% CI -16.1, 28.5)	**Major Limitations:** Homogenous w/r/t nationality and education level No adjustment for time of heel prick (between 4 and 7 days after birth), Small sample size Cross-sectional design prevents causal inference. Power decreased by quartile formation and stratifying, though there was justification for both practices. **Funding Sources:** the European Community's Seventh Framework Programme [FP7/2007-2013] under grant agreement OBELIX n° 227391.

		In girls: Q2 v. Q1, β=-5.9 (95% CI -26.8, 14.9) Q3 v. Q1, β=11.8 (95% CI -19.1, 42.7) **Q4 v. Q1, β=38.6 (95% CI 13.3, 63.8)** No linear associations found for any compound studied.	

Reference and Study Design	Exposure Measures	Results	Comment
(Emmett, Zhang et al. 2006) **Study Design:** Cross-sectional **Location:** Little Hocking water district in SE Ohio **Population:** Stratified random sample of residents (≥2 year residence) of district (N=371, 317 randomly selected, 54 volunteer (18 occupationally exposed)), age range 2.5-89 years **Outcome Definition:** Serum biomarkers and questionnaire of previous clinical diagnoses of liver or thyroid disease.	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Median PFOA: 354 ng/mL, IQR 181 ng/mL to 571 ng/mL	**Stat Method:** Simple regression (liver function/hematologic parameters), binary t-test (normal v. abnormal) blood chemistries or history of clinical diagnoses of interest **Outcome:** Serum chemistry (BUN, creatinine, total protein, albumin, total bilirubin, ASP, AST, ALT, GGR, total cholesterol, TSH) **Major Findings:** No significant positive linear relationships ($p > 0.05$) **Outcome:** Hematologic parameters (WBC, RBC, hemoglobin, hematocrit, MCV, MCH, MCHC, RDW, Platelets, neutrophils, lymphocytes, monocytes, eosinophils, basophils, neutrophils, lymphocytes, monocytes, eos, basos **Major Findings:** **Monocyte (linear) – r=0.13, p-value=0.01** **AST (binary) – p-value=0.03 (inversely)** **% neutrophils (binary) – p-value=0.02 (inversely)** **% lymphocytes (binary) – p-value=0.01 (inversely)** No other significant positive relationships ($p > 0.05$) **Outcome:** Liver disease **Major Findings:** p-value=0.50 Disease (n=13), PFOA=527 ng/mL vs. No disease, PFOA=441 ng/mL **Outcome:** Thyroid disease **Major Findings:** p-value=0.3 Disease (n=40), PFOA=387 ng/mL vs. No disease, PFOA=451 ng/mL	**Major Limitations:** Potential volunteer bias from volunteer group, Cross sectional design precludes causal inference No confounders or covariates were considered beyond stratified analyses by age. Other environmental or lifestyle confounder may be clouding true relationship between exposure and endpoints. **Funding Source:** Grant ES12591 from the Environmental Justice Program and by grant number 1P30 ES013508-01A1 from the National Institute of Environmental Health.

Reference and Study Design	Exposure Measures	Results	Comment
(Eriksen, Raaschou-Nielsen et al. 2013) **Study Design:** Cross-sectional **Location:** Denmark **Population:** Middle aged (50-65 years of age), N=753 (663 male, 90 female) Nested within a larger Danish Diet, Cancer, and Health cohort **Outcome Definition:** Serum, questionnaires	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Mean: 7.1 ng/mL	**Stat Method:** Generalized linear models (crude adjusted for sex only and adjusted). Covariates and confounders considered included sex, age, education, BMI, smoking status, alcohol intake, egg intake, animal fat intake, physical activity. Primary analyses treated PFCs continuously. Additionally, investigated as 8 exposure groups (100 per group), with lowest group as reference. **Outcome:** total cholesterol **Major Findings:** β_{unadj}= 4.1 (95% CI 0.8, 7.4) **β_{adj} = 4.4 (95% CI 1.1, 7.8)** Using the lowest exposure group as a referent group, the level of total cholesterol appears to be higher with higher PFOA plasma levels with a borderline significant p value for trend.	**Major Limitations:** Cross-sectional design precludes causal inference. Other unidentified confounders may exist **Funding Sources**: Danish Cancer Society and the International Epidemiology Institute (IEI), which received an unrestricted research grant from the 3M Company.

Reference and Study Design	Exposure Measures	Results	Comment
(Fisher, Arbuckle et al. 2013) **Study Design:** Cross-sectional **Location:** Canada **Population:** CHMS (Canadian Health Measures Survey, 2007-2009) N=5604, 6-79 year olds **Outcome Definition:** Serum measures and questionnaires	**Exposure Assessment:** Serum concentration **Population-Level Exposure:** Geometric mean = 2.46 µg/L (ng/mL)	**Stat Method:** Multivariate linear and logistic regression models, covariates and confounders considered include cholesterol lowering medications, sampling weights, age, gender, marital status, income adequacy, race, education, BMI, physical activity index, smoking status and alcohol consumption. PFOA analyzed in quartiles, reference=1 ng/mL and dichotomous Outcomes and exposures were log transformed Results shown for most adjusted models by unweighted and weighted.	**Major Limitations:** Cross sectional design precludes causal inference. Reverse causality or unexplored confounder may exist Excluded those on cholesterol-lowering medication, removing some of diseased population Other unidentified confounders may exist. No adjustment for albumin (possible non-casual positive correlation between PFCs and cholesterol)

For all clinical outcomes non-pregnant adults (≥ 18 years of age)		**Outcome:** Cholesterol outcomes **Major Findings:** Linear - unweighted HDL – β=0.007, p-value=0.45 TC/HDL – β=0.02, p-value=0.11 LDL – β=0.008, p-value=0.64 **Non-HDL – β=0.03, p-value=0.003** **TC – β=0.02, p-value=0.001** Triglycerides – β=0.02, p-value=0.49 Linear - weighted HDL – β=0.009, p-value=0.96 TC/HDL – β=0.02, p-value=0.22 LDL – β=0.02, p-value=0.63 Non-HDL – β=0.036, p-value=0.13 TC – β=0.03, p-value=0.22 Triglycerides – β=-0.003, p-value=0.94 (High cholesterol) Quartiles – unweighted Q2 v. Q1, OR=1.26 (95% CI 0.98, 1.62) Q3 v. Q1, OR=1.18 (95% CI 0.91, 1.52) **Q4 v. Q1, OR=1.46 (95% CI 1.13, 1.89)** **P for Trend=0.01** Quartiles - weighted **Q2 v. Q1, OR=1.61 (95% CI 1.02, 2.53)** Q3 v. Q1, OR=1.26 (95% CI 0.76, 2.07) Q4 v. Q1, OR=1.5 (95% CI 0.86, 2.62) P for Trend=0.10 High cholesterol (dichotomous) **Unweighted OR=1.20 (95% CI 1.03, 1.40)** Weighted OR=1.22 (85% CI 0.89, 1.67) **Outcome:** Metabolic outcomes **Major Findings:** Linear - unweighted Insulin – β=-0.003, p-value=0.91 Glucose – β=-0.01, p-value=0.06 HOMA-IR – β=-0.01, p-value=0.63	85% of participants were Caucasian, 60% with post-secondary education, and close to half were in the highest income adequacy category. **Funding Source**: unspec, Authors are employed by Health Canada.

		Linear - weighted Insulin – β=-0.071, p-value=0.12 Glucose – β=-0.04, p-value=0.17 HOMA-IR – β=-0.1, p-value=0.10 Metabolic syndrome (dichotomous) Unweighted OR=1.26 (95% CI 0.92, 1.73) Weighted OR=1.13 (85% CI 0.46, 2.77)	

Reference and Study Design	Exposure Measures	Results	Comment
(Fitz-Simon, Fletcher et al. 2013) **Study Design:** Cohort, Longitudinal, over a 4.4-year period **Location:** Ohio and West Virginia, USA **Population:** n=560, living in areas with PFOA contaminated drinking water (sample derived from C8 short term follow up study), 2005-2006 followed through 2010 [C8 Short-Term Follow-up Study], 20-60 years **Outcome Definition:** Serum concentration (LDL, HDL, total cholesterol, triglycerides)	**Exposure Assessment:** Serum concentration **Population-Level Exposure:** (ng/mL) Baseline (2005/06) Geometric Mean=74.8; Arithmetic Mean=140.1 Follow-up (2010) Geometric Mean=30.8 Arithmetic Mean=68.2	**Stat Method:** adjusted linear regression models. Change-vs-change model, in which the cross-sectional model as baseline is subtracted from the cross-sectional model at follow-up. Covariates and confounders considered include age at baseline, time between measurements, sex, and fasting status (from both baseline and follow-up). PFOS also controlled for in PFOA model. Sensitivity analysis adjusted for baseline BMI, years of schooling, change in smoking, and baseline and changes in GFR. Additionally, a ratio of baseline to follow-up and PFOA tertiles are ordered by decrease. PFOA log transformed. Findings shown below are for most adjusted model and model adjusting for PFOS. Effect estimates are percentage decrease in serum lipid based on halving of PFOA. Estimates are shown as percent decrease. **Outcome:** LDL **Major Findings:** **% ↓=3.58 (95% CI 1.47, 5.66)** **% $\downarrow_{(PFOS)}$=2.92 (95% CI 0.71, 5.09)** **Outcome:** Total cholesterol **Major Findings:** **% ↓ =1.65 (95% CI 0.32, 2.97)** % $\downarrow_{(PFOS)}$= 0.63(95% CI -0.88, 2.12)	**Major Limitations:** Joint models should be assessed with caution because PFOA and PFOS are highly correlated. Only two time points from which to estimate relationship Lag uncertainty Cannot detect any possible irreversible effects of PFOA on serum lipids Low power in LDL analysis – n=56 Exact mechanism unknown – limits causal explanation Caution when extrapolating to general population – this is a highly exposed population **Funding Source**: C8 Class Action Settlement Agreement between DuPont and Plaintiffs

		Outcome: HDL **Major Findings:** % ↓ =1.33 (95% CI -0.21, 2.85) % $\downarrow_{(PFOS)}$=1.24 (95% CI -0.34, 279) **Outcome:** Triglycerides **Major Findings:** % ↓ =-0.78 (95% CI -5.34, 3.58) % $\downarrow_{(PFOS)}$=-1.16 (95% CI -5.85, 3.33)	

Reference and Study Design	Exposure Measures	Results	Comment
(Fletcher, Galloway et al. 2013) **Study Design:** Cross-sectional **Location:** Parkersburg, WV, USA **Population:** Residents for at least one year, 20-60 year olds, N=290 **Outcome definition:** Changes in the expression of genes (13 target genes) involves in cholesterol metabolism in humans. Genes were selected on the basis of roles in cholesterol biogenesis (HMGCR), peroxisome proliferation (PPARA, PPARD, PPARG, and PGC1A), cholesterol transport (ABCA1, ABCG1, and APOA1), downstream transcriptional activation of PPARA (NR1HA, NR1H2, and mobilization of cholesterol (NPC1, ACAT1, and NCEH1)	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Median = 30.1 ng/mL	**Stat Method:** Adjusted linear regression, some models stratify by gender and menopausal status. Covariates and confounders considered include age, sex, BMI, average household income, and smoking status Expression value of each of the target genes and for PFOA/PFOS levels were log transformed. **Outcome:** Candidate gene transcript levels **Major Findings:** Following are statistically significant: **significant:** Gene | Coeff | 95% CI | P **ABCG1** | **-0.29** | **-0.51, -0.06** | **0.014** **NPC1** | **-0.34** | **-0.63, -0.04** | **0.026** **NR1H2 (LXRB)** | **-0.55** | **-0.90, -0.20** | **0.002** ABCA1, ACAT1, APOA1, HMGCR, NCEH1, NR1H3, PPARA, PPARD, PPARGC1A, PPARG – were not statistically significant	**Major Limitations:** Restriction on examining expression in whole blood only – may not correlate with expression in target organs. Limited size of population, power concerns. Cross sectional design precludes causal inference. **Funding Source:** C8 Class Action Settlement Agreement between DuPont and Plaintiffs, funds administered by Garden City Group

Men:

Gene	Coeff	95%CI	P
ABCG1	-0.34	-0.64, 0.047	0.024
NPC1	-0.53	-0.99, -0.06	0.025
PPARA	-0.38	-0.68, 0.07	0.012
NR1H2	-0.71	-1.29, -0.12	0.019

Women:

Gene	Coeff	95% CI	P
NCEH1	**0.44**	**0.03, 0.85**	**0.036**

Premenopausal (inverse effects):

Gene	Coeff	95%CI	P
NR1H2	**-1.47**	**-2.63, -0.32**	**0.013**
PPARD	**-1.06**	**-2.05, -0.07**	**0.036**

Postmenopausal (positive effects):

Gene	Coeff	96% CI	P
APOA1	**0.051**	**0.11, 0.90**	**0.013**

Provides evidence from a human population that PFOA are associated with alterations in the levels of gene transcripts involves in cholesterol transport or mobilization, which appear consistent with PFOA promoting a hypercholesteroleamic environment.

Reference and Study Design	Exposure Measures	Results	Comment
(Frisbee, Shankar et al. 2010) **Study Design:** Cross-sectional **Location:** Mid-Ohio river	**Exposure Assessment:** Serum concentration **Population-Level Exposure:** Mean = 69.2 ng/mL (SD = 111.9)	**Stat Method:** Multiple linear regressions with GLM ANCOVA analysis to estimate predicted lipids with PFOA as quintile. To assess the linearity/nonlinearity of associations, population median for 20-group quantiles were plotted again the PFOA median. Binary	**Major Limitations:** Cross sectional design precludes causal inference. Self-reported survey data not uniformly reliable

valley (OH and WV, USA) **Population:** Children included in C8 Health Project N=12,476, 1-17.9 years **Outcome Definition:** Serum lipids (LDL, total cholesterol, HDL, fasting triglycerides)		logistic regression analysis with outcomes as 'high' or 'low' and PFCs as quintiles. Covariates and confounders considered include age, gender, BMI, time of fasting, and exercise, and sensitivity analyses of fasting and socioeconomic status. Age and gender specific quantiles were created. PFOA was log-transformed. Interaction between PFOA and PFOA was assessed. **Outcome:** Total cholesterol **Major Findings:** *Results not shown but after adjustment **PFOA linearly/positively associated (p<0.0001)** Q2 v. Q1, OR=1.1 (95% CI 1.0,1.3) Q3 v. Q1, OR=1.2 (95% CI 1.0, 1.4) **Q4 v. Q1, OR=1.2 (95% CI 1.1, 1.4)** **Q5 v. Q1, OR=1.2 (95% CI 1.1, 1.4)** **Outcome:** LDL **Major Findings:** *Results not shown but after adjustment **PFOA linearly/positively associated (p<0.0001)** Q2 v. Q1, OR=1.2 (95% CI 1.0,1.5) Q3 v. Q1, OR=1.2 (95% CI 1.0, 1.4) Q4 v. Q1, OR=1.2 (95% CI 1.0, 1.4) **Q5 v. Q1, OR=1.4 (95% CI 1.1, 1.7)** **Outcome:** Triglycerides **Major Findings:** *Results not shown but after adjustment **PFOA linearly/positively associated (p=0.019)** Q2 v. Q1, OR=1.0 (95% CI 0.7,1.5) Q3 v. Q1, OR=1.3 (95% CI 0.9, 1.9) Q4 v. Q1, OR=1.6 (95% CI 1.1, 2.3) Q5 v. Q1, OR=1.0 (95% CI 0.7, 1.6)	Limited availability of known covariates **Funding Source**: C8 Class Action Settlement Agreement between DuPont and Plaintiffs, and contractual relationship between Brookmar, INC. and West Virginia University. Authors are members of the separate C8 Science Panel.

		Outcome: HDL **Major Findings:** *Results not shown but after adjustment PFOA not linearly associated Q2 v. Q1, OR=1.0 (95% CI 0.8,1.2) Q3 v. Q1, OR=1.0 (95% CI 0.8, 1.2) Q4 v. Q1, OR=1.0 (95% CI 0.9, 1.2) Q5 v. Q1, OR=0.9 (95% CI 0.8, 1.1)	

Reference and Study Design	Exposure Measures	Results	Comment
(Fu, Wang et al. 2014) **Study Design:** Cross-sectional **Location:** Henan, China **Population:** Random selection of people coming in for health check-up at Yuanyang hospital, N=133, aged 0-88 years **Outcome Definition:** Serum lipids	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Median = 1.43 ng/mL Quartile / Mean (ng/mL) 1 / 0.71 2 / 1.24 3 / 1.97 4 / 4.88	**Stat Method:** Linear regression, exposure modeled in quartiles with 1st quartile serving as the referent group. Binary logistic regression of abnormal lipids by PFC quartile. Covariates and confounders considered include age, gender, and BMI. Outcomes are based on a change in values. **Outcome:** Total cholesterol **Major Findings:** **↑ (p-value for trend 0.015)** **Outcome:** Triglycerides **Major Findings:** NS (p-value for trend 0.298) **Outcome:** HDLC **Major Findings:** NS (p-value for trend 0.260) **Outcome:** LDLC **Major Findings:** **↑ (p-value for trend 0.022)** *For each of these outcomes there were also binary logistic regression results; however, no results were statistically significant for any level of PFC or outcome.	**Major Limitations:** Cross sectional design precludes causal inference. Did not take into account cholesterol-lowering medications or other environmental factors and contaminants including other co-occurring PFCs. Convenience sample – possible bias. Small sample size. **Funding Source:** National Natural Science Foundation of China (Grant nos. 41371488 and 41071355, the International Scientific Cooperation Program (Grant no. 2012DFA91150), and Key Project of the Chinese Academy of Sciences (Grant no. KZZD-EW-TZ-12).

Reference and Study Design	Exposure Measures	Results	Comment
(Gallo, Leonardi et al. 2012) **Study Design:** Cross-sectional **Location:** United States – West Virginia and Ohio **Population:** Adults (18 years or older). Consumed water for at least 1 year from a water district with known PFOA contamination, n=47,092 **Outcome Definition:** Serum biomarkers of liver function: ALT (alanine transaminase), GGT (gamma-glutamyltransferase), direct bilirubin	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Median: 23.1 ng/mL, IQR 11.3-58.2	**Stat Method:** Linear regression of ln-transformed values, logistic regression fitted (reference levels as cut-offs for outcomes) comparing deciles of exposure, multilevel analysis comparing individual-level associations to population-level associations. Covariates and confounders considered include age, physical activity, BMI, average household income, educational level, alcohol consumption, cigarette smoking, fasting status, SES, race, and month of blood sample collection, and insulin resistance. Results for most adjusted models are presented. **Outcome:** ln-ALT **Major Findings:** **β = 0.022 (95% CI 0.018, 0.025)** **p-value for trend < 0.001** **ln-Unit OR=1.10 (95% CI 1.01, 1.13)** **Relationship consistent for both between water districts and among individuals within districts increases strength of evidence for casual association.** **Outcome:** ln-GGT **Major Findings:** **β = 0.015 (95% CI 0.010, 0.019)** p-value for trend = 0.213 ln-Unit OR=1.01 (95% CI 0.99,1.04) Absence of trend across districts might be indicative of some confounding factor at the individual level. **Outcome:** ln-Direct bilirubin **Major Findings:** β = 0.001 (95% CI -0.002, 0.014) p-value for trend 0.496 ln-Unit OR=0.97 (95% CI 0.90, 1.15) Some evidence of geographic confounding.	**Major Limitations:** Cross sectional design precludes causal inference. Self-reported data of lifestyle characteristics strongly associated with exposures of interest – can hamper confounder adjustment Possible confounding due to unmeasured variables, and other environmental contaminants, including other PFCs. **Funding Source:** C8 Class Action Settlement Agreement between DuPont and Plaintiffs

Reference and Study Design	Exposure Measures	Results	Comment
(Geiger, Xiao et al. 2013) **Study Design:** Cross-sectional **Location:** United States **Population:** NHANES 1999-2000 and 2003-2008, ≤18 years of age, n=1,772 **Outcome Definition:** Hyperuricemia (serum uric acid levels >6 mg/dL)	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Mean = 4.3 ng/mL (SE 0.1)	**Stat Method:** Linear regression models, and multivariable logistic regression. Covariates and confounders considered include age, sex, race/ethnicity, BMI, annual household income, physical activity, total cholesterol, and serum cotinine levels. PFCs were log-transformed in continuous analyses and categorized into quartiles for categorical analysis. Results shown for most adjusted models. **Outcome:** Uric Acid **Major Findings:** **Ln-Unit, mean change=0.20 (0.11, 0.29)** Q2 v. Q1, mean change=0.02 (95% CI -0.10, 0.14) Q3 v. Q1, mean change=0.03 (95% CI -0.11, 0.17) **Q4 v. Q1, mean change=0.30 (95% CI 0.17, 0.43)** **P-value for trend=0.0001** **Outcome:** Hyperuricemia **Major Findings:** **Ln-Unit, OR=1.59 (1.19, 2.13)** Q2 v. Q1, OR=0.94 (95% CI 0.58, 1.53) Q3 v. Q1, OR=1.01 (95% CI 0.62, 1.63) **Q4 v. Q1, OR=1.62 (95% CI 1.10, 0.43)** **P-value for trend=0.0071**	**Major Limitations:** Cross-sectional design precludes causal inference. Spot samples of urinalysis cannot detect changes in response to recent changes in exposure, Possible confounding due to unmeasured variables, and other environmental contaminants, including other PFCs.

Reference and Study Design	Exposure Measures	Results	Comment
(Geiger, Xiao et al. 2014) **Study Design:** Cross-sectional **Location:** United States	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Mean: 4.2 (SE = 0.2) ng/mL	**Stat Method:** Linear regression models, and multivariable logistic regression. Covariates and confounders considered includes age, sex, race/ethnicity, BMI categories, annual household incomes, moderate activity, and serum cotinine.	**Major Limitations:** Cross sectional design precludes causal inference. Possible confounding due to unmeasured variables, and other environmental contaminants, including other PFCs.

Population: NHANES 1999-2000 and 2003-2008, ≤18 years of age, n=815 **Outcome Definition:** Dyslipidemia (TC > 170 mg/dL, HDL < 40 mg/dL, LDL >110 mg/dL, or triglycerides >150 mg/dL)		PFCs were log-transformed in continuous analyses and categorized into tertiles for both continuous and categorical analysis. Results shown for most adjusted models. **Outcome:** Total cholesterol **Major Findings:** Ln-Unit, OR=4.55 (95% CI 0.90, 8.20) Q2 v. Q1, OR=4.72 (95% CI -1.23, 10.67) **Q3 v. Q1, OR=7.00 (95% CI 1.40, 12.60)** **P-value for trend=0.017** **Outcome:** LDL **Major Findings:** **Ln-Unit, OR=5.75 (95% CI 2.16, 9.33)** Q2 v. Q1, OR=3.61 (95% CI -1.13, 8.36) **Q3 v. Q1, OR=8.18 (95% CI 3.04, 13.32)** **P-value for trend=0.0027** **Outcome:** HDL **Major Findings:** Ln-Unit, OR=-1.52 (95% CI -3.02, -0.03) Q2 v. Q1, OR=0.53 (95% CI -1.23, 2.30) Q3 v. Q1, OR=-1.19 (95% CI -2.94, 0.56) P-value for trend=0.1769 **Outcome:** Triglycerides **Major Findings:** Ln-Unit, OR=1.74 (95% CI -2.88, 11.68) Q2 v. Q1, OR=3.00 (95% CI -5.68, 11.68) Q3 v. Q1, OR=0.09 (95% CI -6.11, 6.30) P-value for trend=0.9943	**Funding Source**: Grant numbers 5T32HL090610-04, 1R01ES021825-01 and 5R03ES018888-02 from the National Institutes of Health.

		Outcome: Dyslipidemia **Major Findings:** Findings consistent with findings from linear regression analyses. Positive association between PFOA exposures and high total cholesterol and high LDL, but not with low HDL or high triglycerides.	

Reference and Study Design	Exposure Measures	Results	Comment
(Gilliland and Mandel 1996) **Study Design:** Cross-sectional **Location:** 3M Chemolite plant (PFOA production plant), Cottage Grove, MN **Population:** Occupationally exposed men (currently employed), 1985-1989, n=115 **Outcome Definition:** Hepatic enzyme and serum lipids: Serum glutamyl oxa.oacetic transaminase (SGOT), serum glutamyl pyruvic transaminase (SGPT), gamma glutamyl transferase (GGT), cholesterol, low-density lipoproteins (LDL), high-density lipoproteins (HDL) **Related Studies:** Gilliland and Mandel, 1993	**Exposure Assessment:** Workers with jobs involving direct contact with PFOA = highly exposed. Workers without direct contact with PFOA for at least 5 years = low exposure. Job metrics were not used once determine 'unexposed' groups had levels 20-50 times higher than levels reported for general population. Total serum fluorine used as a surrogate. **Population-Level Exposure:** Mean= 3.3ppm (mg/L)=3300 ng/mL Range 0 – 26 ppm	**Stat Method:** Stratified analysis using ANOVA procedures to assess differences in mean values. Total fluorine divided into 5 groups. Pearson correlation coefficient (univariate), linear multivariate regression. Covariates and confounders considered include age, BMI, alcohol use, and tobacco use. **Outcome:** Serum lipids **Major Findings:** Cholesterol, r=0.07 (NS) LDL, r=0.02 (NS) HDL, r=-0.01 (NS) **HDL, β=-1.61, p-value=0.04** Cholesterol and LDL (NS), results not shown **Outcome:** Hepatic enzymes **Major Findings:** NS SGOT (AST), r=0.01 (NS) SGPT (ALT), r=0.01 (NS) GGT, r=-0.04 (NS) **SGOT (AST), β=-3.23, p-value=0.02** **SGPT (ALT), β=-15.8, p-value=0.0008** GGT, β=-1.93, p-value=0.36 Note: subclinical hepatic toxic effects in obese workers, drinkers and smokers. PFOA may be modulating these effects, i.e. "endocrine disruptor"	**Major Limitations:** Healthy worker effect Cross sectional design precludes causal inference. Self-reported smoking and drinking status – not uniformly reliable Duration of exposure information not available Concerns with using serum fluorine rather than direct PFOA, **Funding Source:** NIOSH grant T150H07098-16 and the 3M Medical Department

Reference and Study Design	Exposure Measures	Results	Comment
(Gleason, Post et al. 2015) **Study Design:** Cross-sectional **Location:** United States **Population:** NHANES 2007-2010, individuals > 12 years of age, n=4,333 **Outcome Definition:** Clinical biomarkers of liver function and uric acid	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Geometric mean=3.5 ng/mL Median=3.7	**Stat Method:** Linear regression, covariates and confounders considered include age, gender, race/ethnicity, BMI group, poverty, smoking, serum creatinine, and/or alcohol consumption. PFCs were natural log transformed. Logistic regression in exposure quartiles. Results are presented for models with most adjustment. **Outcome:** Uric acid (mg/dL) **Major Findings:** LinR: ***β*= .303 (95% CI .238, .367)** LogR: **p-value for trend <.001** **Outcome:** alanine transferase (ALT) (ppb) **Major Findings:** LinR: : ***β*= .038 (95% CI .014, .062)** LogR: **p-value for trend =.007** **Outcome:** gamma-glutamyl transferase (GGT) (ppb) **Major Findings:** LinR: ***β*= .058 (95% CI .021, .096)** LogR: **p-value for trend =.042** **Outcome:** Total bilirubin (mg/dL) **Major Findings:** LinR: ***β*= .048 (95% CI .016, .081)** LogR: **p-value for trend <.001** **Outcome:** asparate aminotransferase (AST) (ppb) **Major Findings:** LinR: : ***β*= .025 (95% CI .007, .043)** LogR: p-value for trend=.058 **Outcome:** alkaline phosphate (ALP) (ppb) **Major Findings:** LinR: : *β*=-.003 (95% CI -.023, .016) LogR: p-value for trend=.528	**Major Limitations:** Modeling techniques may result in model-dependent estimates. PFCs appear to be correlated with each other, making it difficult to clearly distinguish the effects of individual PFCs. Cross sectional design precludes causal inference. **Funding Source:** None, authors employed by NJDEP and NJDOH.

Reference and Study Design	Exposure Measures	Results	Comment
(Grandjean, Andersen et al. 2012) **Study Design:** Prospective birth cohort **Location:** Faroe Islands (National Hospital) **Population:** n=656 consecutive singleton births recruited 1997-2000 and 587 followed-up through 2008. **Outcome Definition:** Serum antibody concentrations against tetanus and diphtheria toxoids at ages 5 years prebooster, approximately 4 weeks after the booster, and at age 7 years.	**Exposure Assessment:** Prenatal exposures to PFCs at week 32 of pregnancy, postnatal exposure at child age 5 years. **Population-Level Exposure:** Geometric means: Maternal PFOA – 3.20 ng/mL Child (age 5) PFOA – 4.06	**Stat Method:** Correlations were determined by pairwise Pearson correlation coefficients. Linear regression, covariates and confounders considered include sex and age. For 5-year pre-booster data models adjusted for time since vaccination, possible PCB exposure, birth weight, maternal smoking during pregnancy, and duration of breastfeeding, and booster type. Structural equation models were generated to determine the joint association of PFCs with the overall antibody concentrations. Also controlled for PFCs in maternal pregnancy serum in some of these structural models. PFCs were also categorized when greater than 0.1 IU/mL – and odds ratios were estimated. PFCs and antibodies were log-transformed. **Outcome:** **Major Findings:** **Tetanus % difference (2-fold)** Maternal PFC (Year 5 Pre): -10.5 (95% CI -28.2, 11.7) (Year 5 Post): 14.5 (95% CI -10.4, 46.4) (Year 7): 7.4 (95% CI -17.1, 39.0) (Year 7 adj. for 5): 12.3 (95% CI -8.6, 38.1) *Structural Eq.* (Year 5 Pre):-20.2 (95% CI -49.2, 25.2) (Age 7): 35.1 (95% CI -25.4, 144.6) Child (age 5) PFC (Year 5 Pre): -13.3 (95% CI -31.6, 9.9) (Year 5 Post): -9.7 (95% CI -30.7, 17.7) **(Year 7): -35.8 (95% CI -51.9,-14.2); OR=3.27 (95% CI 1.43, 7.51)** **(Year 7 adj for 5): -28.8 (95% CI -42.7, -10.1)** *Structural Eq.* (Year 5 Pre):-20.5 (95% CI -44.4, 13.6) (Age 7): -55.2 (95% CI -73.3, 25.0) Child (age 5 adj.) PFC: *Structural Eq.* (Year 5 Pre):-17.2 (95% CI -42.1, 18.5) **(Year 7): -58.8 (95% CI -76.0, 29.3)**	**Major Limitations:** Possible confounding due to unmeasured variables, and other environmental contaminants, including other PFCs.

		<u>Diphtheria % difference (2-fold)</u> <u>Maternal PFC</u> (Year 5 Pre): -16.2 (95% CI -34.2, 6.7) (Year 5 Post): -6.2 (95% CI -22.4, 13.3) **(Year 7): -22.8 (95% CI -39.4, -1.7)** **(Year 7 adj. for 5): -16.8 (95% CI -32.9, 3.8** <u>*Structural Eq.*</u> **(Year 5 Pre): -47.9 (95% CI -67.7, -15.9)** **(Age 7): -42.0 (95% CI -66.1, -0.8)** <u>Child (age 5) PFC</u> (Year 5 Pre): -6.8 (95% CI -28.3, 21.0) (Year 5 Post): -6.1 (95% CI -23.6, 15.5) **(Year 7): -25.2 (95% CI -42.9, -2.0); OR=4.20 (95% CI 1.54, 11.44)** **(Year 7 adj for 5): -23.4 (95% CI -39.3, -3.4)** <u>*Structural Eq.*</u> (Year 5 Pre): -7.9 (95% CI -88.0, 37.0) **(Age 7): -44.4 (95% CI -65.5, -10.5)** <u>Child (age 5 adj.) PFC:</u> <u>*Structural Eq.*</u> (Year 5 Pre): -1.2 (95% CI -33.6, 46.8) **(Year 7): -45.5 (95% CI -66.9, 10.3)** **For the structural equation model the joint change in antibody showed statistical significance with PFCs at age 5 and at age 5 with adjustment for PFC in maternal pregnancy serum.**	

Reference and Study Design	Exposure Measures	Results	Comment
(Granum, Haug et al. 2013) **Study Design:** Prospective birth cohort **Location:** Oslo and Akershus, Norway **Population:** Sub-cohort from Norwegian Mother and Child Cohort Study recruited 2007-2008, n=49-99. **Outcome Definition:** Three annual questionnaire-based follow-ups (infectious diseases, allergies, and asthma) and blood samples collect from the mothers at the time of delivery and from the children at the age of 3 years. Serological outcomes: antibody levels specific for four vaccines (measles, rubella, tetanus, and HiB)	**Exposure Assessment:** Maternal serum concentrations collected at time of delivery. **Population-Level Exposure:** Median 1.1 ng/mL	**Stat Method:** Poisson regression used for health outcomes with count data, otherwise logistic or linear regression analyses were applied. Confounders and covariates considered include maternal allergy, paternal allergy, maternal education, child's gender, and/or age at 3-year follow-up. PFCs categorized into quartiles. **Outcome:** Anti-vaccine antibody levels **Major Findings:** **Rubella: (Bivariate) β=-0.40 (95% CI -0.64, -0.17);** Measles: β=-0.13 (95% CI -0.35, 0.09) Hib: β=-0.05 (95% CI -3.85, 3.74) Tetanus: β=0.01 (95% CI -0.09, 0.10) **(Multivariate) β=-0.40 (-0.64, -0.17)** **Outcome:** Health outcomes **Major Findings:** <u>Common Cold</u> (No. of episodes) **3rd year: β=0.42 (95% CI 0.16, 0.72)** **All 3 years: β=0.17 (95% CI 0.01, 0.33)** (Dichotomous) 3rd year: OR=1.24 (95% CI 0.32, 4.83) <u>Gastroenteritis</u> (No. of episodes) 3rd year: β=0.21 (95% CI -0.21, 0.64) **All 3 years: β=0.31 (95% CI 0.002, 0.61)** (Dichotomous) 3rd year: OR=1.16 (95% CI 0.37, 3.65) All 3 years: OR=3.13 (95% CI 0.37, 3.65)	**Major Limitations:** No direct measures of fetal exposure without cord blood PFCs. Also can not differentiate from prenatal v. postnatal exposure. Possible selection bias due to the low recruitment rate in the original cohort (authors suggest selection bias between participants and non-participants due to self-selection has been evaluated and not likely to be a problem). Small study size. Possible confounding due to unmeasured variables, and other environmental contaminants, including other PFCs.

Reference and Study Design	Exposure Measures	Results	Comment
(Jain 2013) **Study Design:** Cross-sectional **Location:** United States **Population:** NHANES 2007-08, >12 years of age. n=1,540 **Outcome Definition:** Six thyroid function variables (TSH, FT4, TT4, FT3, TT3, thyroglobulin)	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** 4.1 ng/mL 1st tertile ≤3.3 ng/mL 3rd tertile ≥5.1 ng/mL	**Stat Method:** Linear regression, covariates and confounders considered included age, gender, race/ethnicity, smoking, iodine status, C-reactive proteins, BMI, fasting time, and caloric intake. Only select PFCs and outcomes are analyzed following some modeling selection criteria. PFCs and thyroid parameters were log transformed. **Outcome:** TSH **Major Findings:** positively associated β=0.053, p-value=0.098 **↑(tertile) p-value <0.01** **Outcome:** TT3 **Major Findings:** positively associated R^2 = 17.3% **β=0.032, p-value=0.013** **Outcome:** FT3, FT4, TT4, TGN **Major Findings:** FT3: β=0.012, p-value=0.09 FT4: β=0.003, p-value=0.83 TT4: β=0.012, p-value=0.43 TGN: β=0.057, p-value=0.17	**Major Limitations:** 88 participants were on thyroid medication or reported a current thyroid problem (small compared to overall N), Combined effect of persistent organic pollutants not known, Cross sectional design precludes causal inference. **Funding Source:** unspec - Author was publishing regularly for CDC until 2011, then seemed to be researching independently. Address on paper is a residential property in GA. Author subsequently worked for Fort Bragg in NC in 2014 before moving to an independent biostatistics consulting firm, empiristat, in January 2015.

Reference and Study Design	Exposure Measures	Results	Comment
(Ji, Kim et al. 2012) **Study Design:** Cross-sectional **Location:** Sigeung, Korea **Population:** Recruited from cohort, aged greater than 12 years, n = 633 **Outcome Definition:** Thyroid hormones	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** 2.74 ng/mL IQR=2.04-3.64	**Stat Method:** Linear regression, covariates and confounders considered include age and BMI. **Outcome:** T4 **Major Findings:** β=-0.007 (95% CI -0.029,0.015) **Outcome:** TSH **Major Findings:** β=-0.066 (95% CI -0.220,0.089)	**Major Limitations:** One city not representative of all of Korea – very homogenous. Cross sectional design precludes causal inference. Unknown Did not control other environmental pollutants, including other PFCs. **Funding Sources**: This research was supported by Korea Food & Drug Administration (08182KFDA499).

Reference and Study Design	Exposure Measures	Results	Comment
(Jiang, Zhang et al. 2014) **Study Design:** Cross-sectional **Location:** Tianjin, China **Population:** Pregnant women, n=141 **Outcome Definition:** White blood cells, red blood cells, hemoglobin, and platelet, total bilirubin, total protein, albumin, glucose, AST, and ALT.	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Geo Mean n-PFOA = 4.18 ng/mL iso-PFOA = 0.04 ng/mL 5m-PFOA = 0.002 ng/mL Total=4.22	**Stat Method:** Pearson rank-order correlations, PFCs and outcomes were log transformed. Results shown here for total PFOA. **Outcome:** **Major Findings:** WBC, r=-0.042 (NS) RBC, r=0.061 (NS) **Hemo, r=0.192, p<0.05** Platelet, r=-0.004 (NS) Glucose, r=0.103 (NS) Total protein, r=0.092 (NS) **Albumin, r=0.251, p<0.01** Total bilirubin, r=0.135 (NS) AST, r=0.041 (NS) ALT, r=-0.071 (NS)	**Major Limitations:** Cross sectional design precludes causal inference. Third party confounders may exist **Funding Source:** Natural Science Foundation of China (NSFC 21077060, 21325730, 21050110427), Ministry of Education (20130031130005), and the Ministry of Environmental Protection (201009026).

Reference and Study Design	Exposure Measures	Results	Comment
(Kielsen et al., 2015) **Study Design:** Prospective cohort **Location:** Denmark **Population:** Healthy adults (n=12) without a history of tetanus-diphtheria booster vaccination in the past 5, recruited among staff at a hospital, 23 to 66 years of age **Outcome Definition:** Antibody responses followed for 30 days after a booster vaccination (days 2,4,7,10,14 and 30) with diphtheria and	**Exposure Assessment:** Serum PFOA measured prospectively for 30 days after booster vaccination (days 2,4,7,10,14 and 30). **Population-Level Exposure:** Median 1.69 ng/mL, range 1.30-2.79	**Stat Method:** Modeling which assumed a constant concentration level until Day 4 followed by a linear increase between days 4-10 after which the concentration was assumed to be constant, allowing the PFOA concentration to especially affect the intercept of the curve on those days. The analyses estimate how much this factor change, in percent, when the PFOA concentration doubled. Effects were adjusted for sex and age. PFOA and antibody concentrations were log transformed. **Outcome:** Diphtheria Antibody Concentration **Major Findings:** % Change (Day 4-10)=-8.22 (95% CI 6.44, -20.85)	**Major Limitations:** Small sample size Possible confounding due to unmeasured variables, including other environmental contaminant as well as other PFCs.

tetanus. Median antibody concentration pre-vaccination: Diphtheria 1.00 IU/ml Tetanus 4.03 IU/ml		Effects were stronger for diphtheria in general than compared with tetanus, and affects were statistically significant for PFOS, PFNA, and PFDA **Outcome:** Tetanus Antibody Concentration **Major Findings:** % Change (Day 4-10)=0.23 (95% CI 12.1, -10.40)	

Reference and Study Design	Exposure Measures	Results	Comment
(Kim, Choi et al. 2011) **Study Design:** Prospective birth cohort (matching prepartum blood – cord blood- postpartum breast milk) **Location:** Seoul, Cheongju, and Gumi, South Korea **Population:** Pregnant women recruited from three hospitals (n=44), age > 25 years. Paired samples available for n=26 mother-infant pairs. **Outcome Definition:** Serum concentrations mostly sampled in third trimester but some earlier (n=7, week 20-25), cord blood at delivery, and breast milk during mother checkup visit.	**Exposure Assessment:** Maternal blood serum (n=44), fetal cord blood serum (n=43), and breast milk (n=35) **Population-Level Exposure:** Median (maternal prepartum blood serum) = 1.46 ng/mL Median (cord serum) = 1.15 ng/mL Median (breast milk) = 0.05 ng/mL	**Stat Method:** Correlations between exposure and outcomes using Pearson correlation testes performed using the log of thyroid hormones and PFCs with and with-out adjustment for influential covariates. The following covariates and confounders were considered maternal age, gestational age, and maternal BMI. Results shown for adjusted correlations. **Outcome:** fetal cord blood total T3 **Major Findings:** Fetal, r=-0.210 (NS) Maternal, r=-0.238 (NS) **Outcome:** fetal cord blood total T4 **Major Findings:** Fetal, r=-0.157 (NS) Maternal, r=-0.071 (NS) **Outcome:** fetal cord blood TSH **Major Findings:** Fetal, r=0.089 (NS) **Maternal, r=0.443 p<0.05** No significant association with birth weight and length for any measures of PFOA.	**Major Limitations:** Possible confounding due to unmeasured variables, including other PFCs. Plausible competing mechanism involved in transplacental transfer. Cross-sectional design prevents causal inference. Small sample size. **Funding Source**: This research was supported by Korea Food & Drug Administration (08182KFDA499). The research was also supported by a Discovery Grant from the National Science and Engineering Research Council of Canada (Project # 326415-07) and a grant from the Western Economic Diversification Canada (Project # 6578 and 6807). The authors wish to acknowledge the support of an instrumentation grant from the Canada Foundation for Infrastructure.

Reference and Study Design	Exposure Measures	Results	Comment
(Knox, Jackson et al. 2011) **Study Design:** Cross-sectional **Location:** United States – West Virginia and Ohio **Population:** Adults (20 years or older) without thyroid disease, consumed water for at least 1 year from a water district with known PFOA contamination, n=50,113 members of the class of those affected by C8 spill **Outcome Definition:** thyroxine, T3 uptake, TSH,	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** PFOA (ng/mL) Means by age group Women 20-50=52.6 Men 20-50=91.0 Women > 50=98.6 Men > 50=124.3 Total Mean = 86.57 ng/mL	**Stat Method:** Linear regression and ANOVA to estimate gender differences, covariates and confounders considered include age, serum estradiol, and alcohol. **Outcome:** TSH **Major Findings:** No significant associations, results not shown **Outcome:** Thyroxine (T4) **Major Findings:** **Women 20-50, β=0.05, p<0.0001** Men 20-50, (NS) results not provided **Women > 50, β=0.08, p<0.0001** **Men > 50, β=0.06, p=0.0001** **Outcome:** T3 **Major Findings:** **Women 20-50, β=-0.08, p=0.0001** Men 20-50, (NS) results not provided **Women > 50, β=-0.07, p<0.005** **Men > 50, β=-0.04, p=0.037** **Outcome:** albumin **Major Findings:** **Women 20-50, β=0.02, p<0.0001** **Men 20-50, β=0.02, p<0.0001** **Women > 50, β=0.02, p<0.0001** **Men > 50, β=0.02, p<0.0001**	**Major Limitations:** Thyroxin-Binding Globulin production not measured, albumin used instead, Cross sectional design precludes causal inference. Possible confounding due to unmeasured variables, including other PFCs. **Quality of study:** previously unreported gender differences in thyroxine and T3 uptake **Funding Source:** unspec, All authors employed by WVU School of Medicine.

Reference and Study Design	Exposure Measures	Results	Comment
(Lin, Lin et al. 2010) **Study Design:** Cross-sectional **Location:** United States **Population:** NHANES 1999-2000 & 2003-2004, n=2,216, 18 years of age or older	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Gender: Mean PFOA (ng/mL) Male: 5.05 Female: 4.06 Population Mean: 4.54 ng/mL	**Stat Method:** Linear regression, covariates and confounders considered include age, gender, race/ethnicity, smoking, alcohol consumption, education level, BMI, HOMA-IR, metabolic syndrome, and iron saturation. PFCs assessed as quartiles and natural log transformed. Model estimates are shown for most adjusted. PFCs modeled separately and included in a composite analysis with PFOS, PFNA, and PFHxS.	**Major Limitations:** Cross sectional design precludes causal inference. No other environmental chemicals were included, may be important covariates Did not take into account any medications that may cause elevated ALT or GGT Common physiology could influence both serum PFCs and liver enzymes

Outcome Definition: Serum liver enzymes: ALT (alanine transaminase), GGT (gamma-glutamyltransferase), total bilirubin		**Outcome:** ALT **Major Findings:** **Quartiles of PFOA (unadj.), increasing trend (p<0.001)** **Separate, β = 1.86, p=0.005.** **Composite, β = 2.19, p=0.009.** **Outcome:** GGT **Major Findings:** **Quartiles of PFOA (unadj.), increasing trend (p<0.001)** **Separate, β = 0.08, p=0.019.** **Composite, β = 0.15, p=0.001.** **Outcome:** Total bilirubin **Major Findings:** Quartiles of PFOA (Unadj), no trend (p=0.638) Separate, β = -0.09, p=0.645. Composite, β =-0.20, p=0.378.	Status of liver tissue was not available to determine hepatic steatosis, inflammation, or fibrosis. **Funding Source**: This was an investigator-initiated unfunded study. All authors had access to the data and the statistical analysis report. Pau-Chung Chen was the guarantor of the article, from the institute of Occupational Medicine and Industrial Hygiene, National Taiwan University College of Public Health.

Reference and Study Design	Exposure Measures	Results	Comment
(Lin, Wen et al. 2011) **Study Design:** Cross-sectional **Location:** Taipei, Taiwan **Population:** 287 adolescents and young adults, aged 12-30 years of age recruited from a hypertension cohort. **Outcome Definition:** Serum measures Adiponectin is a protein hormone that modulates a number of metabolic processes, including glucose regulation and fatty acid catabolism.	**Exposure Assessment:** Screening of school-age children via urine with subsequent blood serum tests, **Population-Level Exposure:** Median: 2.39 ng/mL Percentile / Median (ng/mL) <50th / 0.75 50-74th / 3.86 75th-89th / 7.89 ≥ 90th / 11.54	**Stat Method:** The relation of PFC variables to categorical variables was tested using the Mann-Whitney U test or Kruskal-Wallis test. Linear regression with adiponectin, glucose homeostasis, lipid profile, inflammatory markers Covariates and confounders: Model 1 (age and gender), Model 2 (age, gender, lifestyle factors), Model 3 (Model 2 + measurement data). Significant only if significant in all models. **Outcome:** Serum adiponectin, glucose, insulin, triglycerides, CRP, HDL **Major Findings:** PFOA levels were significantly lower in subjects with metabolic syndrome (p=0.009)	**Major Limitations:** Cross sectional design precludes causal inference. No inclusion of other environmental chemicals which may be important covariates The study population is made up of adolescents and young adults with abnormal urinalysis results. Study population only adolescents and young adults – not generalizable to larger population Common physiology could influence both serum PFCs and outcome measures **Funding Source:** This study was supported by grants from the National Health Research Institute of Taiwan (NHRI-EX97-9721PC, EX97-9821P C,

		Results in Supporting Information Tables – reported as all non-significant for all 3 model types.	and X97 -9921PC) and (NHRI-EX95-953 I PI, EX95 - 9631PI, and EX95-973IPI) and from the National Science Council of Taiwan (99-2314-B-385 001-MY3).

Reference and Study Design	Exposure Measures	Results	Comment
(Lin, Wen et al. 2013) **Study Design:** Cross sectional **Location:** Taipei, Taiwan **Population:** Young adults with abnormal urinalysis in childhood, 12-30 years old, N=551 (510 with normal blood pressure and 41 hypertensive) **Outcome Definition:** Laboratory, examination, and survey information.	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Geometric mean = 2.67 ng/mL **Quartile** / **Levels ng/mL** < 50th / <3.64 50th-75th / ≤6.66 75th – 90th / ≤9.71 > 90th / >9.71	**Stat Method:** Linear and logistic regression for categories of PFCs (≤50th, ≤50th-75th, ≤75th- 90th, >90th), covariates and confounders considered include age, gender, smoking, and alcohol consumption. TSH was natural log transformed. **Outcome:** Free T4 (ng/dL) **Major Findings:** Means=1.07, 1.08, 1.10, 1.06 (P for Trend NS) **Outcome:** log TSH (m IU/l) **Major Findings:** Means=0.48, 0.45, 0.36, 0.41 (P for Trend NS)	**Major Limitations:** Cross sectional design precludes causal inference. Population is not generalizable – very specific, Did not take into account all medications that may have impact on thyroid functioning, Unmeasured confounders possible, Status of thyroid tissue unavailable to determine thyroid pathology. **Funding Source:** This study was supported by grants from National Health Research Institute of Taiwan (EX97-9721PC, EX97-9821PC, X97-9921PC, EX95-9531PI, EX95-9631PI and EX95-9731PI) and from National Science Council of Taiwan (99-2314-B-385-001-MY3).

Reference and Study Design	Exposure Measures	Results	Comment
(Lin, Lin et al. 2013) **Study Design:** Cross-sectional **Location:** Taipei area, Taiwan **Population:** Taiwanese young adults with abnormal urinalysis in childhood, 12-30 years old, N=664 (246 with elevated blood pressure and 398 with normal blood pressure) **Outcome Definition:** Serum samples, sociodemographic data collected during interview. Clinical outcomes were determined from clinical serum measures. Carotid intima-media thickness (CIMT) is a marker of subclinical atherosclerosis.	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Median: 3.49 ng/mL Range: 0.75-52.2	**Stat Method:** Linear regression and logistic regression for categories of PFCs (≤50th, ≤50th-75th, ≤75th-90th, >90th) covariates and confounders considered include age, gender, smoking status, alcohol consumption, and BMI to estimate association with cardiovascular risk factors, and additionally sBP, BMI, LDL, CRP, TG, and HOMA-IR for CIMT. Logistic regression analysis was conducted to examine the odds ratios of thicker CIMT for PFOS only. Investigators performed a composite analysis with four PFCs modeled together. **Outcome:** Cardiovascular risk factors **Major Findings:** SBP – ↓ (P for trend=0.177) BMI - ↓ (P for trend=0.130) LDL-C - ↓ (P for trend=0.117) **Log-TG - ↓ (P for trend=0.015)** UA - ↑ (P for trend=0.983) Log-HOMA-IR - ↑/↓ (P for trend=0.694) **Outcome:** CIMT **Major Findings:** NS (findings in paper are only PFOS-relevant)	**Major Limitations:** Cross sectional design precludes causal inference. Did not include other environmental factors (ie particulate matter air pollution) Tenuous generalizability due to sample Did not take into account medicines which may affect CIMT Common physiology could influence both things. **Funding Source**: This study was supported by grants from the National Health Research Institute of Taiwan (NHRI EX97-9721PC, EX98-9721PC, EX99-9721PC, and EX100-9721PC; EX95-9531PI, EX96-9531PI and EX95-9731PI) and from the National Science Council of Taiwan (NSC 99-2314-B-385-001-MY3 and NSC 101-2314-B-002-184-MY3).

Reference and Study Design	Exposure Measures	Results	Comment
(Looker, Luster et al. 2014) **Study Design:** Prospective cohort **Location:** U.S., mid-Ohio region of Ohio and West Virginia **Population:** Adults who consumed water (for at least 1 year) from a water district with	**Exposure Assessment:** Serum concentrations at time of vaccination. **Population-Level Exposure:** Median, 31.5 ng/mL	**Stat Method:** Linear regression for PFC (log transformed and quartiles), covariates and confounders considered include age and gender, smoking status, any previous influenza vaccination, specific H1N1 vaccination in previous year, day of serum sample collection, coexisting medical conditions, and common anti-inflammatory, and pain relief medications, as well as mobility. Logistic regression also utilized for dichotomized outcomes. Differences and ratios of antibody titers were log transformed. Titers also dichotomized into seroconversions (4-fold or greater increase in antibody titer following vaccination) and seroprotection (HI titer >	**Major Limitations:** Reporting of previous – potential of recall bias Given the high background rate of respiratory infection, there is not much scope to detect an increase in infections. Not a longitudinal analysis. No vaccination information

known PFOA contamination, n=411. Initial recruitment was 2005-06 and were followed-up in 2010. **Outcome Definition:** Hemagglutionation inhibition tests were conducted on serum samples collected preinfluenza vaccination and 21± 3 postvaccination in 2010. Questionnaires were conducted regarding the occurrence and frequency of recent (last 12 months) of respiratory infections.		1:40). PFCs were untransformed, log transformed, and categorized into quartiles. **Outcome: Geometric Mean antibody titer (GMT) Rise Following Vaccination** **Major Findings:** <u>Influenza Type B</u> Q1: 49.46 (95% CI 38.14, 64.12) Q2: 46.00 (95% CI 35.28, 59.97) Q3: 43.55 (95% CI 33.08, 57.33) **Q4: 20.90 (95% CI 16.58, 28.24)** Titer Rise: Linear - β(adj.)=-0.2 (95% CI -0.13, 0.09) Q2 v. Q1 - β(adj.)=-0.3 (95% CI -0.19, 0.13) Q3 v. Q1 - β(adj.)=-0.2 (95% CI -0.19, 0.15) Q4 v. Q1 - β(adj.)=-0.7 (95% CI -0.24, 0.10) Titer Ratio: Linear - β(adj.)=-0.2 (95% CI -0.11, 0.08) Q2 v. Q1 - β(adj.)=0.5 (95% CI -0.09, 0.19) Q3 v. Q1 - β(adj.)=0.7 (95% CI -0.07, 0.22) Q4 v. Q1 - β(adj.)=-0.3 (95% CI -0.17, 0.12) No statistically significant ORs for seroconversions or seroprotection. <u>Influenza Type A H1N1</u> 476.23 (360.77, 628.65) 352.22 (255.33, 485.88) 306.32 (232.58, 403.44) 274.79 (202.85, 372.23) Titer Rise: Linear - β(adj.)=-0.3 (95% CI -0.14, 0.09) Q2 v. Q1 - β(adj.)=-0.9 (95% CI -0.27, 0.08) Q3 v. Q1 - β(adj.)=-0.10 (95% CI -0.28, 0.09) Q4 v. Q1 - β(adj.)=-0.12 (95% CI -0.30, 0.06) Titer Ratio: Linear - β(adj.)=-0.7 (95% CI -0.06, 0.21) Q2 v. Q1 - β(adj.)=-0.8 (95% CI -0.29, 0.12) Q3 v. Q1 - β(adj.)=-0.04 (95% CI -0.25, 0.18) Q4 v. Q1 - β(adj.)=0.7 (95% CI -0.14, 0.29)	for 2005/2006 such that persons could have been followed as the PFC levels decreased.

		No statistically significant ORs for seroconversions or seroprotection. <u>Influenza A H3N2</u> 228.86 (161.53, 324.27) 125.36 (86.01, 182.73) 104.13 (72.47, 149.64) 183.73 (127.28, 265.23) Titer Rise: Linear - β(adj.)=-0.1 (95% CI -0.17, 0.14) **Q2 v. Q1 - β(adj.)=-0.28 (95% CI -0.51, -0.06)** **Q3 v. Q1 - β(adj.)=-0.37 (95% CI -0.60, -0.13)** Q4 v. Q1 - β(adj.)=-0.12 (95% CI -0.36, 0.13) Titer Ratio: Linear - β(adj.)=-0.127 (95% CI -0.25, 0.02) Q2 v. Q1 - β(adj.)=-0.10 (95% CI -0.30, 0.10) Q3 v. Q1 - β(adj.)=-0.07 (95% CI -0.28, 0.14) **Q4 v. Q1 - β(adj.)=-0.22 (95% CI -0.43, -0.01)** No statistically significant seroconversions. <u>Seroprotection</u> **Q2 v. Q1 - OR=0.34 (95% CI 0.14, 0.83)** **Q3 v. Q1 - OR=0.28 (95% CI 0.11, 0.70)** **Q4 v. Q1 - OR=0.39 (95% CI 0.15, 0.99)** *No statistically significant associations between self-reported colds or influenza and PFOA levels.	

Reference and Study Design	Exposure Measures	Results	Comment
(Lopez-Espinosa, Mondal et al. 2012) **Study Design:** Cross-sectional and birth cohort **Location:** mid-Ohio valley, USA	**Exposure Assessment:** Serum PFOA and modeled in utero PFOA (information on historical releases, environmental distribution, pharmacokinetic modeling, residential histories) **Population-Level Exposure:** Median in utero: 12 ng/mL Median serum PFOA: 29 ng/mL	**Stat Method:** Linear and logistic regression, covariates and confounders considered include age, sex, race/ethnicity, BMI, month of sampling, average household family income, smoking, and alcohol consumption. **Outcome:** ln-TSH **Major Findings:** Modeled (% change 1-17 years old, IQR): %=-0.5 (95% CI -2.4, 1.5)	**Major Limitations:** Mostly cross-sectional design precludes much causal inference, Absence of measurements of child triiodothyronine, Reliance on recall for thyroid diagnosis (though a more stringent case definition produced similar results).

Population: Children (age 1-17) who consumed water (for at least 1 year) from a water district with known PFOA contamination, n=10,725 were included in the analysis. Subsample of children matched to mothers for model in utero PFOA exposure, n=4,713 (pregnancies from 1987-2005) **Outcome Definition:** Serum samples of TSH, TT4, categorized into subclinical hypothyroidism and hyperthyroidism. Also parent self-reported thyroid disease and thyroid disease related medication use.		Measure (%change 1-17 years old, IQR): %=1.0 (95% CI -0.5, 2.7) **Outcome:** TT4 **Major Findings:** Modeled (% change 1-17 years old, IQR): %=-0.1 (95% CI -0.8, 0.6) Measure (%change 1-17 years old, IQR): %=0.1 (95% CI -0.5, 0.6) **Outcome:** Thyroid disease **Major Findings:** Modeled, Reported OR=1.47 (95% CI 0.95, 2.27) **Measured, Reported** **OR=1.44 (95% 1.02, 2.03)** **Outcome:** Hypothyroidism **Major Findings:** Modeled, Reported OR=1.61 (95% CI 0.96, 2.63) **Measured, Reported** **OR=1.54 (95% 1.00, 2.37)** Modeled (in utero), Subclinical OR=0.94 (95% CI 0.76, 1.16) Measure, Subclinical OR=0.98 (95% CI 0.86, 1.15) **Outcome:** Hyperthyroidism **Major Findings:** Modeled, Subclinical OR=1.10 (95% CI 0.69, 1.74) Measure, Subclinical OR=0.81 (95% CI 0.58, 1.15)	No control for other environmental chemicals including other PFCs. Reliant of modeled in utero PFOA exposure for birth cohort. **Quality of study:** first large scale report in children suggesting associations of serum PFOA and hypothyroidism **Funding Source**: C8 class action settlement agreement between DuPont and plaintiffs

Years	Model	Measure
1-5	23.8	33.8
6-10	14.5	32.2
> 10	9.32	26.9
1-17	11.5	29.3

Reference and Study Design	Exposure Measures	Results	Comment
(Melzer, Rice et al. 2010) **Study Design:** Cross-sectional **Location:** United States	**Exposure Assessment:** Serum PFOA **Population-Level Exposure:** Geo mean men = 4.91 ng/mL Geo mean women = 3.77 ng/mL	**Stat Method:** Multivariate logistic regression of thyroid disease by PFC quartile. Sex specific models were used. Covariates and confounders considered include year of NHANES cycle, age, sex, race/ethnicity, education, smoking status, BMI, and alcohol intake.	**Major Limitations:** Investigators based PFOA on a single serum sample, likely to represent medium-term internal dose, samples taken at several time points might be more accurate in classifying exposure. Any misclassification from single measures

Population: NHANES 1999-2000, 2003-2004, and 2005-2006 N=3974; ≥ 20 years of age **Outcome Definition:** Thyroid disease= ever self-reported health professional diagnosis and current diagnosis and currently taking medication for the disease (CURRENT). Also examined other self-reported diseases.		Results shown for most adjusted models. **Outcome:** Thyroid disease **Major Findings:** EVER: Women, Q2 v. Q1, OR=0.95 (95% CI 0.62,1.47) Q3 v. Q1, OR=1.11 (95% CI 0.67, 1.83) **Q4 v. Q1, OR= 1.64 (95% CI 1.09, 2.46)** Men, Q2 v. Q1, OR=1.11 (95% CI 0.62,1.99) Q3 v. Q1, OR=0.57 (95% CI 0.19, 1.66) Q4 v. Q1, OR= 1.58 (95% CI 0.74, 3.39) CURRENT: Women Q2 v. Q1, OR=0.89 (95% CI 0.49,1.59) **Q3 v. Q1, OR=1.86 (95% CI 1.12, 3.09)** **Q4 v. Q1, OR=2.24 (95% CI 1.38, 3.65)** Men, Q2 v. Q1, OR=1.12 (95% CI 0.52, 2.39) Q3 v. Q1, OR=0.49 (95% CI 0.18, 1.38) Q4 v. Q1, OR=1.89 (95% CI 0.60, 5.90) **Outcome:** Arthritis ever, asthma ever, COPD ever, Diabetes ever, Heart disease ever, Liver disease current **Major Findings:** Some evidence of positive association with arthritis (Q2 v. Q1 and Q3 v. Q1 P <0.05, but not Q4). For asthma ever Q2 v. Q1 is statistically significant (p<0.05) but not for other quartile. For other disease in which the percentage of 'disease' become increasingly minimal: COPD (11.2-11.9%), Diabetes (7.0-10.9%), Heart disease (5.4-5.7%), and Liver disease (0.8-1.4%) there are no significant findings.	would tend to decrease power and underestimate the real strengths of association. Cross sectional design precludes causal inference. Confounding is also possible but unlikely. Since associations reported were in people on thyroid medications, which mimic normal thyroid function, reverse causation implausible. **Funding Source:** Peninsula Medical School and University of Exeter

Reference and Study Design	Exposure Measures	Results	Comment
(Nelson, Hatch et al. 2010) **Study Design:** Cross-sectional **Location:** United States **Population:** NHANES 2003-2004, 12-80 year olds, N=416-860 depending on parameter **Outcome Definition:** Serum samples and anthropometric measurements: cholesterol, body weight, insulin resistance	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Median = 3.8 µg/L (ng/mL)	**Stat Method:** Linear regression, covariates and confounders considered include age, sex, race/ethnicity, socioeconomic status, saturated fat intake, exercise, TV time, alcohol consumption, smoking, and parity in women. Also examined PFCs as quartile and performed test for trend. Effect estimates of each quartile to the lowest quartile, Test for trend performed. **Outcome:** Total & non-HDL cholesterol (70-80% of TC) **Major Findings:** **TC, β=1.22 (95% CI 0.04, 2.40)** P for Trend=0.07 **Non-HDL, β=1.38 (95% 0.12, 2.65)** **P for Trend=0.05** **Outcome:** HDL **Major Findings:** β=-0.12 (95% -0.41, 0.16) P for Trend=0.34 **Outcome:** LDL **Major Findings:** β=-0.21 (95% -1.91, 1.49) P for Trend=0.84 **Outcome:** Anthropometric measures **Major Findings:** BMI, P for Trend=096 Waist Circumference, P for Trend=0.84 Log-HOMA, P for Trend=0.31	**Major Limitations:** Cross sectional design precludes causal inference. Differing physiology might affect PFOA behavior (PFCs may bind to beta-lipoproteins and albumin in the blood) Only one measurement of PFCs and cholesterol concentrations Unable to consider other environmental contaminants **Quality of study:** large sample size, ability to account for key covariates like alcohol and cholesterol lowering medications, and consideration of modification by age and/or gender. **Funding Source:** This work was supported in part by grant R21ES013724 from the National Institute of Environmental Health Sciences (NIEHS). J.W.N. was supported in part by award T32ES014562 from NIEHS.

Reference and Study Design	Exposure Measures	Results	Comment
(Olsen, Gillilan et al. 1998) **Study Design:** Two cross-sectional studies (1993 & 1995) **Location:** U.S. – 3M Plant **Population:** 111 and 80 male production workers (68 workers participated in both years) **Outcome Definition:** Eleven hormones were assayed: cortisol, dehydroepiandrosterone sulfate (CHEAS), estradiol, FSH, 17α-hydroxyprogesterone (17-HP), free testosterone, LH, prolactin, thyroid-stimulating hormone (TSH), and sex hormone-binding globulin (SHBG)	**Exposure Assessment:** PFOA serum concentrations **Population-Level Exposure:** Mean by PFOA category 1993 (ppm): 0-1: 0.48 1-<10: 3.34 10-<30: 16.26 >=30: 60.13 1995 (ppm): 0-1: 0.31 1-<10: 3.03 10-<30: 17.11 >=30: 55.96	**Stat Method:** Simple and stratified analyses, analysis of variance (ANOVA), Pearson correlation coefficients, and ordinary multivariable regression were used to evaluate associations between PFOA and each hormone, with adjustment for potential confounding variables. Covariates and confounders considered include age, BMI, alcohol use, and cigarette use. PFOA serum concentrations were divided into four categories (0-1, 1-<10, 10-<30, and >=30 ppm). **Outcome:** Hormones (reproductive mainly) **Major Findings:** (Description is limited since endpoints are not being comprehensively reviewed). "Simple linear regression of the natural log of each hormone with PFOA, treated as a continuous variable, resulted in no statistically significant coefficients in 1993 for any hormone and only one in 1995 (17-HP). " These findings were the same for the multivariable regression also. Univariate analysis (1995) - Statistically increased mean for TSH between 1-<10 and 10-<30 ppm groups (p=0.002), but not for highest PFOA category. Findings were not statistically significant for 1993 but mean TSH increased for the 10-<30 category compared to the lower two categories.	**Major Limitations:** Cross sectional design precludes causal inference. Healthy worker effect Few subjects exposed at highest and lowest levels No females included. **Funding Source**: Not explicitly stated but 3M – corresponding (lead) author is employee of 3M Company

<table>
<tr><th>Reference and Study Design</th><th>Exposure Measures</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>(Olsen, Burris et al. 2000)

Study Design:
Cross-sectional

Location: U.S. – 3M Plant

Population:
Male workers involved in ammonium perfluorooctanate production in 1993 (n=111), 1995 (n=80), and 1997 (n=74); N total=265

Outcome Definition: serum hepatic enzymes, cholesterol, lipoproteins, serum cholecystokinin (CCK, a peptide hormone of the gastrointestinal system responsible for stimulating the digestion of fat and protein) - 1997 only</td>
<td>Exposure Assessment: serum concentrations

Population-Level Exposure:
(ppm)
<table>
<tr><th>Year</th><th>Mean</th><th>Median</th><th>Range</th></tr>
<tr><td>1993</td><td>5.0</td><td>1.1</td><td>0.0-80.0</td></tr>
<tr><td>1995</td><td>6.8</td><td>1.2</td><td>0.0-114.1</td></tr>
<tr><td>1997</td><td>6.4</td><td>1.3</td><td>0-81.3</td></tr>
</table>
Median PFOA: 1.19 ppm
Mean PFOA: 5.93 ppm</td>
<td>Stat Method: Simple and stratified analyses, ANOVA, multivariable regression (linear and nonlinear). Covariates and confounders considered include age, BMI, alcohol use, and cigarette use.

Participants were stratified into 3 categories of PFOA (0-<1ppm), (1-<10ppm), (≥10ppm).

Outcome: Hepatic enzymes
Major Findings:
Simple analysis (p-values)
<table>
<tr><th></th><th>1993</th><th>1995</th><th>1997</th></tr>
<tr><td>ALP</td><td>0.52</td><td>0.25</td><td>0.28</td></tr>
<tr><td>GGT</td><td>0.24</td><td>0.41</td><td>0.78</td></tr>
<tr><td>AST</td><td>0.33</td><td>0.45</td><td>0.83</td></tr>
<tr><td>ALT</td><td>0.82</td><td>0.30</td><td>0.73</td></tr>
<tr><td>T. Bilir</td><td>0.48</td><td>0.11</td><td>0.58</td></tr>
<tr><td>D. Bilir</td><td>0.82</td><td>0.05</td><td>0.74</td></tr>
</table>
Multivariate (β / p-value)
<table>
<tr><th></th><th>1990</th><th>1993</th><th>1995</th><th>1997</th></tr>
<tr><td>ALT</td><td>-15.8/ 0.0008</td><td>0.89/ 0.76</td><td>0.81/ 0.75</td><td>2.77/ 0.03</td></tr>
</table>
Outcome: Serum lipids
Major Findings:
Simple analysis (p-values)
<table>
<tr><th></th><th>1993</th><th>1995</th><th>1997</th></tr>
<tr><td>Choles</td><td>0.45</td><td>0.48</td><td>0.08</td></tr>
<tr><td>HDL</td><td>0.32</td><td>0.70</td><td>0.40</td></tr>
<tr><td>LDL</td><td>0.84</td><td>0.96</td><td>0.11</td></tr>
<tr><td>Trigly</td><td>0.77</td><td>0.07</td><td>0.13</td></tr>
</table>
Multivariate (β / p-value)
<table>
<tr><th></th><th>1990</th><th>1993</th><th>1995</th><th>1997</th></tr>
<tr><td>HDL</td><td>-1.61/ 0.04</td><td>-0.14/ 0.67</td><td>-0.10/ 0.08</td><td>-0.19/ 0.16</td></tr>
</table>
Outcome: CCK (1997 only)
Major Findings:
β=-0.008, p-value=0.07</td>
<td>Major Limitations:
Cross sectional design precludes causal inference.

Healthy worker effect

Only 17 subjects were common in all 3 study years.

Voluntary participation, 50-70% participation

Turnover of employees prevented longitudinal assessment

Changes in laboratory procedures between 1995 and 1997 may have disguised lower exposure doses in earlier years.

Male only

Unable to consider other environmental contaminants

Funding Source: 3M</td>
</tr>
</table>

<table>
<tr><th>Reference and Study Design</th><th>Exposure Measures</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>(Olsen, Burris et al. 2003)

Study Design:
Cross sectional and longitudinal

Location: Antwerp, Belgium, and Decatur, AL, USA

Population:
Cross Sectional: male and female workers at PFC manufacturing locations
N=263 in Decatur
N= 255 in Antwerp
Total N = 421 male, 97 female
Year: 2000

Longitudinal: male workers, both locations n=174
6 year time period

<table>
<tr><td>Worker Cohort</td><td>% involved in production activities</td></tr>
<tr><td>Total Antwerp</td><td>73</td></tr>
<tr><td>Female Antwerp</td><td>12</td></tr>
<tr><td>Total Decatur</td><td>75</td></tr>
<tr><td>Female Decatur</td><td>63</td></tr>
</table>

Outcome Definition:
hematological, lipid, hepatic, thyroid, urinary parameters: Cholesterol, HDL, Triglycerides, ALP, GGT, AST, ALT, total bilirubin, TSH, T4, Free T4, T3</td>
<td>Exposure Assessment:
Serum concentrations

Population-Level Exposure:

Cross-sectional (ppm):
<table>
<tr><td>Site</td><td>Mean (range)</td><td>GeoMean (95%CI)</td></tr>
<tr><td>Antwerp</td><td>0.84 (0.01-7.04)</td><td>0.33 (0.27-0.40)</td></tr>
<tr><td>Decatur</td><td>1.78 (0.04-12.70)</td><td>1.13 (0.99-1.30)</td></tr>
</table>
Longitudinal (ppm): means
<table>
<tr><td>Subgroup</td><td>1994-95</td><td>1997</td><td>2000</td></tr>
<tr><td>A</td><td>1.36</td><td>-</td><td>1.72</td></tr>
<tr><td>B</td><td>-</td><td>1.22</td><td>1.49</td></tr>
<tr><td>C</td><td>1.41</td><td>1.90</td><td>1.77</td></tr>
</table>
Range: 1,220-1,900 ng/mL</td>
<td>Stat Method: ANOVA, multivariable for cross-sectional analyses. Logistic regression for PFOS quartile distribution for those with elevated liver function tests. And linear regression with parameters as continuous.

For longitudinal assessment, repeated measures incorporating the random subject’s effect fitted to a mixed model. Covariates and confounders considered include years of observation, the interaction term of PFOS and years of observation, age, BMI, cigarettes smoked per day, alcohol drinks per day, location, year at first entry, and baseline years worked.

Outcome: Serum lipids
Major Findings:
<u>Longitudinal (n=174 male employees):</u>
Cholesterol
β=0.032 (95% CI 0.013, 0.051)
Triglycerides
β=0.094 (95% CI 0.045, 0.144)

“There were no significant [PFOA] coefficients associated with changes in HDL”

Outcome: Liver function
Major Findings:
“There were no significant [PFOA] coefficients associated with changes in various liver function tests (data not shown) adjusted for potential confounders”.

Outcome: Thyroid hormones
Major Findings:
Results not provided, following description in report: “Multivariate regression analyses of the thyroid hormones resulted in no significant associations with [PFOS or] PFOA except for a positive association with the natural log of T3 (β=0.016, p-value=0.01)</td>
<td>Major Limitations:
Voluntary participation, volunteer bias

Potential environmental confounders
Only 41 employees completed all 3 testing dates (subgroup C)

Healthy worker effect

Cross sectional design of one section precludes causal inference for that section.

Low participation rate in first years of study, participation rates in earlier years were lower, participation rate and date could be related to QC procedures

Unclear mechanisms for PFOA/triglycerides

Unable to consider other environmental contaminants

Funding Source: DuPont/3M</td>
</tr>
</table>

<table>
<tr><th>Reference and Study Design</th><th>Exposure Measures</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>(Olsen and Zobel 2007)

Study Design:
Cross-sectional

Location: Antwerp, Belgium; Decatur, AL; Cottage Grove,, MN

Population:
Voluntary participation of male employees (PFOA production), n=506, from three locations. Exclusion of those taking lipid-lowering medications

Outcome Definition: lipid, hepatic, thyroid parameters: Cholesterol, HDL, Triglycerides, ALP, GGT, AST, ALT, total bilirubin, TSH, T4, Free T4, T3</td>
<td>Exposure Assessment:
Serum concentrations

Population-Level Exposure:

µg/mL (ppm)
<table>
<tr><th>Site</th><th>Mean</th><th>Median</th></tr>
<tr><td>Antwerp</td><td>1.02</td><td>0.65</td></tr>
<tr><td>Cottage Grove</td><td>4.63</td><td>0.95</td></tr>
<tr><td>Decatur</td><td>1.89</td><td>1.51</td></tr>
<tr><td>Total</td><td>2.21</td><td>1.10</td></tr>
</table></td>
<td>Stat Method: Multiple and logistic regression and analysis of covariance. Covariates and confounders considered are age, BMI, and alcohol usage, and serum triglycerides for analysis of hepatic enzymes.

Results shown for most adjusted models (age, BMI, alcohol), and all locations combined.

Outcome: Serum lipids
Major Findings:
<u>All locations</u>
Cholesterol:
β=0.0076, p-value=0.20
LDL:
β=0.0021, p-value=0.81
HDL:
β=-0.0183, p-value=0.01
Triglycerides:
β=0.0711, p-value<0.0001

Findings from cholesterol and LDL were similar for the three locations. HDL associations were not found in any of the other locations. Triglycerides associations only for Antwerp location and Decatur (borderline nonsignificant). Decile analyses suggest decreasing HDL and increasing triglycerides with increasing PFOA.

Outcome: Hepatic enzymes
Major Findings:
<u>All locations</u>
ALP: β=0.0093, p-value=0.25
AST: β=-0.0051, p-value=0.55
ALT: β=0.0249, p-value=0.06
GGT: β=0.0326, p-value=0.05
Total Bili: β=-0.0325, p-value=0.001
Direct Bili: not shown

ALP significant in Decatur, AST borderline insignificant in Cottage Grove, ALT highly significant for unadjusted models for all locations and significant in Decatur, bilirubin significance appears to be driven by Decatur. Decile analyses suggest some</td>
<td>Major Limitations:
Voluntary participation,

50-65% participation,

Demographics of nonparticipants unknown.

They talk about lots of residual confounding when putting locations together – use to explain away HDL effect

Healthy worker effect

Unable to consider other environmental contaminants

Funding Source: unspec, authors employed by 3M Medical Department</td>
</tr>
</table>

		increase in ALT levels with increasing PFOA deciles. There is an inverse association (NS) for GGT in Cottage Grove while other findings suggest a positive association. Model results are not shown for direct bilirubin but decile analyses don't suggest a trend. **Outcome:** Thyroid hormones **Major Findings:** TSH: β=0.0360, p-value=0.08 T4: β=-0.0057, p-value=0.29 **Free T4: β=-0.0117, p-value=0.01** **T3: β=0.0105, p-value=0.05** No significant findings for any of three locations for TSH, TT4 has no significance, FT4 association only overall but Antwerp and Decatur almost reaches significance, and T3 significant except for Cottage Grove. TT4 appears to decrease with increasing deciles of PFOA; no other consistent differences seem to be meaningful for the other thyroid hormones.	

Reference and Study Design	Exposure Measures	Results	Comment
(Olsen, Ehresman et al. 2012) **Study Design:** Longitudinal **Location:** Cottage Grove MN, Decatur AL **Population:** n=204, analyses restricted to 98 to 179 workers not on lipid lowering medication In 2008-2010, 3M employees – general management tasks and Non-3M employees (Contract workers) – specific demolition tasks, demolished a building where 3M produced PFOA. **Outcome Definition:** lipids and hepatic clinical chemistries at baseline and end-of-project	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** (ng/mL) Baseline / Mean Change Mean 3M employee: 881 / -218.3 Mean contract worker: 28.9 / 32.1 Mean time interval between baseline and endpoint assessments: 3M workers = 306 days Contract workers = 152 days	**Stat Method:** Matched-pair and linear regression analyses. Covariates and confounders considered include sex, baseline age, BMI, and alcohol consumption, and time interval between assessments. Stratified by magnitude of change Linear regression results shown for entire database, and adjusted models. **Outcome:** Serum lipids **Major Findings:** Cholesterol: β=-0.003, p-value=0.79 Non-HDL: β=-0.006, p-value=0.54 HDL: β=0.004, p-value=0.35 **Choles/HDL ratio: β=-0.00097, p-value=0.03** **Outcome:** Liver enzymes **Major Findings:** ALP: β=-0.002, p-value=0.67 AST: β=-0.003, p-value=0.29 **ALT: β=-0.0097, p-value=0.05** T. Bilirubin: β=-0.000, p-value=0.60	**Major Limitations:** Healthy worker effect, Exclusion of persons on lipid-lowering medications, Time period of study, Inclusion of largely managerial 3M workers in sample Unable to consider other environmental contaminants **Funding Source:** unspec, authors employed by 3M Medical Department

Reference and Study Design	Exposure Measures	Results	Comment
(Sakr, Kreckmann et al. 2007) **Study Design:** Cross-sectional **Location:** Washington Works production site, WV, U.S.A **Population:** Active workers with potential exposure to APFO, N=1025-excluding those on lipid-lowering medications = 840 **Outcome Definition:** Serum lipids and liver enzymes and other outcomes: Cholesterol, HDL, LDL, triglyceride VLDL, AST, ALT< GGT, CBC (complete blood count), glucose, blood urea nitrogen, creatinine, iron, uric acid, electrolytes, creatinine kinase, lactate dehydrogenase, ALP, protein, albumin, and c-reactive protein, serum PSA in men, and serum hormonal levels TSH, T4, T3 uptake, estradiol, and testosterone.	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Mean = 0.428 ppm = 428 ppb (µg/L) Median = 0.189 ppm (IQR = 0.099-0.381) Range= 0.005-9.55 ppm	**Stat Method:** Linear regression. Covariates and confounders considered include age, BMI, gender, and alcohol consumption, parental history of heart attack, and use of lipid-lowering medication. Log transformation of outcomes as required. **Outcome:** Serum lipids **Major Findings:** **Cholesterol: β=4.04, p-value=0.002** **LDL: β=2.834, p-value=0.005** HDL: β=-0.178, p-value=0.680 **VLDL: β=0.045, p-value=0.031** Triglycerides: β=0.018, p-value=0.384 *findings remained similar with exclusion for lipid-lowering medication use. **Outcome:** Liver enzymes **Major Findings:** AST: β=0.012, p-value=0.317 ALT: β=0.023, p-value=0.124 **GGT: β=0.048,p-value=0.016** Bilirubin: β=0.008, p=value=0.590 *findings remained similar with exclusion for lipid-lowering medication use. **Outcome: others** **Major Findings:** **"Uric acid, iron, lactate dehydrogenase, calcium, and potassium showed statistically significant associations with serum PFOA in linear regression models. In men estradiol and testosterone were significantly associated with serum PFOA..: DuPont – 22458, 2007, internal report.**	**Major Limitations:** Cross sectional design precludes causal inference. 55% voluntary participation rate Healthy worker effect Biological factors not taken into account Unable to consider other environmental contaminants **Funding Source:** DuPont

Reference and Study Design	Exposure Measures	Results	Comment
(Sakr, Leonard et al. 2007) **Study Design:** Longitudinal **Location:** Washington Works site, WV, USA **Population:** n=454 workers with two or more measures of PFOA unless outcomes measures were taken in the same year **Outcome Definition:** Serum lipids and liver enzymes, calculation of LDL based on Friedewald formula	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Overall mean: 1.13 ppm Mean first measurement: 1.04 ppm Mean last measurement: 1.16 ppm	**Stat Method:** Linear mixed effect model including a random effect for subject and fixed effects for age, and other potential confounders. An interaction term gender X age was included. Used a spatial Gaussian covariance structure. Covariates and confounders considered include age, age-squared, gender, BMI, and decade of hire along with all interactions. Number of observations differed for each outcome analyzed. **Outcome:** Serum lipids **Major Findings:** **Choles: β=1.06, (95% CI 0.24, 1.88)** Trigly: β=0.79, (95% CI -5.99, 7.57) HDL: β=0.16, (95% CI -0.39, 0.71) LDL: β=0.46, (95% Ci -0.87, 1.79) **Outcome:** Liver enzymes **Major Findings:** **T. Bilir: β=-0.008, (95% CI -0.0138, -0.0021)** GGT: β=1.24, (95% CI -1.09, 3.57) ALP: β=-0.21, (95% CI -0.60, 0.18) **AST: β=0.35, (95% CI 0.10, 0.60)** ALT: β=0.54, (95% CI -0.46, 1.54)	**Major Limitations:** Healthy worker effect Sample is only 16% of all workers who had ever worked at plant, 10% of workers for over one year. No info on lipid lowering medications or alcohol intake. Exposure and outcome were not measured on same date. LDL calculated based on outdated Friedewald formula (except for 2004 data). Other environmental contaminants, including other PFCs, were not considered. **Funding Source:** DuPont

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

Study Design:
Cross-sectional

Location: USA

Population:
NHANES 1999-2000 & 2003-2006, > 20 years of age, n=3883

Outcome Definition:
Hyperuricemia (serum uric acid levels >6.8mg/dL in men and >6.0 mg/dL in women)</td>
<td>Exposure Assessment:
Plasma concentrations

Population-Level Exposure:
Median = 3.5 ppb (women), 4.6 ppb (men)</td>
<td>Stat Method: Linear regression models with continuous log-transformed PFOA and quartile PFOA. Multivariable logistic regression with PFOA quartiles. Covariates and confounders considered include age, gender, race/ethnicity, education, smoking, alcohol consumption,

Results presented for most adjusted models.

Outcome: Uric Acid
Major Findings:
Mean Change-
Q2 v. Q1: 0.14 (95% CI 0.04, 0.25)
Q3 v. Q1: 0.37 (95% CI 0.25, 0.49)
Q4 v. Q1: 0.44 (95 % CI 0.32, 0.56)
P for Trend < 0.0001

PFOA log transformed: 0.22 (95% CI 0.15, 0.30)

Outcome: Hyperuricemia
Major Findings:
<table>
<tr><td>Quartile</td><td>OR</td><td>95% CI</td></tr>
<tr><td>1</td><td>1</td><td>referent</td></tr>
<tr><td>2</td><td>1.14</td><td>0.78, 1.67</td></tr>
<tr><td>3</td><td>1.90</td><td>1.35, 2.69</td></tr>
<tr><td>4</td><td>1.97</td><td>1.44, 2.70</td></tr>
</table>
P for Trend <0.0001

PFOA log transformed OR=1.43 (95% CI 1.16, 1.76)</td>
<td>Major Limitations:
Cross sectional design precludes causal inference.

Unclear biological mechanism,

Over-adjustment for mediators may have underestimated true association between PFOA and hyperuricemia

Other environmental contaminants, including other PFCs, were not considered.

Funding Source: American Heart Association National Clinical Research Program award and a National Institutes of Health/National Institute of Environmental Health Sciences grant (NIH/NIEHS 5R03ES018888–02)</td>
</tr>
</table>

Reference and Study Design	Exposure Measures	Results	Comment
(Shrestha, Bloom et al. 2015) **Study Design:** Cross sectional **Location:** Upper Hudson River communities, NY **Population:** Older adults 55-74 years of age who lived in three communities adjacent to the Hudson River for 25 years or more, without clinically diagnosed thyroid disease and did not work in PCB related job for a year or more, and other health conditions, n=87 **Outcome Definition:** Thyroid function as measured by serum thyrotropin, free thyroxine, total thyroxine, and total triiodothyronine	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Median = 9.32 ng/mL	**Stat Method:** Multivariable linear regression analyses. Covariates and confounders considered include age, sex, years of education, and serum PCBs PFOA was log transformed. Effects were reported as the changed in thyroid hormone level per interquartile range increase in ln-PFOA. Looked at interactions with other environmental contaminants. **Outcome: Thyroid hormones** **Major Findings:** TSH: β=0.102 (95% -0.023, 0.281) FT4: β=0.016 (95% CI -0.036, 0.069) T4: β=0.380 (95% CI -0.070, 0.830) T3: β=3.032 (95% CI -1.725, 7.789) Statistical interactions between age and PFOA for effect of FT4 and T4 ($p<0.05$). The joint effect of age and PFOA = 7% increase in T4 and 3% for FT4.	**Major Limitations:** Cross sectional design precludes causal inference. No information on serum albumin and TBG Multiple statistical analyses increase probability of chance findings, Small n Unable to analyze joint effects of age and sex, Used archived sera to estimate thyroid biomarkers and PFOA, raising possibility for sample degradation over time (not likely a concern). **Funding Source:** grants provided by the National Institute on Aging (Grant # R15/AG0333700A1) and the Agency for Toxic Substances and Disease Registry (Grant # H75/ATH298312)

Reference and Study Design	Exposure Measures	Results	Comment
(Starling, Engel et al. 2014) **Study Design:** Cross-sectional **Location:** Norway **Population:** Pregnant women enrolled in the Norwegian Mother and Child Cohort Study 2003-04 (n= 891)	**Exposure Assessment:** Plasma concentrations taken at mid-pregnancy **Population-Level Exposure:** Median: 2.25 ng/mL	**Stat Method:** Weighted linear regression + multiple PFAS model, spearman rank-order correlations. Covariates and confounders considered include maternal age, pre=pregnancy BMI, nulliparious or most recent inter-pregnancy interval, duration of breastfeeding most recent child, maternal years of education, current smoking at mid-pregnancy, gestational weeks at blood draw, and amount of oily fish consumed daily, and weight gain, and albumin. PFOA natural log transformed and categorized into quartiles. Results reported for IQR change increase. Outcome log transformed as needed.	**Major Limitations:** Cross sectional design precludes causal inference. Use of nonfasting plasma lipid measurements, No measurement of other environmental contaminants, **Funding Source**: in part by the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences. A.P. Starling was

Outcome Definition: Serum measurements of lipids – measured from same exposure assessment sample.		**Outcome:** Serum lipids **Major Findings:** <u>Total cholesterol</u> Q2 v. Q1 β=1.49 (95% CI -6.49, 9.48) Q3 v. Q1 β=3.54 (95% CI -4.51, 11.59) Q4 v. Q1 β=3.90 (95% CI -5.00, 12.80) Ln-unit β=2.58 (95% CI -4.32, 9.47) IQR β=1.55 (95% CI -2.60, 5.69) Multi-pollutant model brought the already non-significant relationship even closer to the null. <u>HDL</u> Q2 v. Q1 β=0.22 (95% CI -2.38, 2.83) Q3 v. Q1 β=2.31 (95% CI -0.59, 5.20) Q4 v. Q1 β=3.42 (95% CI 0.56, 6.28) Ln-unit β=2.13 (95% CI -0.26, 4.51) IQR β=1.28 (95% CI -0.15, 2.71) <u>LDL</u> Q2 v. Q1 β=0.94 (95% CI -6.08, 7.96) Q3 v. Q1 β=4.16 (95% CI -3.19, 11.50) Q4 v. Q1 β=3.35 (95% CI -4.35, 11.06) Ln-unit β=2.25 (95% CI -3.97, 8.48) IQR β=1.36 (95% CI -2.38. 5.10) <u>Ln-triglycerides</u> Q2 v. Q1 β=0.03 (95% CI -0.04, 0.11) Q3 v. Q1 β=0.01 (95% CI -0.08, 0.09) Q4 v. Q1 β=-0.04 (95% CI -0.12, 0.04) Ln-unit β=0.00 (95% CI -0.07, 0.06) IQR β=0.00 (95% CI -0.04, 0.04)	supported by an extramural award (1-F30-ES022126-01) from the National Institute of Environmental Health Sciences.

<table>
<tr><th>Reference and Study Design</th><th>Exposure Measures</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>(Steenland, Tinker et al. 2009)

Study Design:
Cross-sectional

Location:
OH and WV, USA

Population:
Residents 18 years of age and older who drank water contaminated by nearby plant, for at least one year, n= 46,294

Outcome Definition:
Serum lipids; High cholesterol (>240 mg/dL), serum lipids</td>
<td>Exposure Assessment:
Serum PFOA

Population-Level Exposure:
Mean: 80 ng/mL
Median: 27 ng/mL</td>
<td>Stat Method: Linear regression (continuous and categorical PFOA) and logistic model for “high cholesterol” with PFOA in quartiles. Covariates and confounders considered include age, gender, BMI, education, smoking, regular exercise, and current alcohol consumption, and fasting and PFOS.

Outcomes were natural log transformed.

Outcome: Serum lipids
Major Findings:
A largely monotonic increase in log cholesterol with each decile of PFOA (p<0.0001). Tests for linear trend were highly significant.

Trends for LDL cholesterol, non-HDL cholesterol, and the ratio of total cholesterol to HDL were similar to the nearly monotonic trends for total cholesterol. Therefore was a positive trend with triglycerides <u>but little association with HDL.</u>

The results for unlogged PFOA are test for linear trend for the categorical models; all such linear trends were positive (total cholesterol, LDL, Triglycerides, TC/HDL ratio, non-HDL-C) and significant (p<0.05) <u>except for HDL.</u>

Outcome: “High cholesterol”
Major Findings:
<table>
<tr><td>Quartile</td><td>OR</td><td>95% CI</td></tr>
<tr><td>1</td><td>1</td><td>Referent</td></tr>
<tr><td>2</td><td>1.21</td><td>1.12,1.31</td></tr>
<tr><td>3</td><td>1.33</td><td>1.23,1.43</td></tr>
<tr><td>4</td><td>1.4</td><td>1.28,1.50</td></tr>
</table>
P for trend <0.0001</td>
<td>Major Limitations
Cross-sectional design precludes causal inference.

Lack of a measure of cumulative exposure

Did not control for other environmental contaminants (did control for PFOS).

Funding Source: C8 Class Action Settlement between DuPont and Plaintiffs</td>
</tr>
</table>

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

Study Design:
Cross-sectional

Location:
OH and WV, USA

Population:
Residents 20 years of age and older who drank water contaminated by nearby plant, for at least one year, n= 54,591

Outcome Definition:
Uric acid; hyperuricemia (serum uric acid > 6 mg/dL for women and >6.8 mg/dL for men.</td>
<td>Exposure Assessment:
Serum concentrations

Population-Level Exposure:

(ng/mL)
Mean: 86.4
SD: 261.3
IQR: 13.5 – 71.4
Median: 27.9</td>
<td>Stat Method: Linear regression, some analyses log transformed PFOA, others treated as categorical. Logistic regression for hyperuricemia, PFOA in quintiles.

Covariates and confounders considered include age, sex, BMI, education, smoking, alcohol consumption, serum creatinine (log), and control for PFOS.

Sensitivity analysis – restricting elevated serum creantinine (indicator of kidney disease)

Outcome: Uric acid
Major findings:
Test for linear trend was highly significant (p<0.001).

A close to monotonic increase in uric acid with an increase in PFOA. There is an increase in uric acid of 0.2-0.3 from the lowest to the heist decile for PFOA.

Outcome: Hyperuricemia
Major Findings:
<table>
<tr><td>Quintile</td><td>OR</td><td>95% CI</td></tr>
<tr><td>1</td><td>1</td><td>Referent</td></tr>
<tr><td>2</td><td>1.33</td><td>1.24-1.43</td></tr>
<tr><td>3</td><td>1.35</td><td>1.26-1.45</td></tr>
<tr><td>4</td><td>1.47</td><td>1.37-1.58</td></tr>
<tr><td>5</td><td>1.47</td><td>1.37-1.58</td></tr>
</table>
P for Trend <0.0001

More consistent linear relationship for women than for men.</td>
<td>Major Limitations:
Cross sectional design precludes causal inference.

Mechanism unknown – may have unknown confounder,

No data on blood pressure for population,

Funding Source: C8 Class Action Settlement between DuPont and Plaintiffs</td>
</tr>
</table>

Reference and Study Design	Exposure Measures	Results	Comment
(Steenland, Zhao et al. 2015) **Study Design:** Retrospective occupational cohort **Location:** Washington Works plant, DuPont – WV, USA **Population:** Workers or next of kin in 2008-20011, n=3713. Original DuPont Cohort included 6026 workers with at least 1 day of work between 1948 and 2002. **Outcome Definition:** Self-reported disease – validated by medical record abstraction: all but three.	**Exposure Assessment:** Historical estimates of serum PFOA levels based on job history + nonoccupational exposures ➔ lifetime serum cumulative dose. Investigators used a job exposure matrix validated using worker serum samples from 1979-2004. **Population-Level Exposure:** In 2005, median measured serum level was 113 ng/mL (n=1881)	**Stat Method:** Cox regression models with age as the time scale and time-varying exposure and covariates. Additionally performed parametric tests for trend, categorical trend tests. Looked at lag and 10-year lag. Coronary heart disease, stroke, chronic obstructive pulmonary disease, autoimmune disease, type 2 diabetes, liver disease (hepatitis and non-hepatitis), Parkinson's disease, Alzheimer's disease, thyroid disease, asthma with reported current medication, and cancer. And three self-reported outcomes: osteoarthritis, hypertension, and high cholesterol. All statistically significantly results are listed below ($p < 0.05$). Those not listed mean that outcomes were not significant. **Outcome:** **Major Findings:** **Ulcerative colitis – positive trend using the log cumulative exposure for no lag and a 10-year lag. For 10-year the highest exposure category for had a RR relative to the first quartile of 6.57 ((%CI 1.47, 29.40).** **No other disease showed a statistically significant positive trend using the log transform trend test.** **Rheumatoid arthritis showed a significant positive trend using the trend test based on the quartile midpoints (p=0.04). The highest exposure category (no lag) had a RR relative to the first quartile of 4.45 (95% CI 0.99, 19.9) – p=0.05.** **Type II diabetes (10 year lag) had a significant trend using the categorical trend test.**	**Major Limitations:** Small n compared to whole C8 cohort under representation of hard to trace decedents. Few low-exposed referents. Largely confined to men. Healthy worker effect. Limited sample size for some outcomes. 62% of target population. Failure to obtain medical records for 17% of those self-reporting disease. Survival bias Truncated residential histories at 1970 - underestimated exposures for some participants. **Funding Source:** C8 Class Action Settlement between DuPont and Plaintiffs

		Bladder cancer showed a negative trend using log cumulative dose (for the analysis with no lag) and the categorical trend test (for the analysis with a 10-year lag). **Negative trend for asthma with medication (no lag) in the categorical trend test**	

Reference and Study Design	Exposure Measures	Results	Comment
(Stein et al., 2016) **Study Design:** Cross-sectional **Location:** U.S. general population **Population:** NHANES (1999-2000 and 2003-2005), n=1,191 and (2005-2006), n=640; adolescents aged 12 to 19 years. **Outcome Definition:** Measles, mumps, and rubella antibody concentrations (1999-2000 and 2003-2004 cycles) and allergic conditions and allergic sensitization (2005-2006 cycles). Allergic sensitization defined as sIgE ≥0.35 kU/L	**Exposure Assessment:** PFOA serum concentrations **Population-Level Exposure:** Geometric Mean= 4.13 ng/mL (cycles 1999-2000 and 2003-2005) Geometric Mean=3.59 ng/mL (cycles 2005-2006)	**Stat Method:** Adjusted, survey-weighted regression models. Covariates and confounders considered include age, sex, and race/ethnicity, serum cotinine, BMI, and others. Linear regression was used for the vaccine study with PFC treated continuously and as quartiles. Exposures and outcomes were natural log transformed. All models were run for full population and run again restricted to the seropositive population. Logistic regression for estimates for the allergy study. Odds ratios based on an IQR shift and for quartiles of PFOA and linear regression used to estimates allergen specific IgE antibodies and IgE antibody level by quartiles. Effect estimates shown for doubling of PFC concentration. **Outcome:** Antibody titers **Major Findings:** % Change <u>All</u> Measles=-0.1 (95% CI -13.8, 15.6) Mumps=-6.0 (95% CI -12.4, 0.9) Rubella=-2.5 (95% CI -9.1, 5.3) <u>Seropositive</u> Measles=-3.4 (95% CI -16.7, 11.9) **Mumps= -6.6 (95% CI -11.7, -1.5)** **Rubella=-8.9 (95% CI -14.6, -2.9)**	**Major Limitations:** Cross-sectional design limits causality interpretation. Receipt and/or timing of measles-mumps-rubella vaccination are not available. Other environmental contaminants, including other PFCs not controlled for.

		Outcome: Allergic conditions **Major Findings:** Asthma OR=1.28 (95% CI 0.81, 2.04) Wheeze OR=0.94 (95% CI 0.51, 1.73) Allergy OR=1.12 (95% CI 0.85, 1.47) **Rhinitis OR=1.35 (95% CI 1.10, 1.66)** **Outcome:** Allergic sensitization **Major Findings:** Any, Plants, Dust mites, Pets, Cockroach/shrimp, Rodents, Mold, Food: No findings were statistically significant, confidence intervals appeared wide and findings were both positive or negative depending on the type of allergen. **Outcome:** Serum IgE Antibody Titer **Major Findings:** **%Change=10.5 (95% CI 0.17, 22)**	

Reference and Study Design	Exposure Measures	Results	Comment
(Wang, Rogan et al. 2014) **Study Design:** Cross-sectional and prospective birth cohort. **Location:** Central Taiwan **Population:** Recruited from Taiwan Maternal and Infant Cohort Study (longitudinal birth cohort study of pregnant women and children); n=285 pregnant women in 3rd trimester, n=116 neonates. **Outcome Definition:** Maternal (@ 3rd trimester) and cord serum neonatal thyroid	**Exposure Assessment:** Maternal serum concentrations. **Population-Level Exposure:** Maternal Median = 2.39 ng/mL	**Stat Method:** Linear regression, covariates and confounders considered included maternal age, maternal education, previous live births, family income, maternal pregnancy BMI, and maternal fish consumption during pregnancy. Estimates calculated with and without log-transformation of thyroid hormones. **Outcome:** Thyroid hormones **Major Findings:** <u>Maternal PFOA and maternal:</u> FT4: β=-0.003 (95% -0.012, 0.005) TT4: β=0.011 (95% CI -0.108, 0.130) TT3: β=-0.000 (95% CI -0.002, 0.009) TSH: β=0.011 (95% CI -0.057, 0.078)	**Major Limitations:** Only one sample from mother and neonate was obtained, calls causal inference into question, Measurement of cord blood rather than infant blood may have been more informative, less affected by perturbations of birth, Sample size not sufficient for evaluation of clinical outcomes, Did not have measures of all possible factors that affect thyroid hormones (i.e., iodine) Some aspects of the study were cross sectional, therefore precluding causal inference.

hormone status (TSH, FT4, TT4, T3)		Maternal PFOA and cord: FT4: β=-0.029 (95% -0.062, 0.004) TT4: β=0.128 (95% CI -0.094, 0.350) TT3: β=-0.001 (95% CI -0.004, 0.001) TSH: β=-0.498 (95% CI -1.464, 0.468)	**Funding Source:** National Health Research Institutes, Taiwan (grants EO-101-PP-05,EH-102-SP-02, EO-102-PP-05), the National Science Council, Taiwan (grant NSC101-2325-B-400-008), and in part by the National Institute of Environmental Health Sciences, National Institutes of Health (USA).

Reference and Study Design	Exposure Measures	Results	Comment
(Wang, Zhang et al. 2012) **Study Design:** Cross-sectional **Location:** Jiangsu High-Tech Fluorine Chemical Industrial Zone, Changshu City, Jiangsu Province, CN **Population:** Employees at plant for greater than 2 years, n=55 (male) and nearby residents for at least 5 years, n=132. **Outcome Definition:** serum lipid biomarkers and nine circulating miRNAs (endogenous, small noncoding RNAs. They play an important role in regulating gene expression by base-pairing to complementary sites on target mRNAs to block translation or trigger the degradation of target mRNAs.	**Exposure Assessment:** Serum PFOA **Population-Level Exposure:** Median: 284 ng/mL (residents) 1636 ng/mL (occupational cohort)	**Stat Method:** Described correlations using Spearman non-parametric correlation coefficients. Also linear regression with control for age and BMI, smoking and drinking status. Also did a sensitivity analysis excluding older individuals (residents only) and combined younger (resident and workers). All outcomes and PFOA were natural log transformed. **Outcome:** Serum lipids and liver enzymes **Major findings:** Residents Cholesterol: β=-0.00, p-value=0.85 HDL: β=0.02, p-value=0.39 LDL: β=-0.00, p-value=0.97 HDL/LDL: β=0.02, p-value=0.57 TG: β=0.02, p-value=0.73 ALT: β=-0.10, p-value=0.05 AST: β=-0.04, p-value=0.22 Workers Cholesterol: β=0.02, p-value=0.36 **HDL: β=-0.07, p-value=0.01** LDL: β=0.03, p-value=0.43 **HDL/LDL: β=-0.09, p-value=0.01** TG: β=0.02, p-value=0.73 ALT: β=0.04, p-value=0.38 **AST: β=-0.12, p-value=0.02**	**Major Limitations:** Cross sectional design precludes causal inference. Healthy worker effect Small n. Volunteer bias. Age discrepancy in miRNA cohort. Self-reported weight and height, No info on socioeconomic data. No known covariates for miRNAs, No known exact pathological meanings of altered serum miRNAs **Funding Source:** National Natural Science Foundation of China

<table>
<tr>
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<td>
Outcome: Circulating mrR-26b and miR-199a-3p
Major Findings:
<table>
<tr><th>miRNA</th><th>Residents</th><th>Workers</th><th>Fold change</th><th>P</th></tr>
<tr><td>miR-26b</td><td>4.05</td><td>3.11</td><td>1.9</td><td>0.03</td></tr>
<tr><td>miR-199a-3p</td><td>8.98</td><td>6.9</td><td>3.47</td><td>0.02</td></tr>
</table>
Other miRs were not statistically significant.
</td>
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</table>

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

Study Design:
Prospective cohort study

Location:
Vancouver, Canada

Population:
Euthyroid pregnant women in early 2nd trimester, enrolled in the Chemicals, Health, and Pregnancy study, ≥ 19 years of age, n=152

Outcome Definition:
Repeated measures of maternal thyroid hormones at 15 and 18 weeks gestation.</td>
<td>Exposure Assessment:
Serum concentrations

Population-Level Exposure:

Median = 1.7 ng/mL</td>
<td>Stat Method:
Mixed effects linear regression models with random intercept for individual, Confounders and covariates considered include maternal age, ethnicity, education, household income, current stress levels, smoking, environmental tobacco smoke exposure, drug use, alcohol use, and use of iodized salt and prenatal vitamins containing iodine.

PFCs and hormones not log transformed.

Sensitivity analysis stratified by high v. normal TPOAb (thyroid peroxidase antibody levels, as marker of autoimmune hypothyroidism).

Estimates presented for most adjusted models and an IQR increase.

Outcome: FT4
Major Findings:
Normal TPOAb: β=-0.03 (95% CI -0.3, 0.2)
High TPOAb: β=-0.4 (95% CI -1, 0.5)

Outcome: TSH
Major Findings:
Normal TPOAb: β=0.07 (95% CI -0.1, 0.2)
High TPOAb: β=0.7 (95% CI 0.09, 1)

Outcome: TT4
Major Findings:
No associations found, estimates presented in supplemental material.</td>
<td>Major Limitations:
Small n, especially in high TPOAb group,

Sample unrepresentative of background population of Vancouver,

Use of questionnaires to determine iodine sufficiency and exposure to environmental tobacco smoke,

No control for serum binding protein levels or urinary/blood selenium,

Sightly outdated radioimmunoassay method,

Reverse causation may be a factor

Note:
Associations found early in critical gestational period

Funding Source: Health Canada, BC Medical Services Foundation, BC Environmental and Occupational Health Research Network, UBC Centre for Health and Environment Research, Natural Sciences and Engineering Research Council, Michael Smith Foundation for Health Research, UBC Bridge Program, Interdisciplinary Women’s Reproductive Health Program</td>
</tr>
</table>

Reference and Study Design	Exposure Measures	Results	Comment
(Wen, Lin et al. 2013) **Study Design:** Cross-sectional **Location:** General U.S. population **Population:** NHANES 2007-2008, and 2009-2010, adults > 20 years of age, n=1.180 **Outcome Definition:** Serum concentrations of thyroid measures (TT3, TT4, FT4, thyroglobulin), subclinical hyperthyroidism was defined as TSH < 0.24 mIU/L and subclinical hypothyroidism defined as TSH > 5.43 mIU/L	**Exposure Assessment:** Serum concentrations **Population-Level Exposure:** Geometric mean = 4.15 ng/mL	**Stat Method:** Linear and logistic regression, covariates and confounders considered included age, gender, race, alcohol consumption, smoking, and urinary iodine. Modeled separately for men and women. Outcomes were log transformed if improved model fit, PFOA was log transformed. PFCS were modeled separately and in composite model. Most adjusted (weighted) results shown below. **Outcome:** Thyroid hormones **Major Findings:** <u>TT4</u> Men: β=0.000 (95% CI -0.280, 0.280) Women: β=0.82 (95 % CI -0.369, 0.532) <u>Ln-FT4</u> Men: β=-0.010 (95% CI -0.041, 0.022) Women: β=-0.004 (95 % CI -0.047, 0.039) <u>TT3</u> Men: β=0.775 (95% CI -3.048, 4.598) **Women: β=6.628 (95 % CI 0.545, 12.712)** <u>ln-FT3</u> Men: β=0.013 (95% CI -0.004, 0.031) Women: β=0.016 (95 % CI -0.018, 0.051) <u>Ln-TSH</u> Men: β=0.004 (95% CI -0.081, 0.090) Women: β=-0.030 (95 % CI -0.215, 0.154) <u>Ln-thyroglobulin</u> Men: β=-0.096 (95% CI -0.258, 0.066) Women: β=0.095 (95 % CI -0.111, 0.302)	**Major Limitations:** Cross sectional design precludes causal inference, Common physiology could confound, No accounting for thyroid medications, Data set limited to single time point measures (thyroid hormone levels vary in an individual). **Funding Source:** unspec, authors employed by En Chu Kong Hospital (Wen LL, Lin CY), National Taiwan University Hospital (Lin LY, Su TC), National Taiwan University of Public Health (Chen PC) and Fu Jen Catholic University (Lin CY).

		Outcome: Subclinical thyroid diseases **Major Findings:** Hypothyroidism Men: OR=1.29 (95% CI 0.40, 4.10) **Women: OR=7.42 (95% CI 1.14, 48.12)** Hyperthyroidism **Men: OR=0.38 (95% CI 0.16, 0.95)** Women: OR=0.99 (95% CI 0.13, 7.59)	

Reference and Study Design	Exposure Measures	Results	Comment
(Winquist and Steenland 2014) **Study Design:** Retrospective cohort, prospective analyses in 2005-2006. **Location:** WV and OH, USA **Population:** Community member cohort (recruited from C8 Health Project participants (>20 years of age)), n = 28,541and plant workers in mid-Ohio River Valleys (recruited from a previous occupational mortality study cohort of people who worked at the chemical plant during 1948-2002), n = 3,713, n=32,254 total **Outcome Definition:** Self-reported health professional diagnosis-Questionnaire of demographics, health-related behaviors, and lifetime personal medical history, completed 2008-2010 and 2010-2011.	**Exposure Assessment:** Yearly estimated serum PFOA concentrations starting at birth of 1952, whichever came later. Estimates of yearly air and drinking water PFOA exposure using an environmental fate and transport model were developed. Additional information for community estimates includes residential history and drinking water consumption rates and sources. For workers an occupational exposure model was used, after work, they were decayed until they met the community exposure models. **Population-Level Exposure:** Median 2005-06 = 26.1 ng/mL Median concentrations highest in late 1990s-early 2000s	**Stat Method:** Cox proportional hazard models (survival analysis), stratified by birth year. Covariates and confounders considered include sex, race, education, smoking and alcohol use. Primary models considered patterns of hazard ratios across quintiles of each exposure metric. In tests for trend, natural log-transformed PFOA concentration estimates were used. Retrospective analyses used deciles to explore dose-response curve shape. **Outcome:** Functional thyroid disease (n=2,323) **Major Findings:** Primary retrospective analysis: Quintile / HR 1 / 1 2 / 1.21 3 / 1.12 4 / 1.27 5 / 1.28 HR log µg/ml*yr = 1.03, p-value=0.09 **The trend was more pronounced among women (HR per log µg/ml*yr = 1.04, p-value=0.03),** but was absent among men (HR per log µg/ml*yr = 1.01, p-value=0.85).	**Major Limitations:** Healthy worker effect for worker cohort Population in study area changed over time, no way to identify and include all residents over exposure period. Potential bias if exposure and thyroid conditions were correlated with migration or participation in original C8 study, Possible changes in population susceptibility over time, Potential error in exposure estimates (would bias towards null), Exclusion of people who reported thyroid disease with no type specified, Potential impact of differential access to care (w/r/t thyroid diagnoses) **Funding Source:** C8 Class Action Settlement between DuPont and Plaintiffs

Medical records were reviewed to confirm diagnosis. Functional thyroid disease defined as a report of goiter, Graves' disease, hyperthyroidism, Hashimoto's disease, hypothyroidism, thyroiditis not otherwise specified, or a thyroid function problem of unknown type. Hyperthyroidism defined as report of hyperthyroidism or Graves' disease. Hypothyroidism defined as report of hypothyroidism or Hashimoto's disease.		Yearly exposure metric: Quintile / HR 1 / 1 2 / 1.23 3 / 1.24 4 / 1.10 5 / 1.28 **HR per log µg/ml*yr = 1.03, p-value=0.04** **The trend was more pronounced among women (HR per log µg/ml*yr = 1.04 p-value=0.008),** and was absent among men. Prospective analysis, Bayesian calibration: No clear associations with both sexes combined (smaller number of cases. There was a suggestion of an increasing hazard of function thyroid disease with increasing cumulative exposure among men (p-value=0.09). **Outcome:** Hypothyroidism (n=1,655) **Major Findings:** A trend for increasing hazards with increasing exposure was suggested for both hypothyroidism in relation to cumulative exposure (although log-linear trend p-values were > 0.05). For men considering the cumulative and yearly exposure metrics, and measured PFOA - HR per log µg/ml*yr = 1.24 P=0.021. And for prospective analyses. **Outcome:** Hyperthyroidism (n=456) **Major Findings:** A trend for increasing hazards with increasing exposure was suggested for both. Log-linear trend p-value < 0.05, overall and for women.	

<table>
<tr><th>Reference and Study Design</th><th>Exposure Measures</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>(Winquist and Steenland 2014)

Study Design:
Longitudinal

Location:
WV and OH, USA

Population:
Community member cohort (recruited from C8 Health Project participants (>20 years of age)), n = 28,541and plant workers in mid-Ohio River Valleys (recruited from a previous occupational mortality study cohort of people who worked at the chemical plant during 1948-2002), n = 3,713, n=32,254 total

Outcome Definition:
Self-reported health professional diagnosis- 2008-2011 Questionnaire completion for high blood pressure, high cholesterol, or heart disease.

Questions include diagnosis of hypertension (not during pregnancy), hypercholesterolemia, coronary artery disease</td>
<td>Exposure Assessment:
Yearly estimated serum PFOA concentrations starting at birth of 1952, whichever came later. Estimates of yearly air and drinking water PFOA exposure using an environmental fate and transport model were developed. Additional information for community estimates includes residential history and drinking water consumption rates and sources. For workers an occupational exposure model was used, after work, they were decayed until they met the community exposure models.

Population-Level Exposure:
Median measured in 2005-06: 26.1 ng/mL (24.2 community, 112.7 worker)</td>
<td>Stat Method: Cox proportional hazard models (survival analysis), stratified by birth year. Covariates and confounders considered include sex, race, education, smoking and alcohol use, BMI, self-reported diabetes type 2, family history of coronary disease.

Retrospective analyses started at the late of age 20 years or the age in 1952. Prospective analyses started at the participant’s age 1 year after enrollment in the C8 Health Project (2005-2006).

Primary exposure metric was a measure of cumulative PFOA (sum of all yearly serum concentration estimates). To look at short term effects, secondary analyses looked at yearly concentrations of PFOA estimates. Primary analyses considered the exposure by quintiles. Log of cumulative or yearly concentrations was used for a test of trend.

All analyses performed overall and by age strata. Sensitivity analysis examining only community cohort.

Outcome: Hypertension
Major Findings:
Retrospective analyses of both cumulative and yearly exposure metrics were similar and did no show clear evidence of association.

Cumulative-
<table>
<tr><td>Quintile</td><td>HR</td><td>95% CI</td></tr>
<tr><td>2</td><td>1.10</td><td>1.02, 1.19</td></tr>
<tr><td>3</td><td>1.10</td><td>1.02, 1.18</td></tr>
<tr><td>4</td><td>1.05</td><td>0.97, 1.12</td></tr>
<tr><td>5</td><td>0.98</td><td>0.91, 1.06</td></tr>
</table>

Age- and sex- stratified analyses found some evidence of hypertension with increasing exposure among females, 20 to 39 years and</td>
<td>Major Limitations:
Survivor bias,

Hard to find decedents proxies (workers),

Healthy worker effect (workers)

Self-report bias (towards null)

Funding Source: C8 Class Action Settlement between DuPont and Plaintiffs</td>
</tr>
</table>

		males 40 to 59 but trends were not monotonic or statistically significant. Prospective analysis also did not show clear evidence. **Outcome:** Incident hypercholesterolemia with medication **Major Findings:** **Retrospective analyses showed elevated hazard.** Cumulative- Quintile / HR / 95% CI **2 / 1.24 / 1.15, 1.33** **3 / 1.17 / 1.09, 1.26** **4 / 1.19 / 1.11, 1.27** **5 / 1.19 / 1.11, 1.28** **Test for trend p=0.005** **Yearly exposure showed the same effect as cumulative (Test for trend p<0.001).** **The increased hazard was most pronounced among men 40-59 years of age (Test for trend p<0.001).** Prospective analyses showed no evidence of a positive association. **Outcome:** Coronary artery disease **Major Findings:** Retrospective analyses showed no clear association. Quintile / HR / 95% CI 2 / 1.26 / 1.10, 1.45 3 / 1.17 / 1.02, 1.35 4 / 0.99 / 0.86, 1.14 5 / 1.07 / 0.93, 1.23 Among males 20 to 39 years of age, there appeared to be higher hazards, but log linear test for trend not significant (P for trend = 0.64) Prospective analyses also showed no evidence of association.	

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

Study Design:
Cross-sectional

Location:
Japan (15 prefectures)

Population:
Participants in larger “Survey on the Accumulations of Dioxins and Other Chemical Compounds in Humans” project, ages 16-76, resided in area for at least 10 years with little relocation, n=608

Outcome Definition:
Serum fatty acids and hepatic enzymes: γ-GTP (glutamyl transpeptidase), GOT (glutamic-oxaloacetic transaminase), GPT (glutamic-pyruvic transaminase), EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid)</td>
<td>Exposure Assessment:
Serum concentrations

Population-Level Exposure:
Median: 2.1 ng/mL
IQR: 1.5-3.3 ng/mL</td>
<td>Stat Method:
Spearman rank correlations, covariates and confounders considered included effects of age, sex, BMI, regional block and smoking, and intake of alcoholic beverages.

Most adjusted results presented when available.

Outcome: Liver enzymes and fatty acids
Major Findings:

<table>
<tr><th>Outcome</th><th>r</th><th>P</th></tr>
<tr><td>DHA</td><td>0.12</td><td>0.003</td></tr>
<tr><td>EPA</td><td>0.20</td><td><0.0001</td></tr>
<tr><td>γ-GTP (GGT)</td><td>0.06</td><td>0.12</td></tr>
<tr><td>GOT (AST)</td><td>0.13</td><td>0.002</td></tr>
<tr><td>GPT (ALT)</td><td>0.09</td><td>0.04</td></tr>
</table>
</td>
<td>Major Limitations:
Cross sectional design precludes causal inference.

Subjects engaged in fishing and farming may have been oversampled compared with gen pop

Funding Source: National Natural Science Foundation of China</td>
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<table>
<tr><th>Reference and Study Design</th><th>Exposure Measures</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>(Zeng, Qian et al. 2015)

Study Design:
Cross-sectional

Location:
Taipei, Taiwan

Population:
Taiwanese children ages 12-15 years, n=225
From control group of Genetic and Biomarkers Study for Childhood Asthma

Outcome Definition: Serum lipids.

LDL was calculated using the Friedewald formula for participants when TGs were lower than 400 mg/dl.</td>
<td>Exposure Assessment:
Serum concentrations

Population-Level Exposure:
ng/mL
Mean (boys,) =1.1 (+/- 1.4)
Mean (girls)=0.92 (+/- 0.79)</td>
<td>Stat Method: Linear regression models with continuous log transformed PFOA and as quartiles. Covariates and confounders considered include age, gender, BMI, regular exercise, parental education, and environmental tobacco smoke.

Ordinal variable used to assess trends.

Outcome: Serum lipids
Major Findings:

<table>
<tr><td>Outcome</td><td>β</td><td colspan="2">95% CI</td><td>P for trend</td></tr>
<tr><td>Total Cholesterol</td><td>6.57</td><td>2.72</td><td>10.42</td><td>0.004</td></tr>
<tr><td>HDL</td><td>-1.56</td><td>-3.20</td><td>0.08</td><td>0.36</td></tr>
<tr><td>LDL</td><td>4.66</td><td>1.67</td><td>7.65</td><td>0.002</td></tr>
<tr><td>Triglycerides</td><td>19.63</td><td>14.82</td><td>24.34</td><td>0.01</td></tr>
</table>
</td>
<td>Major Limitations:
Cross sectional design precludes causal inference,

May be unmeasured confounders (dietary sources, etc) or pharmokinetics.

Funding Source: National Natural Science Foundation of China</td>
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</table>

Appendix 5A. Studies that evaluated increased liver weight and provide serum PFOA data from end of dosing period

Note: An additional study (Macon et al, 2011) that includes this information is presented in Appendix 5B

Reference and Study Design	Results	Comment
Lau et al. (2006). Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. ***NOTE:*** *A separate study of pharmacokinetics of PFOA in male and non-pregnant female mice and rats reported in this paper is not included in this table.* **Species and strain:** Timed pregnant CD-1 mice **Test article and vehicle:** PFOA (>98% pure; 98.9% linear, 1.1% branched isomers) Vehicle was NANOpure water. **Route of exposure:** Oral gavage **Exposure levels:** 0, 1, 3, 5, 10, 20, 40 **Exposure regimen:** GD 1-17 **Group size:** Not explicitly stated. Group numbers are reported with data in Results section.	**Maternal body weight gain (GD 1-18)** • Data shown graphically. • n = 9-57 dams per dose level. • Dams were weighted daily on GD 1-18. • Weight gain dramatically decreased during latter part of gestation at 20 mg/kg/day (stated significant on GD 8-18). • Weight loss instead of gain during latter part of gestation at 40 mg/kg/day (weight effects stated significant on GD 5-18). **Maternal liver weight (GD 18)** • Data for absolute liver weight shown graphically. Numerical data for liver weight (absolute, and relative to body weight with gravid uterus removed) provided by investigator. • n = 9-45 dams per dose level. • Absolute liver weight increased (p<0.05) at all doses. Statistical evaluation of liver wt. relative to body wt. minus wt. of gravid uterus not provided by investigator. • Absolute liver weight lower at 20 and 40 mg/kg/day than 10 mg/kg/day, but body weight also decreased at these doses. Dose (mg/kg/day) | Absolute liver weight (g) | Relative liver weight (% of body weight minus weight of gravid uterus) | — 0 | 2.4±0.05 | 7.3±0.14 | 1 1 | 3.4±0.08* | 10.9±0.16 | 1.49 3 | 4.2±0.15* | 12.9±0.41 | 1.77 5 | 4.4±0.17* | 13.8±0.40 | 1.89 10 | 5.1±0.24* | 15.9±0.52 | 2.18 20 | 4.2±0.20* | 16.9±0.51 | 2.32 40 | 4.0±0.16* | 18.9±0.63 | 2.59 *p<0.05 **Maternal PFOA serum levels (GD 18)**. Dose (mg/kg/day) | n | Serum level (µg/ml) 0 | 58 | 0.51±0.12 1 | 6 | 21.93±1.71 3 | 7 | 40.5±1.89 5 | 33 | 71.91±8.33 10 | 30 | 116.12±16.18 20 | 54 | 180.55±19.05 40 | 14 | 271.14±19.15 • Shown graphically; numerical data provided by investigator. • n = 6-58 per dose level.	

Reference and Study Design	Results	Comment
Lau et al. (2006). (continued)		

- Increased in a dose-related fashion, but less than proportionally

Reproductive outcomes

Data presented in tabular form.

- Parameters examined include full litter resorptions, # of implants per live litter and per litter with full litter resorption, # live fetuses per litter, % prenatal loss, and fetal body weight.
- n = 9-45 dams per dose group
- Data in table below for parameters with statistically significant (p<0.05) effects. No statistically significant effects for parameters now shown.

Dose (mg/kg/day)	# of dams	% full litter resorptions	# live fetuses per litter	% prenatal loss per live litter	Fetal body weight (g)
0	45	6.7	12.5±0.4	4.1±1.4	1.05±0.02
1	17	11.8	13.0±0.4	1.0±0.7	0.98±0.03
3	17	5.9	10.8±0.9	7.4±2.5	1.03±0.04
5	27	25.9*	11.1±0.4	2.4±0.8	1.03±0.04*
10	26	46.1*	11.7±0.8	7.7±3.3	0.98±0.05
20	42	88.1*	7.2±2.0*	25.9±11.7*	0.86±0.11*
40	9	100*	---	---	----

*p<0.05

  - Full litter resorptions increased at 5 mg/kg/day and above. NOAEL – 3 mg/kg/day.
  - Number of live fetuses and fetal body weight decreased, % prenatal loss increased at 20 mg/kg/day. NOAEL – 10 mg/kg/day.

Fetal teratology

- Data shown in tabular form.
- Extent of ossification assessed in sternebrae, caudal vertebrae, metacarpals, metatarsals, proximal phalanges of forelimb and hindlimb, calvari, supraoccipital, and hyoid.
  - Statistically significant (p<0.05) reduction in ossification at one or more sites at all doses (1 mg/kg/day or above). No NOAEL identified.
  - Reduced ossification effects at some sites. Do not increase in a dose-dependent fashion.
- Tail defects significantly (p<0.05) increased at 5, 10, and 20 mg/kg/day and limb defects at 5 and 20 mg/kg/day. NOAEL – 3 mg/kg/day.
- Microcardia significantly increased at 10 and 20 mg/kg/day. NOAEL – 5 mg/kg/day.

Reference and Study Design	Results	Comment

Lau et al. (2006).
(continued)
	<u>Neonatal survival</u>
	Data shown graphically for birth - PND 23.
	n = 8-22 litters per dose group.
	Stillbirth and neonatal mortality increased (p<0.05) at 5 mg/kg/day and above. Effect most severe (>50% stillbirth; >70% neonatal mortality at 10 and 20 mg/kg/day.
	
	<u>Offspring body weight</u>
	• Birth – PND 23: Data shown graphically.
	  ○ n = 7-30 litters per dose group.
	  ○ Body weight decreased (p<0.05) at 3 mg/kg/day and higher. At PND 23, body weight 25-30% lower than controls at 3 mg/kg/day and higher. NOAEL – 1 mg/kg/day
	• 6.5 – 60 weeks: Data shown in tabular form (male and female separately).
	  ○ n = 4-11 litters per dose group.
	  ○ Significant (p<0.05) dose-related effect at 6.5 weeks in males.
	  ○ Trend to higher body weights in treated than control at later time points.
	
	<u>Developmental landmarks</u>
	• Data shown in tabular form. n=4-56 pups from 2-22 litters.
	• *Day of eye opening* – Dose-related delay at all doses 1 mg/kg/day and higher. Significant (p<0.05) at 3 mg/kg/day and higher. Delayed by 3.1 days at 20 mg/kg/day.
	• *Day of vaginal opening* – Significant delay (p<0.05) of 2.9 days at 20 mg/kg/day.
	• *Day of first estrus* – Significantly delayed at 5 and 20 mg/kg/day, but not 10 mg/kg/day
	• *Day of preputial separation* – Significantly accelerated compared to controls at 1 – 10 mg/kg/day, with greatest effect at 1 mg/kg/day (3.8 days). Significantly delayed at 20 mg/kg/day.	

Reference and Study Design	Results	Comment
Loveless et al. (2006). Comparative responses of rats and mice exposed to linear/branched, linear, or branched ammonium perfluorooctanoate (APFO) **Species and strain:** Male rats and mice, approximately 8 weeks old, strain not stated. **Test article and vehicle:** Linear/branched APFO, 77.6% linear. 97.99% pure. Linear APFO, purity not stated. Branched APFO, purity not stated. Vehicle was NANOpure water. **Route of exposure:** Oral gavage **Exposure levels:** 0, 0.3, 1, 3 or 1 mg/kg/day **Exposure regimen:** 14 days **Group size:** 10 per species per dose group.	**RAT STUDY** **Hepatic endpoints**	Data for parameters for hepatic effects and dosimetry are presented in detail. Data for other parameters are presented in summary form.

Dose (mg/kg/day)	Linear/Branched			Linear			Branched		
	Serum PFOA (µg/ml)	Relative Liver Weight (g/100g)	Peroxisomal beta-oxidation (palimitoyl CoA) (nM/min/mg protein)	Serum PFOA (µg/ml)	Relative Liver Weight (g/100g)	Peroxisomal beta-oxidation (palimitoyl CoA) (nM/min/mg protein)	Serum PFOA (µg/ml)	Relative Liver Weight (g/100g)	Peroxisomal beta-oxidation (palimitoyl CoA) (nM/min/mg protein)
0	0.37±0.14	3.43±0.40	18.3±3.8	0.39±0.51	3.63±0.35	15.7±3.6	0.11±0.24	3.38±0.29	13.0±3.3
0.3	19±2.5	3.63±0.23	18.0±3.7	20±3.2	3.86±0.14	16.0±3.1	16±3.2	3.58±0.27	12.9±3.8
1	51±10	3.94±0.20*	27.0±4.3*	65±11	4.13±0.33*	29.8±5.6*	48±12	3.91±0.33*	14.4±3.1
3	106±10	3.94±0.20*	57.6±10.0	137±18	3.94±0.20*	67.5±14.3*	73±25	3.94±0.20*	17.4±3.5*
10	183±46	5.67±0.52*	82.2±18.1*	206±65	6.03±0.41*	74.8±10.1*	92±20	5.77±0.75*	28.7±8.0*
30	208±51	6.82±0.56*	84.7±16.3*	223±77	6.64±0.51*	89.0±13.6*	124±33	6.37±0.70*	37.6±12.2*

*p<0.05. n=10/group.

- Data shown in tabular form
- NOAEL for increased relative liver weight: 0.3 mg/kg/day;16 ug/ml serum. LOAEL for increased relative liver weight; 1 mg/kg/day; 27 ug/ml serum.
- Linear/branched ratio data in linear branched/group shown in tabular form. Linear/branched ratio in serum increased with dose, suggesting preferential elimination of branched form
- The authors state that, when analyzed by serum levels, branched caused greater increase in liver weight than linear/branched and linear. Also, and peroxisomal beta-oxidation (palmitoyl Co-A oxidation; a marker of PPAR-alpha activity) lower in branched than in linear/branched and branched on a serum level basis., This indicates that PPAR-alpha independent increases in liver weight occurred. These data are shown graphically in the paper.

Other Endpoints

Mortality/clinical signs: No mortality or clinical signs of toxicity during study.

Body weight/body weight gain: Data for body weight on day 0, 7, and 13, and for body weight gain on day 13; shown in tabular form.
Body weight decreased (p<0.05) compared to control on days 7 and 13 in linear/branched and linear 30 mg/kg/day groups.
Body weight gain on days 0-13 decreased (p<0.05) compared to control at 3 mg/kg/day in linear, 10 mg/kg/day in branched/linear, and 30 mg/kg/day in all treated groups.

Reference and Study Design	Results	Comment
Loveless et al. (2006). (continued)		

Hematology: Data not shown. Treatment related increases in acanthocytes in linear/branched and linear at 3, 10, and 30 mg/kg/day. Treatment related poililocytes and schistocytes in linear/branched at 30 mg/kg/day, and linear at 3,10, and 30 mg/kg/day. Dose-related increases, but not present at high numbers.

Serum lipids: Data shown in tabular form. Cholesterol, HDL, Non-HDL, and triglycerides decreased by all 3 forms at multiple doses. Linear caused statistically significant (p<0.05_decreases on cholesterol, non-HDL, and triglycerides at lowest dose (0.3 mg/kg/day and above). All 3 forms caused statistically significant effects on one or more parameter at 1 mg/kg/day and above.

MOUSE STUDY

Hepatic endpoints

Dose (mg/kg/day)	Linear/Branched			Linear			Branched		
	Serum PFOA (µg/ml)	Relative Liver Weight (g/100g)	Peroxisomal beta-oxidation (nM/min/mg protein)	Serum PFOA (µg/ml)	Relative Liver Weight (g/100g)	Peroxisomal beta-oxidation (nM/min/mg protein))	Serum PFOA (µg/ml)	Relative Liver Weight (g/100g)	Peroxisomal beta-oxidation (nM/min/mg protein))
0	0.04±0.02	5.14±0.27	17.9±5.7	0.07±0.06	5.52±0.24	17.3±4.1	0.02±0.03	5.14±0.31	18.2±4.3
0.3	10±1.4	6.12±0.25	34.4±7.4*	13±2.4	6.45±0.23*	42.0±10.8*	14±3.5	6.12±0.34*	24.2±3.7
1	27±5.0	7.92±0.49	48.8±8.9*	32±5.2	8.33±0.31*	49.6±11.4*	34±10	7.85±0.85*	36.9±10.2*
3	66±8.6	10.72±0.63*	47.1±7.1*	69±10	11.67±1.06*	48.5±10.0*	82±10	11.94±0.83*	44.5±8.5*
10	190±29	16.27±1.05*	31.6±7.4*	225±68	17.18±1.13*	37.2±9.0*	172±29	17.71±1.81*	42.5±4.4*
30	241±28	18.28±1.57*	30.7±8.2*	259±34	17.96±1.06*	31.9±4.5*	244±50	21.01±1.40*	35.4±9.7*

*p<0.05.

- LOAEL for increased relative liver weight: 0.3 mg/kg/day; serum PFOA 13 µg/ml, linear, 14 µg/ml branched.
- No NOAEL identified.
- Beta-oxidation similar in all 3 forms. Did not continue to increase with dose, although liver weight increase continued to increase with dose. Maximum beta-oxidation increase less than 3-fold.

Reference and Study Design	Results	Comment
Loveless et al. (2006). (continued)	**Other Endpoints** *Mortality/clinical signs:* One mouse in 30 mg/kg/day linear group died suddenly of unknown cause. The incidence of enophthalmos and lethargy was significantly increased in 30 mg/kg/day linear group. *Body weight/body weight gain*: Data for body weight on day 0, 7, and 13, and for body weight gain on day 13; shown in tabular form. Data were also analyzed on the basis of subtraction of liver weight, to account for liver weight gain. In summary, there was a dose-related decrease in body weight gain and/or loss of body weight (adjusted for liver weight) in all 3 forms. Linear/branched and linear affected body weight gain more than branched. *Hematology:* Data not shown. Treatment related increases in acanthocytes in linear/branched and linear at 3, 10, and 30 mg/kg/day. Treatment related poiliocytes and schistocytes in linear/branched at 30 mg/kg/day, and linear at 3,10, and 30 mg/kg/day. Dose-related increases, but not present at high numbers. *Serum lipids:* Data shown in tabular form. Cholesterol and HDL decreased at all doses in all treatment groups. Cholesterol significant ($p<0.05$) at 3 and 30 mg/kg/day (except branched) for cholesterol. HDL significant at 3, 10, and 30 mg/kg/day for all treatment groups. Non-HDL generally not affected. Triglycerides significantly increased in linear /branched at 0.3, 1, 3, and 10 mg/kg/day.	

Reference and Study Design	Results	Comment
Perkins et al. (2004). 13-week dietary toxicity study of ammonium perfluorooctanoate (APFO) in male rats. **Species and strain:** Males CR:CD BR rats, approximately 41 days old. **Test article and vehicle:** Ammonium perfluorooctanoate (98% pure) in feed **Route of exposure:** Dietary **Exposure levels:** 0, 0 (pair fed with 100 ppm), 1, 10, 30, 100 ppm. Mean doses: 0, 0.06, 0.64, 1.94, 6.50 mg/kg/day. (Doses in mg/kg/day are provided for each time point). **Exposure regimen:** 4, 7, and 13 weeks exposure, and 8 weeks post-exposure. **Group size:** For each dose group; 15 per group for 4, 7, and 13 week exposure; 10 per group for 8 week recovery (week 22) sacrifice.	**LIVER** *Liver weight relative to body weight (g/100 g) and brain weight (g/g) and PFOA dose (mg/kg/day) & serum levels at weeks 4, 7, and 13*	NOAEL for increased relative liver weight – 1 ppm (0.07 mg/kg/day; 6.51 µg/ml in serum; Week 4). LOAEL for increased relative liver weight – 10 ppm (0.71 mg/kg/day; 55 µg/ml in serum; Week 4). Serum levels increased with dose, but did not increase over time. Steady-state was apparently reached by Week 4.

Diet Conc. (ppm)	Mean Dose (mg/kg/day)	Week 4				Week 7				Week 13			
		Dose (mg/kg/day)	PFOA serum level (µg/ml)	Relative to body weight (g/100 g)	Relative to brain weight (g/g)	Dose (mg/kg/day)	PFOA serum level (µg/ml)	Relative to body weight (g/100 g)	Relative to brain weight (g/g)	Dose (mg/kg/day)	PFOA serum level (µg/ml)	Relative to body weight (g/100 g)	Relative to brain weight (g/g)
0	-	--	ND	3.97±0.37	7.88±0.94	--	ND	3.75±0.29	8.50±1.07	--	ND	3.53±0.28	9.03±1.20
0-pair fed**		---	ND	4.07±0.27	7.87±0.70	--	ND	3.76±0.37	8.12±1.14	--	ND	3.24±0.23	7.64±0.77
1	0.06	0.07	6.51±1.0	3.73±0.23	7.63±0.92	0.06	7.5±1.3	3.64±0.33	8.53±1.29	0.05	7.1±1.2	3.24±0.30	8.19±1.56
10	0.64	0.71	55±8.1	4.49±0.32*	8.77±1.13	0.60	46±16	4.12±0.37	9.33±1.10	0.47	41±13	3.69±0.32	9.41±1.33
30	1.94	2.14	104±14	5.77±0.60*	11.3±1.35*	1.85	87±28	5.14±0.53*	11.4±1.58*	1.44	70±16	4.21±0.56*	10.8±1.96
100	6.50	7.39	159±30	6.73±0.49*	12.5±1.10*	6.21	149±35	6.06±0.72*	13.3±1.52*	4.97	138±34	5.50±0.84*	12.6±2.88*

Liver weight data: n=15 per data point. Serum data: n=8-10 per data point.
*Increased compared to ad libitum controls at p<0.05 using two-tailed test. ND: <0.7 µg/ml.
**Pair fed based on 100 ppm group.

- No effect on relative liver weight in recovery group sacrificed at Week 21. PFOA serum levels in recovery groups were 1.2 – 2.5 µg/ml.
- NOAEL for increased liver relative liver weight – 1 ppm (0.07 mg/kg/day; 6.51 µg/ml in serum (Week 4)).
- LOAEL for increased relative liver weight – 10 ppm (0.71 mg/kg/day; 55 µg/ml in serum (Week 4).
- Serum levels increased with dose, but did not increase over time. Steady-state serum level was reached by Week 4.
- Relative liver weight increases were similar at all 3 time points.

Liver histopathology
Data shown in tabular form. Minimal to slight hepatocellular hypertrophy in 10, 30, and 100 ppm groups at 4, 7, and 13 weeks. Severity increased with dose, but not with duration of exposure.
It is stated that the histopathology of the livers in the recovery group (sacrificed at week 21 after 8 weeks of recovery) was similar to controls. The data are not shown.

Palimitoyl CoA oxidase activity
Discussed in detail in Mode of Action section.

MORTALITY, CLINICAL OBSERVATIONS, BODY WEIGHT
Only mortality was one 100 ppm rat sacrificed during week 4 due to severe neck sores.
No clinical signs observed.
Body weight data shown in tabular form. Body weight affected only at 100 ppm (high dose). At 100 ppm, body weight decreased (p<0.05) compared to ad libitum controls at all time points (weeks 1,4,7,10,13). Body weight gain during exposure period and recovery period decreased (p<0.05) at 100 ppm.

Reference and Study Design	Results	Comment
Perkins et al. (2004). (continued)	**<u>OTHER ORGANS</u>** <u>*Organ weight and histopathology*</u> It is stated that organ weight, organ to body weight, organ to brain weight were assessed for brain, lungs, testes, and accessory sex organs, and that these organs were examined microscopically. Data for organ weights is not shown or discussed. It is stated that there was an increase in testis to body weight ratio in the 100 ppm recovery group that was not statistically significant. It is assumed that no effects were seen on these parameters for other organs or other treatment groups, although this is not stated. It is stated that histopathology of tissues from treated groups (other than liver) did not differ from controls. **<u>SERUM HORMONE LEVELS (ESTRADIOL, LUTEINIZING HORMONE, TESTOSTERONE)</u>** Assessed at weeks, 4, 7, 13, and 21 (recovery). Data shown in tabular form. No statistically significant effects. For estradiol, most results were below detection level. More detectable levels in treated groups (and elevated levels at some timepoints) suggest that PFOA increased estradiol.	

Reference and Study Design	Results	Comment
Six month oral capsule study in Cynomolgus monkeys. ***Citations*: Thomford (2001); Butenhoff et al. (2002); Butenhoff et al. (2004)** **Species and strain:** Male Cynomolgus monkeys, estimated age 3 – 9 years, mean 6 years. **Test article and vehicle:** Ammonium perfluorooctanoate (95.2% pure; impurities included perfluorhexanoate, 0.73%; perfluoroheptanoate, 3.76%). Vehicle was gelatin capsule. **Route of exposure:** Oral in capsule. **Exposure levels:** 0, 3, 10, and 30/20 mg/kg/day (30 mg/kg/day suspended on Day 12, resumed at 20 mg/kg/day on Day 22 due to toxicity at higher level). **Exposure regimen:** Daily for at least 26 weeks. Recovery animals observed for 13 weeks following 26 weeks dosing **Group size:** Control – 4 plus 2 recovery. 3 mg/kg/day – 4 10 mg/kg/day – 4 plus 2 recovery 30/20 mg/kg/day - 6 (*Note: some animals did not complete the study.)*	***Note:* Results presented here focus primarily on hepatic effects, since this endpoint has been the focus of other evaluations and risk assessments based on this study. A summary of the entire study is presented in the text.** **Animals sacrificed or removed from study before end of dosing period** • *Low dose (3 mg/kg/day) group*: In this group, 3 monkeys were dosed for the full 26 week study period. A monkey in this group (which entered the study on Day 17 to replace another monkey which died of an infection) was sacrificed in moribund condition on day 137,, having lost 10% of its body weight in one week and showing other signs of toxicity, and this mortality was possibly treatment-related. The relative liver weight of this monkey (2.37%) was much higher than for the others in this dosing group, and was comparable to those in the high dose (30/20 mg/kg/day) group. • *High dose group (30/20 mg/kg/day)*: In this group, 2 monkeys were dosed for the full 26 week study period. One monkey in this group was sacrificed in moribund condition on Day 29, having lost 12.5% of its body weight and showing other signs of toxicity, and this death was considered likely to be treatment-related. Dosing was stopped for 3 other monkeys on Days 43, 66, and 81 due to dramatic weight loss, and low or no food consumption and feces. **Body weight** • Similar in the control, 3, and 10 mg/kg/day groups (19-20% during 26 wk study period). • In high dose (30/20 mg/kg/day) group, one of 2 monkeys that completed the study had weight gain similar to controls (18%) and the other lost 5% of body weight. **Relative liver weight, PFOA serum concentrations, and hepatic palmitoyl CoA** Dose (mg/kg/day) | n | Serum PFOA (µg/ml)[a] | Relative Liver Weight: % of body weight[c] | Relative Liver Weight: Comparison to control | Palmitoyl Co-A: µmol/min/ g liver[e] | Palmitoyl Co-A: Comparison to control 0 | 4 | 0.16±0.15 | 1.53±0.08 | 1 | 0.53±0.12 | 1 3 | 3 (4)[d] | 72±47[b] | 1.82±0.05* (1.96)[d] | 1.19 (1.28)[d] | 0.47±0.13 | 0.89 10 | 4 | 85±20 | 1.87±0.06* | 1.23 | 0.90±0.29 | 1.70 20/30 | 2 | 155±102 | 2.41±0.05* | 1.58 | 1.36±1.36 | 2.60* *p<0.05 compared to control. Data comes from tables in the three publications cited. [a]From Butenhoff et al. (2004). Data from weeks 20, 22, 24, and 26. [b]3 mg/kg/day data includes one measurement from monkey removed from study in moribund condition on Day 137. [c]From Thomford (2001). Same data at 3 and 10 mg/kg/day is not stated to be statistically significant in Butenhoff et al. (2002). [d]Includes data from monkey sacrificed in moribund condition on Day 137. Relative liver weight of this monkey was 2.37 % of body weight. [e]From Butenhoff et al. (2002).	The results of this study are of limited use in risk assessment because of the small number of animals per group, the heterogeneity of the monkeys (e.g. widely different ages, potential for having been exposed to other chemicals in previous studies, etc.), possible treatment-related mortality in the low dose group, and because internal dose of PFOA (levels in serum and liver) did not differ in the low and mid-dose groups. Only two high dose monkeys completed the study, while 4 were sacrificed and/or removed due to toxicity. The 2 high dose animals that completed the study appeared to react very differently to PFOA. One monkey gained weight similarly to those in the control and lower dose groups, and its liver PFOA concentration was also similar to the low dose groups. The other monkey lost weight during the study and its PFOA liver concentration was about 4-fold higher than that of any other high dose monkey completing the study. Because of the potential mortality at the low dose, and the fact that the serum and liver PFOA levels did not differ the low and mid doses, the results of this study

Reference and Study Design	Results	Comment
Six month oral capsule study in Cynomolgus monkeys. (continued). ***Citations*: Thomford (2001); Butenhoff et al. (2002); Butenhoff et al. (2004)**	**<u>Pathological observations</u>** Discussed in text. In the 30/20 mg/kg/day monkey sacrificed in moribund condition, had liver lesions including hepatocellular degeneration and necrosis and other changes indicative of liver regeneration. The liver of the 3 mg/kg/day monkey sacrificed in moribund condition appeared normal, although this monkey had a highly elevated liver to body weight ratio. No other notable pathological findings were reported. **<u>Hepatic and non-hepatic clinical endpoints</u>** • Data for liver enzymes, serum lipid, bilirubin, glucose, thyroid hormones, and other hormones is shown in tabular form for before dosing began and weeks 5, 10, 14, and 27. Comparisons are made with baseline values and with controls at same timepoint. For comparisons with baseline values, data from monkeys remaining in the study are compared with data from all monkeys who began the study. • *3 and 10 mg/kg/day*: No effects on clinical parameters including hematology, clinical chemistry, and urinalysis except in 3 mg/kg/day monkey sacrificed moribund on Day 137. This monkey had increased plasma fibrinogen, and decreased lymphocytes, albumin, and cholesterol. • *30/20 mg/kg/day*: The 2 monkeys that completed the study had increased triglycerides, and decreased neutrophils, total protein, and albumin. The 3 monkeys removed from the study due to toxicity also had decreased neutrophils and albumin. Liver enzymes were extremely elevated (10-30 fold) in the monkey sacrificed moribund on Day 29 and the monkey removed from the study due to toxicity on Day 66. • Levels of several thyroid hormones appeared to be decreased in the two higher dose groups. **<u>Biochemical markers for subcellular fractions in liver</u>** • The data are shown in tabular form. • Peroxisomal oxidation (palmitoyl CoA oxidation) was increased in a dose dependent manner (shown in table above0. • Activity of succinate dehydrogenase, a mitochondrial enzyme, was increased in treated groups. • There was no effect on acid phosphatase, a lysosomal marker, or glucose-6-phosphatase, a marker of endoplasmic reticulum. **<u>Liver PFOA concentrations</u>** Shown in table and discussed in text. Concentrations were within a similar range (6.3 -18.8 ug/g) and did not increase with dose in the monkeys that completed the study and the 3 mg/kg/day monkey sacrificed in moribund condition, with the exception of one monkey in the 30/20 mg/kg/group (83.3 ug/g). The monkey in this group sacrificed in moribund condition in the high dose group had a very high concentration (154 ug/g).	are not informative as to the NOAEL for PFOA in this species of monkey. An additional major uncertainty is lack of information from female monkeys.

Appendix 5B. Studies that evaluated mammary gland development in mice

Notes:

- **Tables include 9 publications on this endpoint. The tables are arranged in two groups:**

  Appendix 5B-1: Studies of exposure to PFOA during the perinatal period (pregnant dams, and offspring during gestation and/or nursing) – 6 publications.

  Appendix 5B-2: Studies of exposure to PFOA during the peripubertal period – 3 publications. .

- **Some of the publications include several separate studies.**

- **Many of the studies evaluated endpoints in addition to mammary gland development. Data on all endpoints are summarized in the tables.**

Appendix 5B-1: Publications/studies of mammary gland development after perinatal (gestational and/or lactational) exposure to PFOA in mice

<table>
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Albrecht et al. (2013). A species difference in the peroxisome proliferator-activated receptor α-dependent response to the developmental effects of perfluorooctanoic acid.

Species and strain:
Sv/129 (obtained from NIH) wild type (WT), PPAR-alpha null (KO), and PPAR-alpha humanized (H) pregnant female mice and their offspring.

Test article and vehicle:
PFOA (provided by DuPont Haskell Laboratories, purity not specified)

Route of exposure:
Oral gavage

Exposure levels: 0 or 3 mg/kg/day. Preliminary studies reported in methods section used 0.6 and 1.0 mg/kg/day.

Exposure regimen:
GD 1-17.

Group size:
5-8 per strain per dose group

Timepoint for mammary gland (MG) evaluation:
Offspring on PND 20.

Related studies:
Comparisons with Abbott et al. (2007)

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MAMMARY GLAND DEVELOPMENT

Whole mounts of MG from female pups on PND 20 were assessed. There were no significant differences in number of terminal end buds (TEB) or ductal length in control versus PFOA treated mice in any of the 3 strains.

Effect of Prenatal PFOA Exposure on Mammary Gland Development in PND20 Female Offspring

Genotype	Treatment	TEB/gland	Ductal length (mm)	*N*
Wild-type (WT)	Control	2.1±0.1	2.4±0.3	3
	PFOA	2.2±0.2	2.4±0.4	4
*Ppar*α-null (KO)	Control	2.1±0.2	2.5±0.1	9
	PFOA	2.6±0.6	1.7±0.4	3
*hPPAR*α (H)	Control	2.1±0.3	2.7±0.3	4
	PFOA	1.5±0.3	2.6±0.2	5

Note. Values represent the litter mean ± SEM.

OTHER ENDPOINTS

Effect of PFOA on maternal and fetal reproductive outcomes on GD 18

● Tabular data. N = 5-8 dams or litters per group. Significance evaluated at p<0.05 level
● No effect on maternal weight gain, gravid uterus weight, # of implantations per dam, # of resorptions per litter in any of the 3 strains of mice
● No effect on number of fetuses per litter, fetus length or body weight, or number of live or dead fetuses per litter in any strain.

Effect of PFOA on maternal liver on GD18

● Tabular data. Absolute and relative maternal liver weight increased significantly and to a similar degree in all 3 strains. (photomicrographs and discussion in text; N = 5-8 dams per group. Significance evaluated at p<0.05 level)
● Hepatocellular hypertrophy in all 3 strains, with some differences in morphological features among the 3 strains. (n not provided)
● Gene expression in maternal liver (Data presented in bar graphs. n not provided; significance at p<0.05):
Acox1 (PPAR alpha target gene) increased in WT, not KO or H
Cyp4a10 (PPAR alpha target gene) increased in WT and H, but not KO
Cyp2b10 (CAR target gene) and Cyp3a11 (PXR target gene) increased in all 3 strains.

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● The authors conclude that the study confirms the PPAR alpha dependent postnatal lethality of PFOA previously reported by Abbott et al. (2007). They conclude that this effect occurs only in WT mice, but not in PPAR alpha null (KO) or humanized PPAR alpha (H) mice, and that this suggests a strain difference in developmental toxicity of PFOA.

● They also conclude that PFOA did not affect MG development in WT, KO, or H mice at the single dose tested. However, there are several issues with the data and presentation of this study that are problematic and that limit the consideration of its results.

- The study used only one dose of PFOA, and it is unclear whether that dose was sufficient to cause developmental toxicity in the WT strain. For this reason, the basis for the conclusion that WT, but not H, mice are sensitive to developmental effects of PFOA is uncertain.

- It is stated in the text that elevated PFOA levels (up to > 1000 ng/ml) were found in liver and serum from some control fetuses, pups, and dams. However, no further information as to which groups of animals these samples came from, how many samples were elevated, or statistics for serum levels in the control samples is provided. Data from control animals with elevated PFOA exposures were not excluded from the comparisons of endpoints of toxicity in control and treated groups. Including the data from these control animals could have affected the results of the study, especially since serum levels in some of the treated groups were only a few fold higher than those in some of the controls.

- The serum PFOA data for WT dams on PND 20 appear to be inconsistent within the paper. Maternal serum levels in WT dams on PND 20 are stated to range from 2066 – 6812 ng/ml. No statistical parameters (e.g. median, mean, S.D.) are provided numerically. However, the estimated serum level from the bar graph of maternal serum levels is 6700±3600 in WT (higher than what would be expected from the range provided in the text).

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</table>

Reference and Study Design	Results	Comment
Albrecht et al. (2013). (continued)	**<u>Effect of PFOA on fetal liver on GD18</u>** ● Relative liver weight increased in WT and H fetuses, but not KO (Data presented in bar graph. n not provided; p<0.05). ● Microscopic changes consistent with peroxisome proliferation in 2/5 of livers from WT fetuses. ● No effects in other strains (n not provided). Gene expression in fetal liver (Data presented in bar graphs; n not provided; significance at p<0.05): • Acox1 and Cyp4a10 (PPAR alpha target genes) increased in WT and H but not KO. • Cyp2b10 (CAR target gene) not changed in all 3 strains. • Cyp3a11 (PXR target gene) increased in H, but no effect in WT and KO. **<u>Effect of PFOA on reproductive and developmental parameters in offspring of dams allowed to deliver their litters</u>** ● N = 8-14 dams and litters per group. Significance at p<0.05 ● Day of parturition slightly but significantly increased by PFOA in H (numerical data). ● No effect on number of pups per litter at birth (numerical data). ● Percentage of male pups significantly increased by PFOA in H (numerical data). ● Day of eye opening not affected by PFOA in any strain (bar graph). ● Pup wt. gain through PND 20 not affected by PFOA in any strain (numerical data & bar graph). ● **At 3 mg/kg/day, post-natal lethality at PND 20 reported significantly increased in WT, but not KO or H (numerical data). This conclusion appears to be based on an invalid statistical comparison. See comments.** ● Text states that no post-natal lethality was seen in WT dosed with 0.6 or 1 mg/kg/day PFOA, but numerical data for this effect and serum levels at these doses are not provided. **<u>Effect of PFOA on pup liver on PND20</u>** ● Pup liver weight on PND 20 increased in WT, but not KO or H (n not provided; bar graph). ● Microscopic changes consistent with peroxisome proliferation in WT livers on PND 20 (n not stated). Equivocal evidence of centrilobular hypertrophy in 1/5 KO pup livers on PND 20. No definitive liver changes in H (n not stated). ● Gene expression in fetal liver (Data presented in bar graphs. n not provided; significance at p<0.05): • Acox1 (PPAR alpha target gene) increased in WT, not KO or H • Cyp4a10 (PPAR alpha target gene) increased in WT and to a lesser degree in KO, but not H • Cyp2b10 (CAR target gene) increaaed in all 3 strains. • Cyp3a11 (PXR target gene) increased in WT and H, but no effect in KO.	• The post-natal lethality in WT mice on PND 20 reported as statistically significant (p<0.05) appears to be based on an inappropriate statistical comparison. Number of pups per litter on PND 20 in the control and PFOA-treated groups of WT mice were compared. However, this comparison does not appear to be valid because the control and PFOA treated litters had different numbers of pups on PND 0. This parameter should be evaluated by comparing number of pups in the same litter on PND 0 and PND 20 (i.e. percent mortality within the litter between PND 0 and PND 20). In WT pups, 96% of controls and 70% of PFOA-treated survived from PND 0 to PND 20. From the analysis presented, it is unclear whether post-natal lethality was actually significantly increased by PFOA in WT pups. • The authors note that PFOA serum levels in this study are lower than in the same strain of WT mice given comparable PFOA doses in Abbott et al. (2007). For example, the WT maternal serum PFOA levels on PND 20 are comparable to those in WT dams similarly dosed with lower levels of PFOA (0.3 – 1 mg/kg/day) in Abbott et al (2007). Although both studies used SV/129 mice, this study obtained them from NIH and Abbott et al. (2007) obtained them from Jackson Laboratories. The authors suggest that pharmacokinetic differences in the mice used may explain the differences in effects of PFOA in WT mice in the two studies. However, a close review of the data from the two studies indicates that Albrecht et al. (2013) did not observe developmental effects of PFOA in WT pups that were seen at similar or lower PFOA levels in WT mice by Abbott et al. (2007). Abbott et al. (2007) reported significantly increased post-natal lethality in WT pups exposed gestationally to 0.6 and 1 mg/kg/day PFOA. In both of these dose groups, pup survival to PND 22 was 43%. WT pup serum levels on PND 22 in the 0.6 and 1 mg/kg/day dose groups in Abbott et al. (2007) were 3810 and 9860 ng/ml (respectively). PFOA serum level in treated WT pups on PND 22

Reference and Study Design	Results	Comment
Albrecht et al. (2013). (continued)	**PFOA LEVELS IN SERUM AND LIVERS FROM DAMS, FETUSES, AND PUPS** ● Dams – n=5 per strain on GD18 and PND 20. Fetuses and pups – n= 7-25 fetuses or pups from 3 – 7 litters on GD 18 and PND 20. It is not clear if this represents 7-25 fetuses/pups altogether at each time point or 7-25 pups per strain at each time point. ● Text states that PFOA levels from control dams, fetuses, and pups ranged from <5 to 1060 ng/ml in liver and from <5 to 1370 ng/ml in serum. This is described as a "small cohort" of samples, and it is stated that concentrations in control samples "typically" were ND (<5 ng/ml). However, the number/percentage of control samples with elevated levels (including how many from fetuses, pups, and dams), and data or statistics on the levels found in controls, are not provided numerically or in bar graphs. Additionally, the data on endpoints of toxicity from control animals with elevated PFOA levels were not excluded from the analysis (see comments). ● Maternal serum levels in WT dams on PND 20 are stated to range from 2066 – 6812 ng/ml. No statistics (median, mean, S.D., etc.) are provided numerically. However, the estimated serum level from the bar graph of maternal serum level s is 6700±3600 in WT (higher than what would be expected from the range provided in the text). The WT maternal serum PFOA levels on PND 20 are comparable to those in WT dams similarly dosed with lower levels of PFOA (0.3 – 1 mg/kg/day) in Abbott et al (2007). See comments. ● In dams, serum PFOA levels were lower in WT than in KO or H on GD 18, and were higher in WT than KO or H on PND 22 (shown in bar graph).). ● In pups and fetuses, PFOA serum levels were higher in H than in KO or WT on GD 18, and were lower in H than in KO or WT on PND 22. Liver PFOA levels in dams, pups, and fetuses did not necessarily follow the same relative pattern among strains as serum PFOA.	in Albrecht et al. (2013; this study) are estimated as 10,000 ng/ml (presented in bar graph), yet post-natal mortality (70% survival to PND 20) was much lower than in Abbott et al. (2007) and may not be statistically significant (see above). Similarly, eye opening was significantly delayed in the 0.6 and 1 mg/kg/day WT pups in Abbott et al. (2007) but not in the treated pups with similar and higher PFOA serum levels in Albrecht et al. (2013). ● In summary, this study is problematic for several reasons. The inclusion of data from controls with elevated PFOA exposures may have influenced the comparisons of effects in control and treated groups. Important data on serum levels is not presented or is presented inconsistently. It is unclear whether post-natal mortality actually was significantly increased in WT pups, and the delayed eye opening seen in WT pups in Abbott et al. (2007) was not observed. The differences in developmental effects in WT pups in Albrecht et al. (2013) versus Abbott et al. (2007) cannot be explained on the basis of pharmacokinetic differences in the two studies, since effects were seen at pup serum levels in Abbott et al. (2007) that are lower than the pup serum levels in Albrecht et al. (2013). For these reasons, the conclusion that developmental toxicity occurs in WT mice but not humanized PPAR-alpha mice, indicating that humans may be less sensitive to developmental toxicity of PFOA, is not clearly supported by this study. In this study, no effect on mammary gland development was reported in pups from the 3 strains on PND 20. The effects of PFOA on mammary gland development in SV/129 mice have not been evaluated in other studies. **It is possible that this strain may be less sensitive to this effect than the other strains in which it has been reported. However, the general issues with this study discussed above create uncertainty about its conclusions, including those related to mammary gland development.**

Reference and Study Design	Results	Comment
Macon et al (2011). Prenatal perfluorooctanoic acid exposure in CD-1 mice: low-dose developmental effects and internal dosimetry. **Species and strain:** Timed pregnant CD-1 mice and their offspring **Test article and vehicle:** PFOA (ammonium salt, >98% pure) in deionized water **Route of exposure:** Oral gavage ***Full-gestation exposure study*** **MG Assessment:** Female offspring **Exposure levels:** 0, 0.3, 1.0, or 3.0 mg/kg/day **Exposure regimen:** Gestational. GD 1-17. **Group size:** 13 dams per treatment group. Litters equalized to 10 pups, with equal M & F when possible. **Timepoint(s) for mammary gland (MG) evaluation:** PND 7, PND 14, PND 28, PND 42, PND 63, and PND 84	***General note:*** The litter was the unit used for statistical analysis in this study. Thus, when multiple pups from the same litter were assessed at a given time point, the individual pup data were averaged to give a single data point for that litter. **Full-gestation exposure study (GD 1-17)** ***MG developmental scores in whole mounts (1-4 scale) from female offspring after exposure on GD 1-17*** Dose (mg/kg/day) / PND 7 (n) / PND 14 (n) / PND 21 (n) / PND 28 (n) / PND 42 (n) / PND 63 (n) / PND 84 (n) Control: 3.3 ± 0.2 (5) / 3.2 ± 0.3 (4) / 3.4 ± 0.3 (3) / 3.4 ± 0.3 (4) / 3.8 ± 0.1 (4) / --- / 4.0 ± 0.0 (2) 0.3: 2.4 ± 0.4 (4) / 1.5 ± 0.2*** (6) / 1.9 ± 0.2** (5) / 2.8 ± 0.2 (6) / 2.8 ± 0.3* (5) / 2.4 ± 0.6 (3) / 2.4 ± 0.3* (3) 1.0: 2.2 ± 0.3* (5) / 1.5 ± 0.1*** (6) / 1.3 ± 0.1*** (5) / 2.3 ± 0.3** (4) / 2.0 ± 0.3*** (6) / 2.9 ± 0.4 (5) / 2.2 ± 0.2** (4) 3.0: 1.6 ± 0.2** (4) / 1.7 ± 0.3** (4) / 1.6 ± 0.4** (3) / 1.8 ± .2*** (6) / 2.8 ± 0.2 (4) / 1.8 ± 0.5 (2) / 2.9 ± 0.4 (2) [a] Significance at PND 63 could not be determined due to absence of controls at this time point (because of low # of female pups in control group). * p≤0.05, ** p≤0.01, *** p≤0.001 as compared to controls. ● MG development scores were decreased in all treated groups compared to controls at all time points. ● MG development score decreases were significant (p< 0.05, p < 0.01, or p < 0.001) at 4 or 5 of the 7 time points at each dose. ● The authors state that the mammary glands from PFOA treated pups displayed histological characteristics of delayed epithelial growth, and that histological observations at PND 63 and PND 84 are indicative of substantially delayed MG development, although comparison to controls could not be made on PND 63. Terminal end buds persisted at PND 63 and 84 (9 and 12 weeks), while normally they differentiate into terminal structures starting at PND 63 and disappear by PND 84. At PND 84, epithelial branching did not fill the fat pads as completely as in the controls. The decreases were statistically significant at all doses on PND 14 and 21, at 1 and 3 mg/kg/day on PND 28, at 0.3 and 1 mg/kg/day on PND 42, and at 0.3 and 2.2 mg/kg/day on PND 84, while other decreases were not significantly significant, possibly due to the small numbers (n=2-6) for each data point. ● The LOAEL for delayed MG development was 0.3 mg/kg/day, and a NOAEL was not identified. ***Other endpoints (full gestation exposure study)*** ● *Body weight* - No effect in M or F pups (with or without liver weight subtracted) at any time point. ● *Relative liver weight* – Increased (p< 0.05, p < 0.01, or p < 0.001) at all doses in M & F pups on PND 7, in 1 mg/kg/day F pups on PND 14, and in 3 mg/kg/day M & F pups on PND 14, PND 21, and PND 28. ● *Brain weight* – No effect on relative brain weight. Absolute brain weight decreased in 1 and 3 mg/kg/day M pups on PND 63 only (p<0.05)	In the full gestation exposure study (exposure on GD 1-7 to 0.3, 1.0, and 3 mg/kg/day), delayed MG development, as assessed by developmental score, occurred in female offspring the absence of effects on body weight. The LOAEL was 0.3 mg/kg/day and no NOAEL was identified. Delays were evident, and were statistically significant (with the exception of the 3 mg/kg/day dose group with n of only 2), at the end of the study on PND 84. In the late gestation exposure study (exposure on GD 10-17 to 0.01, 0.1, and 1 mg/kg/day), individual parameters of MG development were evaluated in addition to the overall developmental score used in the full gestation study and earlier studies from this laboratory (White et al., 2007, 2009, 2011). MG development, based on numerous endpoints, was assessed in female pups on PND 1, 4, 7, 14, and 21. The authors state that the mammary glands from the treated mice displayed aberrant morphology, with delays most evident on PND 21. Only PND 21 data are shown in tabular form and some PND 14 data are presented in the text, while data from other time points are not shown. On PND 21, the overall developmental score was significantly decreased in all dose groups compared to controls in a dose-related fashion. Other MG parameters were also decreased on

Reference and Study Design	Results	Comment

Macon et al. (2011) (cont.)

Late-gestation exposure study

MG Assessment: Female offspring

Exposure levels: 0, 0.01, 0.1, or 1.0 mg/kg/day

Exposure regimen:
Gestational.
GD 10-17.

Group size:
Block 1: 5 dams per treatment group.
Block 2: 8 dams per treatment group.
Pups pooled on PND 1 and redistributed to dams within treatment group. n=7-9 pups per dam, including 4-7 female pups per dam.

Timepoint(s) for mammary gland (MG) evaluation:
Offspring – PND 1, PND 4, PND 7, PND 14, PND 21.

Related studies: Post et al. (2012)

Late-gestation exposure (GD 10-17) study

MG development parameters in whole mounts from female offspring at PND 21 after exposure on GD 10-17

Dose (mg/kg/day)	*Developmental score (1-4) (n)*	*Longitudinal growth (µm) (n)*	*Lateral growth (µm)[a] (n)*	*Δ Longitudinal growth (µm)[a] (n)*	*Δ Lateral growth (µm) (n)*	*# Terminal end buds(n)*	*# Terminal ends (n)*
Control	3.3 ± 0.3 (5)	4321 ± 306 (5)	5941 ± 280 (5)	3394 ± 306 (5)	4358 ± 280 (5)	40 ± 4 (5)	81 ± 12 (5)
0.01	2.2 ± 0.2* (4)	3803 ± 386 (4)	5420 ± 326 (4)	3087 ± 386 (4)	3899 ± 326 (4)	33 ± 4 (4)	61 ± 8 (4)
0.1	1.8 ± 0.3** (3)	3615 ± 320 (3)	4822 ± 672 (3)	2370 ± 320 (3)	3035 ± 672 (3)	24 ± 4* (3)	58 ± 4 (3)
1.0	1.6 ± 0.1*** (5)	2775 ± 285** (5)	4822 ± 313 (5)	1553 ± 301** (5)	3380 ± 313 (5)	15 ± 2***(5)	47 ± 11 (5)

[a] Change in lateral or longitudinal epithelial growth based on comparison to mean values on PND 1.
Significant effects compared with controls, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

- At PND 14, longitudinal epithelial growth in the 0.1 and 1.0 mg/kg/day groups reduced compared with controls by 14.4% (p = 0.04) and 37.3% (p = 0.01), respectively, and the change in longitudinal growth from PNDs 1 to 14 was reduced by 27.4% (p = 0.005) and 56.5% (p = 0.002), respectively. Other PND 14 data not shown.
- Developmental delays were most evident at PND 21. Numerical data presented in table. MG development scores in all treated groups were significantly lower than in controls (p < 0.02; Table 1). Effects for developmental score and other endpoints (with the exception of change in lateral growth) occurred in a dose-related fashion.
- LOAEL for delayed MG development was 0.01 mg/kg/day. No NOAEL was identified.

Other endpoints (only female pups assessed) (late gestation exposure study)

- *Body weight* - No effect (with or without liver weight subtracted) at any time point.
- *Liver weight* – Absolute liver weight Increased (p<0.001) in 1 mg/mg/day group on PND 4 and PND 7. Relative liver weight increased (p< 0.05, p < 0.01, or p < 0.001) in 1 mg/kg/day group on PND 4 – PND 14. No effect in 0.01 or 0.1 mg/kg/day groups.
- *NOAEL for increased relative liver weight based on PFOA serum level* - In the 1 mg/kg/day group during the period when liver weight was increased, serum PFOA levels were 16,305 ng/ml on PND 1 and had decreased to 6084 ng/ml on PND 14. The NOAEL for increased relative liver weight was 2303 ng/ml, the highest serum level in the 0.1 mg/kg/day group, which occurred on PND 1. This is consistent with the serum level in the 1 mg/kg/day group on PND 21 (when liver weight was no longer increased), which was 2683 ng/ml.

PFOA levels in serum, liver, and brain
Numerical data are presented for serum, liver, and brain in M & F pups in the full gestational (GD 1-17) study. Data from PND 7 (earliest time point assessed) shows that serum levels were similar in M&F pups, with slightly higher values at all doses in males. Serum levels in the late gestational (GD 10-17) study are for combined M&F pups on PND 1, and for F pups at other times. Serum data for late gestational study (exposure on PND 10-17) shown below.

PND 21 in all treated groups in a dose-related fashion, but were significant only for change in longitudinal growth and number of terminal end buds in the highest dose group (possibly due to the small n (3-5) at each dose.

There were no effects on body weight, and liver weight was not affected at 0.01 or 0.1 mg/kg/day.

Serum levels were highest in the treated pups on PND 1 and had decreased to 6-12% of the PND 1 levels by PND 21. The serum level on PND 1 in the 0.01 mg/kg/day group was 285 ng/ml.

Serum levels in the dams were not measured but are expected to be similar to those in the pups. Serum levels in non-pregnant adult CD-1 mice exposed to 0.1 and 1 mg/kg/day for 8 days were 3200, and 31,000 ng/ml, from preliminary data of Das et al. (2010), and the serum level after dosing with 0.01 mg/kg/day for 8 days can be estimated at 320 ng/ml from these data. Earlier studies showed pup serum levels 2-fold or less higher than maternal serum levels at PND 1. Thus, levels in the dams at delivery would also be expected to be around 300 ng/L or less in the 0.01 mg/kg/day group.

Summary: Gestational exposure to ≥ 0.01 mg/kg/day PFOA caused dose-related delays in MG development in female offspring. MG development was delayed by doses that did not

Reference and Study Design	Results	Comment
Macon et al. (2011) (cont.)	***PFOA serum levels on PND 1-21 in offspring exposed on GD 10-17 (late gestation exposure study)***	affect body weight or liver weight. The LOAEL for delayed MG development in the pups is a serum level of 285 ng/ml (measured) and is also estimated as about 300 ng/ml or lower in the dams, and no NOAEL was identified. This LOAEL is based on the serum level on PND 1, and the actual LOAEL may be lower, since effects occurred at later time points when serum levels had decreased. These findings indicate that delayed mammary gland development is a more sensitive endpoint for PFOA's effects in female mouse pups than decreased body weight or increased liver weight.

Serum (ng/ml)	PND 1 (n)	PND 4 (n)	PND 7 (n)	PND 14 (n)	PND 21 (n)
Control	22.6 ± 5.5 (4)	8.6 ± 0.5 (2)	7.8 ± 2.1 (5)	7.8 ± 1.5 (8)	4.1 ± 0.6 (7)
0.01 mg/kg	284.5 ± 21.0 (3)	184.1 ± 12.1 (2)	150.7 ± 20.9 (7)	80.2 ± 13.9 (8)	16.5 ± 2.1 (10)
0.1 mg/kg	2303.5 ± 114.1 (2)	-	1277.8 ± 122.6 (8)	645.4 ± 114.2 (7)	131.7 ± 24.5 (7)
1.0 mg/kg	16305.5 ± 873.5 (7)	-	11880.3 ± 1447.6 (11)	6083.7 ± 662.6 (11)	2025.1 ± 281.9 (11)

- Serum levels increased with dose and generally decreased over time.

Reference and Study Design	Results	Comment
Tucker et al. (2015). The mammary gland is a sensitive pubertal target in CD-1 and C57Bl/6 mice following perinatal perfluorooctanoic acid (PFOA) exposure. **Species and strain:** Timed pregnant CD-1 and C57Bl/6 mice and their offspring **MG Assessment:** Female offspring **Test article and vehicle:** PFOA (ammonium salt, >98% pure) in deionized water **Route of exposure:** Oral gavage **Exposure levels:** 0, 0.01, 0.1, 0.3, or 1.0 mg/kg/day **Exposure regimen:** Gestational. GD 1-17. **Group size:** CD-1: 3 blocks of pregnant dams (n=97, 40, and 26) C57Bl/6: 41 pregnant dams **Timepoint(s) for mammary gland (MG) evaluation:** CD-1: PND 21, PND 35, PND 56 C57Bl/6: PND 21 and PND 61	**MG DEVELOPMENT** **MG development scores (1-4) in whole mounts from CD-1 and C57Bl/6 female offspring exposed on GD 1-17** Control (*n*) / 0.01 mg/kg (*n*) / 0.1 mg/kg (*n*) / 0.3 mg/kg (*n*) / 1.0 mg/kg (*n*) CD-1 PND 21: 2.9 ± 0.1 (19) / 2.4 ± 0.1 (22) / 2.3 ± 0.1 (22)** / 2.0 ± 0.1 (21)*** / 1.7 ± 0.1 (21)**** PND 35: 3.1 ± 0.1 (16) / 2.3 ± 0.2 (17)** / 2.2 ± 0.2 (14)** / 2.3 ± 0.1 (16)** / 1.9 ± 0.2 (14)**** PND 56: 3.3 ± 0.1 (9) / 2.3 ± 0.2 (13)** / 2.5 ± 0.2 (8)* / 2.2 ± 0.1 (10)** / 1.9 ± 0.2 (9)**** C57Bl/6 PND 21: 2.9 ± 0.2 (7) / 2.5 ± 0.4 (5) / 2.1 ± 0.7 (2) / 1.8 ± 0.3 (6)* / 1.8 ± 0.2 (5)* PND 61: 2.8 ± 0.2 (10) / 2.2 ± 0.2 (5) / 2.6 ± 0.1 (3) / 2.1 ± 0.1 (10)* / 1.7 ± 0.1 (8)*** *p≤0.05; **p≤0.01, ***p≤0.001, ****p≤0.0001. Means represent averages of individual pup scores. ● Authors report that delayed MG development was apparent at lowest dose (0.01 mg/kg/day) in both strains. ● MG development scores were lower than controls at all doses, including lowest (0.01 mg/kg/day) and timepoints in both strains. ● Lack of significance in C57Bl/6 at lower doses may be due to lower PFOA serum levels and smaller n, as compared to CD-1. ● Specific observations indicating delayed MG development at different time points in each strain are described in the text. ● Delayed MG development persisted through early adulthood (end of study) in both strains. **OTHER ENDPOINTS** Body weight: Assessed at same time points as MG development. No effect on absolute body weight. Body weight with liver weight subtracted decreased (p<0.05) only in 1 mg/kg/day CD-1 on PND 21 and PND 35, but not PND 56. Liver weight: Assessed at same time points as MG development. No effects on absolute liver weight. Relative liver weight increased (p<0.05) on PND 21 only in 1 mg/kg/day CD-1. Pubertal events: PFOA treatment had no effect on day of vaginal opening, body weight at vaginal opeing, or day of first estrus in either strain. Serum estradiol and progesterone: Assessed at same time points as MG development. PFOA treatment had no effect on hormone levels at any time point in either strain.	MG development was delayed on PND 21 until PND 56 or 61 in CD-1 and C57Bl/6 mice exposed to 0.01- 1 mg/kg/day PFOA on GD 1-17. No NOAEL for delayed MG development was identified in CD-1 mice. The results in CD-1 mice are consistent with delayed MG development after exposure to 0.01 mg/kg/day and higher doses on GD 10-17 (Macon et al., 2011). Lack of significant effects at lower doses in C57Bl6 mice as compared to CD-1 mice may be due the small number of mice per dose group and/or lower serum levels (faster excretion) in C57Bl/6. Serum PFOA levels were first measured on PND 21. After gestational exposure, PFOA serum levels in CD-1 mice were highest on PND 1 and decreased thereafter (Macon et al., 2011). Since the effects seen on PND 21 and later times could be the result of higher internal doses earlier in life, these serum data are not suitable as the basis for quantitative risk assessment. Summary: Delayed MG development in both strains occurred in the absence of effects on body weight, liver weight, timing of pubertal events, and serum estradiol and progesterone. This suggests that MG development is a more sensitive endpoint for effects of PFOA than the other endpoints assessed.

Reference and Study Design	Results	Comment
Tucker et al. (2015) (cont.)	**PFOA SERUM LEVELS (ng/ml)**	

	Control (*n*)	0.01 mg/kg (*n*)	0.1 mg/kg (*n*)	0.3 mg/kg (*n*)	1.0 mg/kg (*n*)
CD-1					
PND 21	<LOQ (6)	74.8 ± 16.9 (10)	457.3 ± 91.0 (9)	904.8 ± 131.5 (10)*	3119.0 ± 396.4 (5)**
PND 35	<LOQ (11)	14.3 ± 2.3 (12)	61.7 ± 6.5 (9)	200.9 ± 32.2 (11)*	889.8 ± 117.5 (11)**
PND 56	<LOQ (5)	< LOQ (5)	15.0 ± 5.0 (4)	46.2 ± 7.5 (6)	200.2 ± 21.1 (7)**
C57Bl/6					
PND 21	<LOQ (2)	26.1 ± 19.0 (2)	247.1 ± 11.4 (2)	891.3 ± 528.7 (3)	2141.67 ± 666.8 (2)
PND 61	<LOQ (6)	<LOQ (2)	27.7 ± 10.4 (3)*	9.3 ± 2.2 (5)	22.0 ± 7.6 (5)

- Serum PFOA levels in both strains increased with dose and decreased over time.
- Serum PFOA was below LOQ or very low (9-27 ng/L) at some doses and time points when MG development was significantly delayed.
- Serum PFOA levels were lower in C57Bl/6 than CD-1 mice on PND 21, and generally lower at later time points (PND 56 in CD-1 versus PND 61 in C57Bl/6).

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comments</th></tr>
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White et al. (2007).
Gestational PFOA exposure of mice is associated with altered mammary gland development in dams and female offspring.

Species and strain:
Timed pregnant CD-1 mice and their offspring

Test article and vehicle:
PFOA (ammonium salt, >98% pure) in deionized water

Route of exposure:
Oral gavage

Exposure levels:
0 or 5 mg/kg/day

Study 1
MG assessment:
Dams

Exposure regimen:
Gestational.
GD 1-17

Group size:
5

Timepoint(s) for mammary gland (MG) evaluation:
GD 18

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Study 1

MG development in dams on GD 18 after treatment on GD 1-17
Whole mounts were examined microscopically and scored on a scale of 1-4. Delay in 5 mg/kg/day group.

<table>
<tr><th colspan="2">MG Development Score (mean ± SE)</th></tr>
<tr><td>Control (n=4)</td><td>5 mg/kg/day (n=3)</td></tr>
<tr><td>3.9±0.1</td><td>2.0±0.6*</td></tr>
</table>
*p<0.015

Study 2

MG DEVELOPMENT (Study 2)

Lactating dams on PND 10 or PND 20 after treatment during gestation (Study 2)

MG development scores
All treated groups were dosed with 5 mg/kg/day on stated gestational days (GD). Whole mounts/H&E slides were scored on a scale of 1-4. Numerical data shown only for whole mount scores. Representative H&E slides from control and treated mice are shown.

- PND 10: MG development was delayed in all exposure groups (significant for GD 8-17 and 1-17).
- PND 20: MG in all PFOA-exposed groups resembled MG from PND 10 controls, indicating delayed development.

<table>
<tr><th colspan="5">MG Development Score in Whole Mounts (mean ± SE)</th></tr>
<tr><td></td><td>Control</td><td>GD 12-17</td><td>GD 8-17</td><td>GD 1-17</td></tr>
<tr><td>PND 10 (n=4-6)</td><td>4.0±0.0</td><td>3.7±0.1</td><td>3.2±0.2*</td><td>1.8±0.5**</td></tr>
<tr><td>PND 20 (n=3-6)</td><td>2.7±0.2</td><td>2.8±0.2</td><td>3.2±0.1</td><td>3.3±0.2</td></tr>
</table>
*p<0.05; **p<0.001

- Non-pregnant adult females dosed with PFOA for up to 17 days have no effects on MG (data not shown)

Expression of milk proteins
Gene expression profiles of four milk protein genes (beta-casein, EGF, alpha-Lac, and LactoF) in maternal mammary gland tissue on PND 10 and 20 showed that expression of genes for all 4 proteins was significantly (p<0.05 or p<0.001) ↑ or ↓ in at least one exposure group (GD 12-17, 8-17, and 1-17) on PND 10 and/or PND 20. Some of the changes did not follow a consistent pattern.

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Study 1
The authors interpreted the delayed MG development in treated dams on PND 18 (prior to parturition) as indicating that the delayed MG development in dams at later time points in Study 2 was not due to a deficiency in stimulation of lactation due to decreased suckling ability of pups.

Study 2
Lactating dams on PND 10 and 20
MG development in lactating dams
Delayed MG development in dams occurred in the absence of decreased maternal weight gain. Exposure during the second half of pregnancy was sufficient to cause this effect. MG development in non-pregnant females was not affected by similar exposures.

On PND 10, normally the peak of lactation in rodents, MG from treated mice on PND 10 resembled those normally seen earlier in lactation. On PND 20, immediately prior to weaning, MG are normally involuted, but MG from PFOA-treated mice resembled PND 10 (peak of lactation) MG from controls. This suggests a delay of up to 10 days in MG differentiation due to PFOA exposure.

Expression of milk proteins in lactating dams
The authors stated that the decrease in LactoF at PND 20, when a peak normally occurs, is consistent with the observed structural delays in mammary gland development seen at PND 20.

Decreased pup body weight at birth
The authors state that this effect was not due to general maternal toxicity, since maternal body weight and reproductive parameters were not affected.

MG development in female offspring
Delayed MG development occurred after exposure during the final 6 days of gestation. MG development was arrested between PND 10 and 20, although pups grew isometrically during this time period.

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</table>

Reference and Study Design	Results	Comments
White et al. (2007) continued ***Study 2*** **MG assessment:** Dams and female offspring **Exposure regimen:** Gestational. GD 1-17; GD 8-17; or 12-17. Controls dosed with water GD 1-17. **White et al. (2007) (continued).** **Group size:** 14-16 dams 10 offspring per litter (litters equalized on PND 1) **Timepoint(s) for mammary gland evaluation:** PND 10 - Half of dams and litters PND 20 - Half of dams and litters	LactoF expression was significantly (about 50%-80%) ↓ compared to controls in all 3 exposure duration groups on PND 20, the time point when a peak in this protein normally occurs. LactoF levels in the treated groups on PND 20 were similar to the controls on PND 10. **Female Offspring on PND 10 or PND 20 (Study 2)** Whole mounts were examined microscopically and scored on a scale of 1-4. All treated groups were prenatally exposed to 5 mg/kg/day on stated gestational days (GD). • MG development was delayed (p<0.001) in all groups at both time points. • MG development was not correlated with body weight. • Virtually no MG development occurred in treated pups between PND 10 and 20, although relative body weight gain was not decreased in treated pups during this period. • PFOA serum levels in serum and liver decreased from PND 10 to PND 20, but MG development remained arrested. *MG Development Score (mean ± SE)* Control: PND 10 (n=4-6 litters) 3.1±0.2; PND 20 (n=3-6 litters) 3.3±0.2 GD 12-17: PND 10 1.7±0.1*; PND 20 1.4±0.1* GD 8-17: PND 10 1.4±0.1*; PND 20 1.5±0.1* GD 1-17: PND 10 1.6±0.2*; PND 20 1.8±0.3* P<0.001 **OTHER REPRODUCTIVE AND DEVELOPMENTAL PARAMETERS (Study 2)** *Maternal body weight gain* – No effect. *Reproductive parameters* – No effect on number of implants, live fetuses, or % prenatal loss per live litter. *Offspring body weight* – Significantly ↓ (p<0.05; p<0.01, or p<0.001) in all treated exposure duration groups at all timepoints assessed (At birth [PND 1], PND 5, PND 10, and PND 20). **PFOA LEVELS IN DAMS (BLOOD) AND OFFSPRING (BLOOD, LIVER)** • PFOA was determined semiquantitatively in blood and quantitatively in liver (n=3-6 for each treatment duration group at each time point). • Dam blood - PND 10 and 20: ↑ with exposure duration; similar levels on PND 10 and 20; differences may have been masked by semiquantitative determination. • Offspring blood – PND 10 and 20: ↑ with exposure duration; ↓ between PND 10 and 20. • Offspring liver – PND 1, 10, and 20: ↑ with exposure duration; similar on PND 1 and 10, ↓ on PND 20. • Offspring liver:blood ratio: Levels in liver approximately 2 – 3 times higher than in blood in all treated groups at all time points.	

White et al. (2009). Effects of perfluorooctanoic acid on mouse mammary gland development and differentiation resulting from cross-foster and restricted gestational exposure

Species and strain:
Timed pregnant CD-1 mice and their offspring,

Test article and vehicle:
PFOA (ammonium salt, >98% pure; 98.9% straight chain, 1.1.% branched isomers) in deionized water

Route of exposure:
Oral gavage

Early-life effects cross-foster study
MG assessment: Dams and female offspring

Exposure levels: 0 and 5 mg/kg/day

Exposure regimen:
Gestational.
GD 8-17.
Pups were cross-fostered at birth. Four groups of pups, with exposures to 0 or 5 mg/kg/day in utero (U), during lactation (L), or both (U, L), designated as follows: 0U/0L, 5U/5L, 5U/0L, and 0U/5L.

Group size:
56 per dose level on GD 8-17.
10 pups per foster litters (M and F equal when possible).

Timepoint(s) for mammary gland (MG) evaluation:
PND 1, PND 3, PND 5, and PND 10

EARLY-LIFE EFFECTS CROSS-FOSTER STUDY

MG development (Early effects cross foster study)

Dams: Whole mounts assessed microscopically. Numerical data not shown.

- MG in treated dams nursing treated or untreated pups had profoundly altered differentiation on PND 1. The MG appeared immature, resembling normal late pregnancy MG. Deficits persisted until end of study (PND 10).
- MG in untreated dams nursing pups exposed in utero showed delayed MG morphology on PND 3-10. PFOA serum levels in these dams was about 2000 ng/ml on PND 3, presumably from exposure through maternal behavior such as grooming of pups and ingestion of waste excreted by pups.

Female offspring:

MG development scores of whole mounts from female offspring exposed on GD 1-17				
Treatment group	*PND 1*	*PND 3*	*PND 5*	*PND 10*
Control	3.3 ± 0.2	3.5 ± 0.2	3.0 ± 0.1	2.8 ± 0.2
5L	1.7 ± 0.4*	1.8 ± 0.2*	1.5 ± 0.1*	2.3 ± 0.2*
5U	1.9 ± 0.2*	1.9 ± 0.3*	2.0 ± 0.2*	1.4 ± 0.1*
5U/5L	1.5 ± 0.2*	1.7 ± 0.3*	1.1 ± 0.1*	1.5 ± 0.2*

*p<0.05. n=4 litters, 3 pups per litter, per treatment group per timepoint.
Scored on a 1-4 scale adjusted for stage of development and age.

● MG development was delayed on PND 1 (after less than 1 day of exposure in 5L group), and in the absence of effects on body weight or liver weight. See Comments for more detail.

Other Effects (Body Weight and Relative Liver Weight) (Early effects cross foster study)

Dams:
● No effect on body weight at any time point (PND 1-10).
● ↑ relative liver weight (p<0.05) at all time points (PND 1, 3, 5, 10) in treated dams (5U; 5U/5L).

Offspring:
● No effect on body weight on PND 1. ↓ body weight (p<0.05) in 5U/5L on PND 3, and in all treated groups (5U, 5L, 5U/5L) on PND 5 and PND 10.
● ↑ relative liver weight (p<0.05) PND 1 and PND 3 in pups exposed *in utero* (5U, 5U/5L) and in all treated groups (5U, 5L, 5U/5L) on PND 5 and PND 10. Relative liver weight not affected with lactation only exposure (5L group).

Offspring
MG development was delayed in female offspring after gestational and/or lactational exposure.

MG development delay in female offspring occurred at timepoints at which body weight was not decreased and/or relative liver weight was not increased, suggesting that MG development may be a more sensitive endpoint in offspring than decreased growth or increased relative liver weight.

MG development delay occurred as early as PND 1, including in PND 1 female offspring exposed only lactationally with less than 1 day of exposure and serum levels of ~2000 ng/ml.

MG development delay in female offspring persisted until PND 63 (9 weeks) after gestational and/or lactational exposure. At this time point, serum levels were <1000 ng/ml in treated groups, and as low as 350 ng/ml.

MG developmental deficits after gestational and/or lactational exposure persisted until age 18 months, a time point when PFOA had essentially been completed eliminated from the body.

Exposure on GD 15-17, or longer portions of gestation, caused MG development delay in female offspring on PND 29 and 32, and persistent changes in MG morphology at age 18 months.

White et al. (2009) (continued).

Late-life effects cross-foster study
Note: This study is an additional component of Wolf et al. (2007).

MG Assessment: Female offspring only.

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

Exposure regimen:
Gestational.
GD 1-17.
Pups were cross-fostered at birth. Seven groups of pups, with exposures to 0, 3, or 5 mg/kg/day in utero (U), during lactation (L), or both (U, L), designated as follows: Control (0U/0L), 3U/3L, 5U/5L, 3U, 5U, 3L, and 5L.

Group size:
28-48 dams per dose level on GD 1-17.
10 pups per foster litter (M and F equal when possible).

Timepoint(s) for mammary gland (MG) evaluation:
Offspring - PND 22, PND 43, PND 63, and 18 months

LATE-LIFE EFFECTS CROSS-FOSTER STUDY

MG development (Late-life effects cross foster study)

MG development scores of whole mounts from female offspring exposed on GD 1-17			
Treatment group	*PND 22 (weaning)*	*PND 42*	*PND 63*
Control (0U/0L)	3.7 ± 0.1	3.2 ± 0.2	3.1 ± 0.2
3L	3.0 ± 0.2*	2.5 ± 0.2*	2.6 ± 0.2*
5L	2.1 ± 0.2*	2.5 ± 0.1*	2.4 ± 0.2*
3U	1.8 ± 0.2*	2.2 ± 0.1*	2.6 ± 0.2*
5U	2.1 ± 0.3*	2.2 ± 0.1*	1.9 ± 0.2*
3U/3L	1.8 ± 0.2*	2.7 ± 0.1	2.6 ± 0.3*
5U/5L	1.2 ± 0.2*	1.9 ± 0.2*	1.9 ± 0.2*

*p<0.05. n=9-18 per treatment group at each time point.
Scored on a 1-4 scale adjusted for stage of development and age.

- MG development delayed until PND 63 (9 weeks of age) in all treatment groups. See Comments.

MG development at 18 months:
- MG examined both as whole mounts and as histological sections stained with H & E; n= 5-12 per group. (Could not be assessed with scoring criteria used at earlier timepoints.)
- Epithelial density appeared reduced in treated animals.
- Higher densities of darkly staining foci in glands of treated animals. Mean # foci/gland: Control, 6.9; 3 mg/kg/day groups, 34.3; 5 mg/kg/day groups, 38.6. No statistical parameters presented.
- Foci appear due to hyperplasia of ductal epithelium, infiltration of inflammatory cells into ductal regions, increased stromal density surrounding the ducts, and/or inappropriate differentiation of ductal epithelium. Some ductal inflammation observed in controls.

Other endpoints in female offspring (Late-life effects cross foster study; from Wolf et al., 2007)
- ↓ body weight and/or body weight gain on PND 22 (weaning) in all groups except 3L (not significant at p<0.05 in 3U). p<0.05 or 0.001 in other groups.
- ↓ body weight persisted to PND 85 in 5U and 5U/5L (p<0.05).
- ↓ survival until PND 22 (weaning) (65%) in 5U/5L (p<0.001).
- Delayed eye opening in 3U/3L, 5U, and 5U/5L (p<0.05).
- ↑ relative liver weight on PND 22 in all exposed groups (p<0.001).

Dams
MG development was profoundly delayed in treated dams on PND 1, and delays persisted until PND 10.

MG development was also delayed beginning on PND 3 in untreated dams with exposure through nursing of treated pups. The exposure presumably was through maternal behavior and ingestion of excreted PFOA in pups' waste.

White et al. (2009) (continued).

Restricted exposure study
Note: This study is an additional component of Wolf et al. (2007).

MG Assessment: Female offspring

Exposure levels: 0, and 5 mg/kg/day

Exposure regimen:
Gestational.
GD 7-17, GD 10-17, GD 13-17, or GD 15-17.

Group size:
12-14 dams per group.
10 pups per litter (M and F equal when possible).

Timepoint(s) for mammary gland (MG) evaluation:
PND 29, PND 32, and 18 months

Related studies:
Wolf et al. (2007)

RESTRICTED-EXPOSURE STUDY

MG development (restricted exposure study)

MG development scores of whole mounts from female offspring exposed to 0 or 5 mg/kg/day during gestation

Treatment group	*PND 29*	*PND 32*
Control	3.6 ± 0.1	3.6 ± 0.1
GD 7-17	2.1 ± 0.1*	2.2 ± 0.2*
GD 10-17	2.0 ± 0.2*	2.2 ± 0.2*
GD 13-17	2.2 ± 0.1*	2.5 ± 0.1*
GD 15-17	2.0 ± 0.2*	2.3 ± 0.3*

*p<0.05. n=10-21 per treatment group at each time point.
Scored on a 1-4 scale adjusted for stage of development and age.

- At 18 months, MG examined both as whole mounts and as histological sections stained with H & E; n= 4-11 per group. (Could not be assessed with scoring criteria used at earlier time points.)
- Higher densities of darkly staining foci in glands of treated animals. Mean # foci/gland: Control, 1.5; GD 15-17, 29.8; GD 13-17, 17.9; GD 10-17, 32.8; GD 7-17, 25.5. No statistical parameters presented.
- Foci appear due to hyperplasia of ductal epithelium, infiltration of inflammatory cells into ductal regions, increased stromal density surrounding the ducts, and/or inappropriate differentiation of ductal epithelium. Some ductal inflammation in controls.
-Peripheral localized increases in epithelial density in whole mounts from many treated animals.

Other endpoints in female offspring (restricted exposure study; from Wolf et al., 2007)
- No effect on pup survival to weaning.
- No effect on body weight at birth.
- ↓ body weight starting on PND 2 in longest exposed groups (GD 7-17, GD 10-17) and in all groups on PND 7-22 (p<0.05 or lower).
- No ↓ body weight after PND 29.
- Delayed eye opening in all groups, significant (p<0.01) in longest exposed (GD 7-17, GD 10-17
- ↑ relative liver weight on PND 22 in all exposed groups (p<0.001).

PFOA SERUM LEVELS
- Data for 5 mg/kg/day dams from early-life exposure study, and 5 mg/kg/day offspring from early-life exposure and late-life exposure studies presented graphically together for comparison. (3 mg/kg/day data not shown).
- Levels in treated dams decreased from LD (lactation day) 1-10.
- Levels in control dams nursing treated pups increased from LD 1-5.
- Levels in gestationally exposed pups were higher than in dams on PND 1.
- Levels in gestationally exposed (5U and 5U/5L) pups decreased from PND 1-63, with 5U (no lactational exposure) decreasing more quickly.
- Levels in lactation-only group (5L) offspring stated to be ~2000 ng/ml on PND 1 in text. Levels in 5L increased from PND 1-10, and then decreased from PND 22-42. Levels in 5L were similar to 5U on PND 10-22.
- Levels in all offspring groups had decreased to <1000 ng/ml at PND 63.
- Data from restricted exposure study from Wolf et al. (2007) discussed in text, not shown.

Reference and Study Design	Results	Comments
White et al. (2011). Gestational and chronic low-dose PFOA exposures and mammary gland growth and differentiation in three generations of CD-1 mice. **Species and strain:** Timed pregnant CD-1 mice and their offspring **MG assessment:** Dams and female offspring **Test article and vehicle:** PFOA (ammonium salt, >98% pure) in deionized water or drinking water **Route of exposure:** Oral gavage and/or drinking water **Exposure levels:** 0, 1, or 5 mg/kg/day and/or 5 ppb in drinking water **Exposure regimen (see study design figure in 2nd column, below):** Gestational through PND 63. 5 groups as follows: - Control - GD 1-17: 1 mg/kg/day (P0 dams) - GD 1-17: 5 mg/kg/day (P0 dams) - 5 ppb in drinking water (P0 dams on GD 7-17 and continuing for P0 dams, F1, and F2 until end of study on F2 PND 63). - 1 mg/kg/day (P0 dams, GD 1-17) + 5 ppb in drinking water (P0 dams on GD 7-17 and continuing for P0 dams, F1, and F2 until end of study on F2 PND 63).	***MG DEVELOPMENT and LACTATIONAL EFFECTS (P0 and F1 dams; F1 and F2 female offspring)*** ***MG Development Scores of Whole Mounts from Female Dams and Offspring (1-4 scale)***** *Treatment group* / *PND 10* / *PND 22* / *PND 42* / *PND 63* ***P0 dams (1 or 5 mg/kg/day, P0 GD 1-17 and/or 5 ppb in drinking water, P0 GD 7- F1 PND 22)*** Control: ---------- / 2.4 ± 0.2 / ------- / ------- 5 ppb in dw: ---------- / 3.4 ± 0.1* / ------- / ------- 1 mg/kg/day: ---------- / 3.0 ± 0.2* / ------- / ------- 1 mg/kg/day + 5 ppb in dw: ---------- / 3.2 ± 0.2* / ------- / ------- 5 mg/kg/day: ---------- / 3.9 ± 0.1* / ------- / ------- **p<0.05 compared with control; n=7-11 per group.* *Stated to be consistent with observations from H&E stained slides (data not shown)* ***F1 female offspring (1 or 5 mg/kg/day, P0 GD 1-17 and/or 5 ppb in drinking water, P0 GD 7- F1 PND 63)*** Control: ------- / 3.8 ± 0.1 / 3.8 ± 0.1 / 3.8 ± 0.2 5 ppb in dw: ------- / 2.5 ± 0.2* / 3.3 ± 0.2* / 2.6 ± 0.4* 1 mg/kg/day: ------- / 2.3 ± 0.2* / 2.6 ± 0.4* / 2.9 ± 0.2* 1 mg/kg/day + 5 ppb in dw: ------- / 2.2 ± 0.1* / 2.2 ± 0.3* / 2.0 ± 0.3*# 5 mg/kg/day: ------- / 1.6 ± 0.1* / 2.0 ± 0.3* / 2.2 ± 0.2* **p<0.05 compared with control; #p<0.05 compared with 1 mg/kg/day. n=4-10 per group.* ***F1 dams (1 or 5 mg/kg/day, P0 GD 1-17 and/or 5 ppb in drinking water, P0 GD 7- F2 PND 22)*** Control: 4.0 ± 0.1 / 2.1 ± 0.3 / ------- / ------- 5 ppb in dw: 2.8 ± 0.5* / 2.2 ± 0.2 / ------- / ------- 1 mg/kg/day: 2.5 ± 0.2* / 1.9 ± 0.4 / ------- / ------- 1 mg/kg/day + 5 ppb in dw: 2.0 ± 0.2* / 1.5 ± 0.2* / ------- / ------- 5 mg/kg/day: 2.5 ± 0.2* / 3.2 ± 0.3* / ------- / ------- **p<0.05 compared with control; n=4-10 per group. Bred to F1 males at age 7-8 weeks (PND 49-56).* *Evaluation of H&E stained slides showed compromised lactational morphology in all treated groups on PND 10. On PND 22, only 5 mg/kg/day group differed from controls & did not show normal regression at weaning.* ***F2 female offspring (1 or 5 mg/kg/day, P0 GD 1-17 and/or 5 ppb in drinking water, P0 GD 7- F2 PND 63)*** *Control: 2.8 ± 0.1 / 3.1 ± 0.4 / 3.5 ± 0.2 / 3.4 ± 0.2* *5 ppb in dw: 3.0 ± 0.2 / 1.9 ± 0.3 / 2.5 ± 0.4* / 3.5 ± 0.2* *1 mg/kg/day: 1.9 ± 0.3 / 2.3 ± 0.1 / 3.4 ± 0.2 / 2.4 ± 0.2** *1 mg/kg/day + 5 ppb in dw: 2.6 ± 0.2 / 2.3 ± 0.2 / 2.4 ± 0.2*# / 2.6 ± 0.5* *5 mg/kg/day: 2.0 ± 0.2 / 2.0 ± 0.2 / 3.3 ± 0.4 / 2.6 ± 0.4* **p<0.05 compared with control; #p<0.05 compared with 1 mg/kg/day. n=4-8 per group.* ***PFOA exposure through drinking water was 50-100 ng/day in pregnant P0 dams and lower in F1 offspring.*	- In **P0 dams**, MG development was delayed in the absence of overt maternal toxicity (decreased body weight or effects on reproductive parameters), suggesting that MG development is a more sensitive endpoint. Higher MG development scores of whole mounts in all treated **P0 dams** and 5 mg/kg/day **F1 dams** on PND 22 is indicative of delayed MG development. In these treated dams, MG at PND 22, when MG involution is normally seen at weaning, resembled normal MG on PND 10, the peak of lactation. This is consistent with observations in MG from these animals in H&E stained slides. Results are also consistent with White et al. (2007), but also occurred in this study after 34 days of exposure to 5 ppb in drinking water. - In the **F1 female pups**, MG development was significantly delayed in all dosed groups including 5 ug/L in drinking water at PND 22, 42, and 63. MG development was delayed in 5 ppb drinking water group in the absence of effects on body weight or relative liver weight at any timepoint, indicating that it is a more sensitive endpoint. The PFOA serum levels in the **F1 pups** exposed to 5 ppb in drinking water ranged from 21.3 ng/ml on PND 22 to 66.2 ng/ml on PND 63 (compared to 0.6 and 3.1 ng/ml, respectively, at these times in the controls). Thus the LOAEL for delayed mammary gland development in the F1 pups is 21.3 ng/ml (the level on PND 22), and no NOAEL was identified. - In the **F1 dams** that were bred at 7-8 weeks of age, MG development was significantly delayed in all treated groups on PND 10 and in the 1 mg/kg/day + 5 ppb drinking water group on PND 22. Maternal toxicity (effects on reproductive parameters) occurred only in the 5 mg/kg/day group. These MG development delays are consistent with the delayed MG development in the virgin F1 siblings up to PND 63, the latest time point assessed (see above).

White et al. (2011) (cont.)

Group size:
P0 dams: 7-12 per treatment group
F1 offspring: 12-13 per litter
F1 dams: 7-10 per treatment group
F2 offspring: 10 per litter

Timepoint(s) for mammary gland (MG) evaluation:

F0 dams: PND 22
F1 offspring: PND 22, PND 42, PND 63
F1 dams: PND 10, PND 22
F2 offspring: PND 10, PND 22, PND 42, PND 63

Lactational challenge in F1 dams and F2 litters on PND 10

Treatment group	*Milk produced in 30 min (g)*	*Time to initiate (sec)*
Control	*2.10 ± 0.20*	*267 ± 38*
5 ppb in dw	*1.80 ± 0.35*	*384 ± 55*
1 mg/kg/day	*2.08 ± 0.25*	*307 ± 114*
1 mg/kg/day + 5 ppb in dw	*1.40 ± 0.44*	*351 ± 86*
5 mg/kg/day	*1.73 ± 0.51*	*279 ± 30*

n=7-10 litters per group; n=10 offspring per litter.

- *Dams were separated from offspring for three hours, then returned to their litters and allowed to nurse for 30 minutes. The time between reunion and initiation to nurse and the weight of the 10-pup litter were recorded before and after precisely 30 minutes of nursing, in order to estimate the volume of milk produced during the nursing period.*
- *Although not statistically significant, milk volume was ↓ and time to initiate nursing was ↑ in all treated groups compared to controls. High variability limited power to detect statistically significant differences.*

OTHER ENDPOINTS (n=4-10 per treatment group)

Maternal body weight gain – No significant effect in P0 dams (data not shown for F1 dams).

Reproductive parameters (P0 and F1 dams)
- *No significant ↓ in number of implants or live fetuses, or ↑ prenatal loss or postnatal survival except in 5 mg/kg/day group.*
- *In 5 mg/kg/day group, ↓ in number of implants (F1) and live fetuses (P0, F1), and ↑ prenatal loss (P0) and postnatal survival (F1).*

Female offspring (F1 and F2) - body weight, body weight excluding liver weight, and relative liver weight:
F1 offspring: All parameters assessed on PND 22, PND 42, and PND 63:
- *↓ Body weight, and body weight excluding liver weight - 5 mg/kg/day (compared to control) on PND 42 ($p<0.05$).*
- *↓ Body weight in 1 mg/kg/day + 5 ppb in drinking water group compared to 1 mg/kg/day on PND 63 ($p<0.05$).*
- *↑ Relative liver weight - 1 mg/kg/day on PND 22, and 5 mg/kg/day (compared to control) on PND 22 and PND 42 ($p<0.05$).*

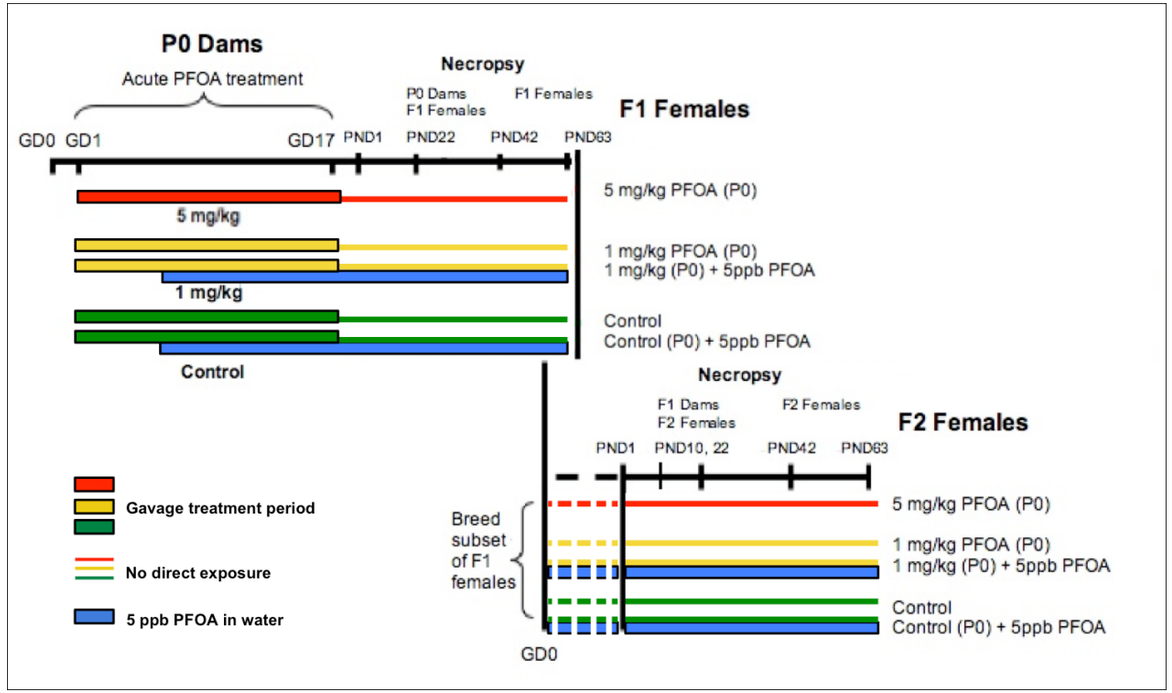
F2 offspring: Body weight assessed on PND 1, PND 3, PND 5, PND 10, PND 14, PND 17 and PND 22); Body weight excluding liver weight, and relative liver weight assessed on PND 22, PND 42, and PND 63):
- *No consistent effects on body weight. ↓ Body weight only in 5 ppb in drinking water group on PND 1 ($p<0.05$).*
- *↑ Body weight (or body weight excluding liver weight) ↑ at a few timepoints in 1 mg/kg/day (with or without 5 ppb in drinking water) groups ($p<0.05$).*
- *Relative liver weight was not affected.*

PFOA SERUM LEVELS
- *Serum levels assessed in P0 and F1 dams on PND 22; in F1 and F2 female offspring on PND 22, 42, and 63.*
- *Serum levels in dams exposed only to 5 ppb in drinking water were 75-87 ng/mL. Serum levels in F1 and F2 offspring were 21–69 ng/ml.*

- In the lactational challenge experiment with **F1 dams and F2 litters** on PND 10, milk produced in 30 minutes was decreased, and time to initiate nursing behavior was increased, in all treated groups compared to controls. However, none of the changes were statistically significant, possibly due to high variability and the small number of animals assessed. Additionally, postnatal survival and body weight were not affected in the treated F2 offspring, suggesting that the ability of the F1 dams to provide nutritional support was not decreased. However, it is not known if there were deficits in lactational function that were compensated for by increased frequency or longer duration of nursing events, since these parameters were not assessed.

- The **F2 pups** showed a trend toward delayed mammary gland development, with decreased scores in all treated groups compared to controls at all time points (PND 10, 22, 42, and 63) except for the 5 ug/L drinking water group on PND 63. However, decreases were statistically significant only on PND 42 in the 5 ppb drinking water group and the 1 mg/kg/day + 5 ppb drinking water groups, and on PND 63 in the 1 mg/kg/day group. Serum levels in F2 pups exposed to 5 ppb in drinking water were 26.6 to 68.5 ng/ml, similar to the F1 pups with the same exposure (see above). In the F2 pups which were the offspring of dams exposed during development to 1 mg/kg/day or 5 mg/kg/day, but not exposed later through drinking water, serum levels at the various time points (PND 22, 42, and 63) ranged from 0.4 ng/ml (below controls) to 7.8 ng/ml.

Summary: Chronic exposure to 5 ppb PFOA in drinking water caused delayed MG development in both P0 and F1 dams and F1 and F2 offspring. MG development was delayed by exposure to 5 ppb in drinking water at all timepoints in F1 offspring and on PND 42 in F2 offspring. Delayed MG development was a more sensitive endpoint than decreased body weight in dams and offspring, reproductive effects in dams, and increased liver weight in offspring. The LOAEL based on serum PFOA levels for delayed MG development in the F1 pups is 21.3 ng/ml (the level on PND 22). No NOAEL was identified in this study.

White et al. (2011) (cont.)	 **White et al. (2011) study design and experimental timeline:** Bar color denotes dose: green, 0 mg PFOA/kg body weight/day; yellow, 1 mg PFOA/kg body weight/day; red, 5 mg PFOA/kg body weight /day; blue, 5 ppb PFOA in drinking water. Bar thickness denotes timing of treatment: thick bars denote on-going direct treatment, thin bars denote only group identity subsequent to treatment.	

Appendix 5B-2: Publications/studies of mammary gland development after peribubertal exposure to PFOA in mice

Reference & Study Design	Results	Comment
Yang et al. (2009). Differential effects of peripubertal exposure to perfluorooctanoic acid on mammary gland development in C57Bl/6 and Balb/c mouse strains. **Species and strain:** Female Balb/C and C57Bl/6 mice **Test article and vehicle:** PFOA (ammonium salt, >98% pure) in deionized water **Route of exposure:** Oral gavage **Exposure levels:** 0, 1, 5 or 10 mg/kg/day **Exposure regimen:** Peripubertal. Starting at age 3 weeks for 4 weeks (5 days per week). **Group size:** 5 per strain per dose group **Timepoint for mammary gland (MG) evaluation:** Age 7 weeks **Related studies:** Zhao et al. (2010). Zhao et al. (2012).	**MAMMARY GLAND DEVELOPMENT** **MG development in whole mounts from female 7 week old Balb/C and C57BL/6 mice treated with PFOA for 4 weeks (*SEE ALSO SUMMARY TABLE AND GRAPH OF ALL 3 STUDIES FROM THIS LAB)*** | Treatment (mg/kg/day)	PFOA serum level (ug/ml)[a]	Ductal Length (relative units)	# Terminal End Buds (TEBs)	# Stimulated Terminal Ducts (TDs)
---	---	---	---	---
Balb/c				
0	--	7.60 ± 0.94	8.20 ± 1.92	6.20 ± 2.39
1	29 ± 4	7.28 ± 0.73	6.00 ± 3.74	3.80 ± 1.64
5	109 ± 12	3.99 ± 0.36*	3.99 ± 0.36*	1.20 ± 1.30*
10	---	3.40 ± 4.77*	0.00 ± 0.00*	0.00 ± 0.00*
C57BL/6				
0	---	4.76 ± 1.19	7.80 ± 2.39	6.60 ± 2.88
1	26 ± 6	4.06 ± 0.85	10.40 ± 3.36	9.80 ± 2.77
5	68 ± 10	4.01 ± 0.71	11.20 ± 1.30*	16.60 ± 5.90*
10	96 ± 10	0.30 ± 0.67*	0.00 ± 0.00*	0.00 ± 0.00* | [a] Not reported in this study. Reported in Zhao et al. (2012) in bar graph form. Levels estimated from bar graph. n = 5 per group. * p<0.05 compared to control. n=5 per strain per dose group. ● MG development was delayed at 1, 5, and 10 mg/kg/day, in a dose-related fashion in Balb/C strain (p<0.05 at two highest doses). ● A lower dose, 0.1 mg/kg/day, had no effect on MG development in Balb/C mice (data not shown). ● In C57BL/6 strain, MG development was stimulated at 5 mg/kg/day and totally arrested at 10 mg/kg/day. **MG epithelial cell proliferation in TEBs/stimulated TDs and ducts in 7 week old Balb/C and C57BL/6 mice treated with PFOA for 4 weeks** ● Assessed in 1 and 5 mg/kg/day groups as % Brdu positive cells after treatment with Brdu 2 hours before sacrifice. ● Data shown graphically and discussed in text. ● In Balb/C, ↓ % proliferating cells in TEBs/stimulated TDs at 1 and 5 mg/kg/day (dose-related) and ducts at 5 mg/kg/day. ● In C57BL/6, % proliferating cells in TEBs/stimulated TDs and ducts at both doses. Since # of TEBs/stimulated TDs was ↑ at 5 mg/kg/day, results indicate overall ↑ in MG epithelial cell proliferation at this dose.	After peripubertal exposure to PFOA, MG development was delayed at 1-10 mg/kg/day in Balb/C mice. In C57BL/6 mice, MG development was stimulated at 1 and 5 mg/kg/day and delayed at 10 mg/kg/day. The range of serum PFOA levels was similar in the two strains of mice within the dose ranges mentioned above. The dose-response curve for MG development in the C57Bl/6 mice was non-monotonic, and the change from a large stimulatory effect to a large inhibitory effect occurred within a relatively small range of serum PFOA levels (68 µg/ml to 96 µg/ml). . Uterine development followed a similar pattern of delay/simulation as MG development in the two strains. However, in C57BL/6, stimulation of uterine development occurred at 1 mg/kg/day but not 5 mg/kg/day. Vaginal opening was delayed at all doses in both strains. Liver weight was increased at all doses in both strains. In this study, PFOA exposure was during the peripubertal period, while exposure was prenatal and/or neonatal in White et al., 2007, 2009, 2011; Macon et al., 2011; and Tucker et al., 2015. PFOA serum levels from this study are not reported in this publication, but are reported in Zhao et al. (2012).

Reference & Study Design	Results	Comment
Yang et al. (2009). (continued)	**OTHER ENDPOINTS** Body weight: ● Numerical data provided. ● Body weight was unaffected except for decreased body weight at 10 mg/kg/day of both strains on the final 2 days of dosing (Day 19 and 20). Liver weight and histology: ● Numerical data provided for liver weight. ● In both strains, dose-related increase in absolute and relative weight. Significant ($p<0.05$) compared to control at all doses. Principal change seen in histological examination was dose-related increase in hepatocellular hypertrophy. Uterine weight and histology: ● Numerical data provided for uterine weight. ● In Balb/C, dose-related decrease in absolute and relative weight. Significant ($p<0.05$) compared to control at all doses. Histological evaluation also showed decreased uterine development. ● In C57BL/6, absolute and relative uterine weight ($p<0.05$) and histological development were increased at 1 mg/kg/day, no effect at 5 mg/kg/day, and delayed at 10 mg/kg/day. ● Effects were not due to differences between groups in stage of estrus cycle at sacrifice. Day of vaginal opening ● Numerical data provided. ● Delayed in a dose-related manner at all doses in both strains. ● In Balb/C, delayed ($p<0.05$) compared to control at 1 mg/kg/day and did not occur by Day 50 (end of study) in 5 and 10 mg/kg/day. ● In C57BL/6, delayed ($p<0.05$) compared to control at 1 and 5 mg/kg/day and did not occur by Day 50 (end of study) in 10 mg/kg/day.	

Reference & Study Design	Results	Comment

Zhao et al. (2012). Perfluorooctanoic acid effects on ovaries mediate its inhibition of peripubertal mammary gland development in Balb/c and C57Bl/6 mice

Species and strain:
Female Balb/C, C57Bl/6 Wild Type (WT), and C57Bl/6 PPAR-alpha null (KO) mice

Test article and vehicle:
PFOA (ammonium salt, >98% pure) in deionized water

Route of exposure:
Oral gavage

Exposure levels: 0 or 2.5 mg/kg/day (Balb/C)

0 or 7.5 mg/kg/day(C57BL/6 WT & KO)

PFOA exposure regimen:
Peripubertal.
Starting at age 3 weeks for 4 weeks (5 days per week)).

In study of effect of estradiol and progesterone, starting at age 3 weeks for 2 weeks. Estradiol and progesterone treatment during 2nd week of PFOA treatment.

Group size: 5-10

Timepoint for MG evaluation:
50 days (7 weeks). 356 days (5 weeks) for studies of effect of estradiol and progesterone.

MAMMARY GLAND DEVELOPMENT

MG development in whole mounts from female 7 week old Balb/C, C57BL/6 WT, and C57BL/6 PPAR-null (KO) mice treated with PFOA for 4 weeks (*SEE ALSO SUMMARY TABLE AND GRAPH OF 3 STUDIES FROM THIS LAB BELOW*)

Treatment (mg/kg/day)	PFOA serum level (ng/ml)[a]	Ductal Length (relative units)	# Terminal End Buds (TEBs)	# Stimulated Terminal Ducts (TDs)	Absolute MG Weight (g)	Relative MG Weight (%)
Balb/c						
0	--	8.17 ± 1.08	7.40 ± 1.14	6.11 ± 2.52	0.09±0.004	0.51±0.04
2.5	51,000± 8000	4.99 ± 0.67*	3.25 ± 0.95*	2.63 ± 1.41*	0.11±0.024	0.63±0.13
C57BL/6 WT						
0	--	6.30 ± 0.96	6.25 ± 2.06	6.50 ± 2.89	0.15±0.035	0.77±0.18
7.5	93,000±11,000	2.10 ± 1.98*	1.00 ± 1.41*	0.80 ± 1.31*	0.11±0.026	0.66±0.13
C57BL/6 PPAR-null (KO)						
0	--	8.90 ± 1.04	10.00 ± 4.36	6.20 ± 1.64	0.17±0.035	0.88±0.21
7.5	38,000 ± 7000	8.43 ± 1.08	9.43 ± 1.90	7.02 ± 2.16	0.14±0.026	0.78±0.11

*p<0.05 compared to control. n=5 per strain per dose group. [a] Levels estimated from bar graph. n = 5 per group.

- Doses of PFOA (2.5 mg/kg/day in Balb/C mice and 7.5 mg/kg/day in WT and KO mice) were chosen because they were lower than the LOAELs for delayed MG development in Balb/C (5 mg/kg/day) and WT (10 mg/kg/day) strains in the previous study (Yang et al., 2009). The goal was to further study the delayed MG development caused by PFOA in these strains.
- MG development was inhibited in Balb/C and WT mice, and was not affected in KO mice, by the PFOA doses used in this study..

In Balb/C mice, the PFOA serum level and the MG development delay at 2.5 mg/kg/day were intermediate to the serum PFOA levels and MG development delays at 1 mg/kg/day and 5 mg/kg/day in this strain in Yang et al. (2009).

In WT mice, the PFOA serum level (93,000±11,000 ng/ml) at 7.5 mg/kg/day in this study is very close to the PFOA serum level at 10 mg/kg/day (96,000±10,000 ng/ml) in the earlier study (Yang et al., 2009). MG development was delayed at both of these doses in these two studies. The serum level (68,000±10,000 ng/ml) at which MG development was reported to be greatly stimulated in Yang et al. (2009) is relatively close to the serum levels where MG development was delayed.

In KO mice, PFOA (7.5 mg/kg/day) did not affect MG development. However, serum PFOA levels in KO mice (38,000 ± 7000 ng/ml) were much lower than the serum level where MG was inhibited in WT mice. It is stated that in KO mice treated with 10 mg/kg/day, the serum levels were similar to those at 7.5 mg/kg/day and there was no effect on MG development (data not shown). Because the serum levels in KO mice were lower than those in WT mice where MG development was delayed, no conclusions about PPAR-alpha dependence of delayed MG development can be made from these data.

The purpose of this study was to evaluate effects on MG development from peripubertal exposure to doses lower than those that caused delayed MG development in Balb/C and C57Bl/6 WT mice in an earlier study (Yang et al., 2009). Effects in PPAR-alpha null mice were also studied.

In Balb/C mice, inhibition of MG development occurred at 2.5 mg/kg/day, lower than the LOAEL of 5 mg/kg/day identified in Yang et al.(2009).

In C57BL/6 WT mice, inhibition of MG development occurred at 7.5 mg/kg/day, lower than the LOAEL of 10 mg/kg/day identified in Yang et al. (2009). In Yang et al. (2009), 5 mg/kg/day was reported to stimulate MG development in this strain.

In C57BL/6 PPAR alpha null (KO) mice, 7.5 mg/kg/day PFOA had no effect on MG development. Lack of inhibition of MG development in C57Bl/6 KO, as compared to the other two strains, in this study, may be due to kinetic factors unrelated to PPAR status. PFOA serum levels in KO mice were below the serum levels at which MG development was delayed in the other two strains of mice.

Reference & Study Design	Results	Comment
Zhao et al. (2012). (continued) **Related studies:** Yang et al. (2009). Zhao et al. (2010).	● PFOA had no effect on absolute or relative weight of mammary glands/fat pads. • Absence of corpus lutea and uterine changes in Balb/C and WT stated to be indicative of absence of estrous cycling. **<u>MG development in whole mounts from 5 week old Balb/C and C57BL/6 WT mice after 2 weeks of PFOA treatment, with or without estradiol and/or progesterone treatment during final week</u>** ● Balb/C mice were dosed with PFOA at 2.5 mg/kg/day, and WT mice with 7.5 mg/kg/day, for 2 weeks. KO mice were not included in this component of the study. ● During the second week, 5 mice from PFOA-treated groups in each strain were either not treated with hormones, or dosed with estrogen (0.1 ug/mouse), progesterone (0.1 mg/mouse), or both, by subcutaneous injection. Data on effects of hormones in controls (no PFOA treatment) are not shown. These doses of hormones were stated to be physiological levels of these hormones. n=5 per group. Mice were sacrificed 24 hours after last dose. ● MG data are shown numerically for ductal length, number of TEBs, and absolute and relative mammary gland weight. ● In mice from both strains that were not dosed with hormones, PFOA caused delayed MG development (decreased ductal length and number of TEBs), and there was no effect on MG weight. These results are consistent with the MG gland development delays at the same administered doses in these strains of mice in the first component of study (above). Serum PFOA levels were not measured in this second component of the study. ● Hormone treatment (estradiol, progesterone, or both) prevented the delayed MG development caused by PFOA in both strains. Hormone treatment is stated to have no effect on MG development in mice not treated with PFOA (data not shown). *<u>Levels of growth factors, receptors, and cell proliferation marker in MG</u>* ● MG levels of 2 growth factors (Areg and HGF alpha), two receptors (ER alpha and EGFR), and a cell proliferation marker (PCNA) were also assessed. ● PFOA (in the absence of hormone treatment) decreased the levels of Areg, EGFR, and PCNA in both strains of mice compared to untreated control (n = 3 per group; data shown in bar graphs). Hormone treatment in mice dosed with PFOA (Balb/C and WT) increased the levels of these factors above those in untreated controls not given PFOA or hormones ($p<0.05$ for all effects). **<u>OTHER ENDPOINTS (*in 7 wk old mice after 4 wks of PFOA treatment (Balb/C - 7.5 mg/kg/day; WT & KO - 2.5 mg/kg/day))*</u>** **<u>Body weight</u>:** ● PFOA treatment caused no significant effects except decrease ($p<0.05$) in WT treated with 7.5 mg/kg/day during 4th (last) week of dosing (n=5 per group). Data shown graphically.	In Zhao et al. (2010), 5 mg/kg/day PFOA was reported to stimulate MG development similarly in KO and WT mice. The authors concluded that the stimulatory effect on MG development was PPAR alpha independent (Zhao et al., 2010). However, in Zhao et al. (2012) presented here, there was no effect on MG development in KO mice dosed with 7.5 mg/k/day at a PFOA serum level of 38 ppb (Zhao et al., 2012). This serum level is within the range of PFOA serum levels (26 to 68 ppb) at which MG development was stimulated in WT mice (Yang et al., 2009). Considering these data together, a definitive conclusion about whether the stimulation is PPAR-dependent or independent cannot be made. There are no numerical data or concurrent comparisons with WT for the single data point where stimulation in KO mice was reported. Alternatively, the dose-response for stimulation of MG development may be very different in KO mice than WT mice.

Reference & Study Design	Results	Comment
Zhao et al. (2012). (continued)	**Day of vaginal opening, presence of corpus lutea in ovary, and uterine histology:** ● Vaginal opening data presented numerically, other information presented in text. n=5 per group. • PFOA treatment delayed vaginal opening in Balb/C and WT (p<0.05), but not KO. • PFOA prevented development of corpus lutea present in Balb/C and WT, but had no effect in KO. • PFOA caused thinning of uterine myometrium and endometrium thinned Balb/C and WT; no changes in KO. • Absence of corpus lutea and uterine changes in Balb/C and WT stated to be indicative of absence of estrous cycling. **Levels of ovarian enzymes related to steroid hormone synthesis and PPAR alpha** ● PFOA treatment in both Balb/C and WT mice decreased (p<0.05) levels of 4 ovarian enzymes related to synthesis of steroid hormones (StAR, CYP11A1, HSD3beta1, HSD17beta1) and had no effect on aromatase. PFOA did not affect these enzymes in KO mice (n=3 per group). ● PFOA treatment did not affect ovarian PPAR alpha levels. Ovarian PPAR alpha was higher (p<0.05) in Balb/C (1.00 ± 0.20) than WT (0.43 ± 0.19), and was not measured in KO mice. Units not stated. **MG levels of ovarian growth factors, receptors, and cell proliferation marker in Balb/C, WT, and KO mice** ● Levels of 3 growth factors (Areg, IGF-I, HGF), 2 receptors (ERalpha, EGFR), and the cell proliferation marker PCNA were assessed in ovaries. (Data shown in bar graphs. n = 3 per group). ● In Balb/C and WT mice, PFOA decreased 2 growth factors (Areg and HGF), decreased both receptors (ERalpha, EGFR), and decreased PCNA (p<0.05) in Balb/C and WT mice. PFOA had no effect on IGF-I. ● PFOA had no effect on any parameter in KO mice. • These results are not consistent with the results in WT and KO mice dosed with 5 mg/kg/day PFOA for 4 weeks from Zhao et al. (2010). Ovarian levels of 3 growth factors (Areg, IGF-I, HGF), 2 receptors (ERalpha, EGFR), and 2 cell proliferation markers (cyclin D1, PCNA) were assessed (see above). • 5 mg/kg/day PFOA increased all 3 growth factors in KO mice and 2 growth factors in WT mice (1.5-2.7 fold, p<0.05); increased both receptors in WT and KO mice , and increased both markers of cell proliferation) in both WT and KO mice **Estrogen and progesterone levels** ● It is not known if estrogen and progesterone levels were affected by PFOA treatment. The authors state that this could not be assessed due to insufficient numbers of animals in each stage of estrous. However, delayed vaginal opening, absence of corpus lutea, and abnormal uterine histology suggest abnormal ovarian function and decreased hormone levels. **Kidney organic anion transporters (OATs):** ● Expression of 7 OATs was measured in kidneys from the three strains of mice (control and PFOA-treated). ● In control & PFOA-treated mice, levels of 3 OATs were higher in KO than Balb/C, & 2 of these OATs were higher in WT than Balb/C. ● PFOA treatment did not affect levels of OATs expression. ● This endpoint is of interest in evaluation of strain differences in serum PFOA levels from same administered dose, but is not directly relevant to the evaluation of effects of PFOA on MG development.	Additionally, it is difficult to interpret the effects seen in Yang et al. (2009) and in this study in C57BL/6 WT mice because the dose-response between 5 and 7.5 mg/kg/day was not evaluated within the same study. The 5 and 10 mg/kg/day doses were not included along with 7.5 mg/kg/day dose in this study. The two doses and serum levels are close together (68 versus 93 µg/ml), yet opposite effects on MG development (stimulation versus strong inhibition) were reported at the lower versus higher dose. This implies that that a very steep dose-response curve exists for stimulation versus inhibition, and that there is a dose between 5 and 7.5 mg/kg/day and a serum level between 68 and 93 µg/ml where no effect occurs. These data cannot be validly interpreted in the absence of data at both doses from the same study. Similarly, it is difficult to interpret the opposite effects of PFOA at 5 versus 7.5 mg/kg/day on ovarian enzymes related to hormone synthesis, and MG growth factors, receptors, and markers of proliferation, since the data at each dose comes from a different study.

Reference & Study Design	Results	Comment
	<u>SERUM PFOA LEVELS</u> ● Serum PFOA levels from 3 studies (this study; Yang et al., 2009; and Zhao et al., 2010) are presented in this study. Numerical data are not shown and were estimated from the bar graph. n = 5 per group. These estimated numerical data are shown in the table on MG developmental effects above, and in the tables presenting the other two studies. ● Serum PFOA levels were not measured in the component of this study in which the effects of estradiol and progesterone given along with PFOA were studied.	

SUPPLEMENTAL TABLE: Mammary gland development parameters and serum PFOA levels from female 7 week old Balb/C and C57BL/6 mice treated with PFOA for 4 weeks

Treatment (mg/kg/day) *Balb/c*	Citation[a]	Serum PFOA[b] (µg/ml)	Ductal Length (relative units)	% Control	# Terminal End Buds (TEBs)	% Control	# Stimulated Terminal Ducts (TDs)	% Control
Balg/C								
0	1	--	7.60 ± 0.94	--	8.20 ± 1.92	--	6.20 ± 2.39	--
0	3	--	8.17 ± 1.08	--	7.40 ± 1.14	--	6.11 ± 2.52	--
1	1	29 ± 4	7.28 ± 0.73	96%	6.00 ± 3.74	73	3.80 ± 1.64	61
2.5	3	51 ± 8	4.99 ± 0.67*	61	3.25 ± 0.95*	44	2.63 ± 1.41*	43
5	1	109 ± 12	3.99 ± 0.36*	53%	3.99 ± 0.36*	49	1.20 ± 1.30*	19
10	1	---	3.40 ± 4.77*	45	0.00 ± 0.00*	0	0.00 ± 0.00*	0
C57BL/6 Wild Type								
0	1	--	4.76 ± 1.19	--	7.80 ± 2.39	--	6.60 ± 2.88	--
0	3	--	6.30 ± 0.96	--	6.25 ± 2.06	--	6.50 ± 2.89	--
1	1	26 ± 6	4.06 ± 0.85	85	10.40 ± 3.36	133	9.80 ± 2.77	148
5	1	68 ± 10	4.01 ± 0.71	84	11.20 ± 1.30*	144	16.60 ± 5.90*	252
7.5	3	93 ±11	2.10 ± 1.98*	33	1.00 ± 1.41*	16	0.80 ± 1.31*	12
10	1	96 ±10	0.30 ± 0.67*	6	0.00 ± 0.00*	0	0.00 ± 0.00*	0
C57BL/6 PPAR-null (KO)								
0	3	---	8.90 ± 1.04	---	10.00 ± 4.36	---	6.20 ± 1.64	---
5	2	28 ± 2	Terminal end buds and stimulated terminal ducts stated to be **INCREASED**. Numerical data not shown.					
7.5	3	38 ± 7	8.43 ± 1.08	95	9.43 ± 1.90	94	7.02 ± 2.16	113

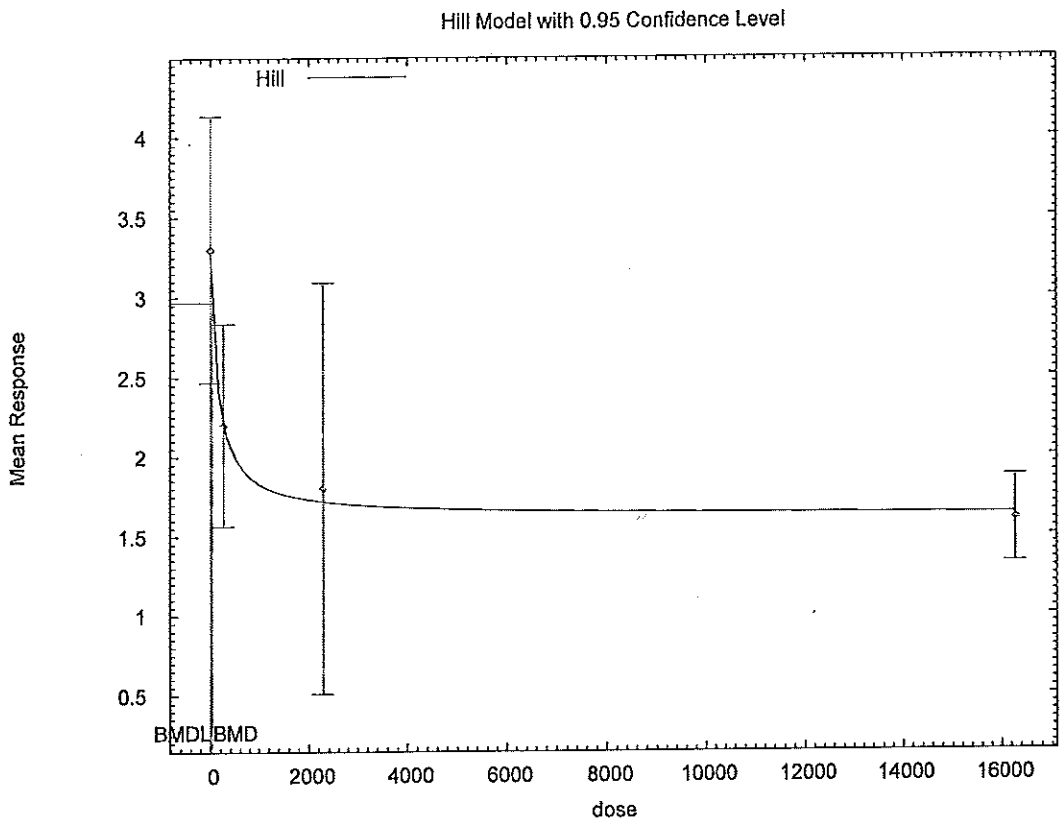
[a] Citations are: 1. Yang et al. (2009); 2. Zhao et al. (2010); 3. Zhao et al. (2012).

[b] PFOA serum data from all 3 studies are presented in a bar graph in Zhao et al. (2012). Numerical values were extrapolated from the bar graph.

*P<0.05 compared to control. n=5 per strain per dose group.

Appendix 6: Outputs of Benchmark Dose Modeling for Mammary Gland Development Endpoints (Macon et al., 2011)

Appendix 6A: Benchmark Dose Modeling of Mammary Gland Developmental Score in Response to PFOA Using BMR of 10% Decrease Relative to Controls (Macon et al., 2011)




```
================================================================
        Hill Model. (Version: 2.15;  Date: 10/28/2009)
        Input Data File: C:/USEPA/BMDS212/Data/hil_pfoa mammary_Opt.(d)
        Gnuplot Plotting File:  C:/USEPA/BMDS212/Data/hil_pfoa mammary_Opt.plt
                                          Wed May 04 14:10:58 2011
================================================================

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

  The form of the response function is:

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


  Dependent variable = dev_score
  Independent variable = zeroed_serum
  rho is set to 0
  Power parameter restricted to be greater than 1
  A constant variance model is fit

  Total number of dose groups = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008
```

Default Initial Parameter Values

alpha =	0.231538	
rho =	0	Specified
intercept =	3.3	
v =	-1.7	
n =	1.59127	
k =	202.223	

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho -n
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	intercept	v	k
alpha	1	-2.2e-011	-9.9e-008	6.4e-008
intercept	-2.2e-011	1	-0.74	-0.27
v	-9.9e-008	-0.74	1	-0.17
k	6.4e-008	-0.27	-0.17	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	0.178604	0.0612607	0.0585353	0.298673
intercept	3.29893	0.189163	2.92818	3.66968
v	-1.67528	0.250648	-2.16654	-1.18402
n	1	NA		
k	141.808	97.0679	-48.4413	332.058

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	5	3.3	3.3	0.67	0.423	0.00565
261.7	4	2.2	2.21	0.4	0.423	-0.0587
2281	3	1.8	1.72	0.52	0.423	0.321
1.628e+004	5	1.6	1.64	0.22	0.423	-0.202

Model Descriptions for likelihoods calculated

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

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

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that were specified by the user

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

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	6.215823	5	-2.431646
A2	8.999248	8	-1.998497
A3	6.215823	5	-2.431646
fitted	6.141961	4	-4.283923
R	-4.983702	2	13.967403

Explanation of Tests

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

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	27.9659	6	<.0001
Test 2	5.56685	3	0.1347
Test 3	5.56685	3	0.1347
Test 4	0.147723	1	0.7007

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

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

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

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

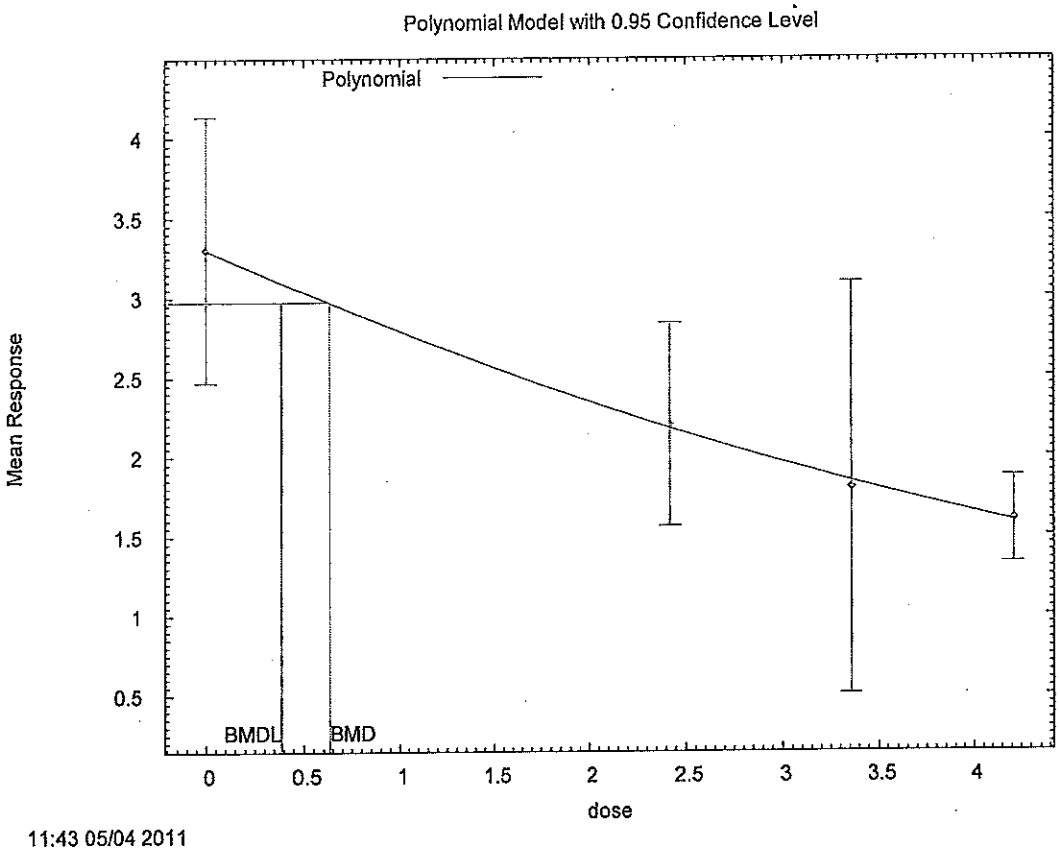
```
Specified effect =          0.1

Risk Type        =     Relative risk

Confidence level =          0.95

             BMD =       34.7717

            BMDL =      5.51541
```




```
==================================================================
        Polynomial Model. (Version: 2.16;  Date: 05/26/2010)
        Input Data File: C:/USEPA/BMDS212/Data/ply_pfoa mammary_Opt.(d)
        Gnuplot Plotting File:  C:/USEPA/BMDS212/Data/ply_pfoa mammary_Opt.plt
                                          Wed May 04 11:43:35 2011
==================================================================

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

  The form of the response function is:

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


  Dependent variable = dev_score
  Independent variable = zeroed_log_serum
  rho is set to 0
  Signs of the polynomial coefficients are not restricted
  A constant variance model is fit

  Total number of dose groups = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008
```

Default Initial Parameter Values

alpha =	0.231538	
rho =	0	Specified
beta_0 =	3.30302	
beta_1 =	-0.544614	
beta_2 =	0.032366	

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	beta_0	beta_1	beta_2
alpha	1	3.7e-008	8.9e-008	-1e-007
beta_0	3.7e-008	1	-0.51	0.32
beta_1	8.9e-008	-0.51	1	-0.96
beta_2	-1e-007	0.32	-0.96	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	0.177536	0.0608942	0.0581851	0.296886
beta_0	3.30204	0.188191	2.93319	3.67089
beta_1	-0.541826	0.230176	-0.992962	-0.0906905
beta_2	0.0320545	0.0550625	-0.075866	0.139975

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	5	3.3	3.3	0.67	0.421	-0.0108
2.42	4	2.2	2.18	0.4	0.421	0.102
3.36	3	1.8	1.84	0.52	0.421	-0.178
4.21	5	1.6	1.59	0.22	0.421	0.0579

Model Descriptions for likelihoods calculated

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

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

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that were specified by the user

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

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	6.215823	5	-2.431646
A2	8.999248	8	-1.998497
A3	6.215823	5	-2.431646
fitted	6.192967	4	-4.385934
R	-4.983702	2	13.967403

Explanation of Tests

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

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	27.9659	6	<.0001
Test 2	5.56685	3	0.1347
Test 3	5.56685	3	0.1347
Test 4	0.0457118	1	0.8307

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

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

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

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

Specified effect = 0.1

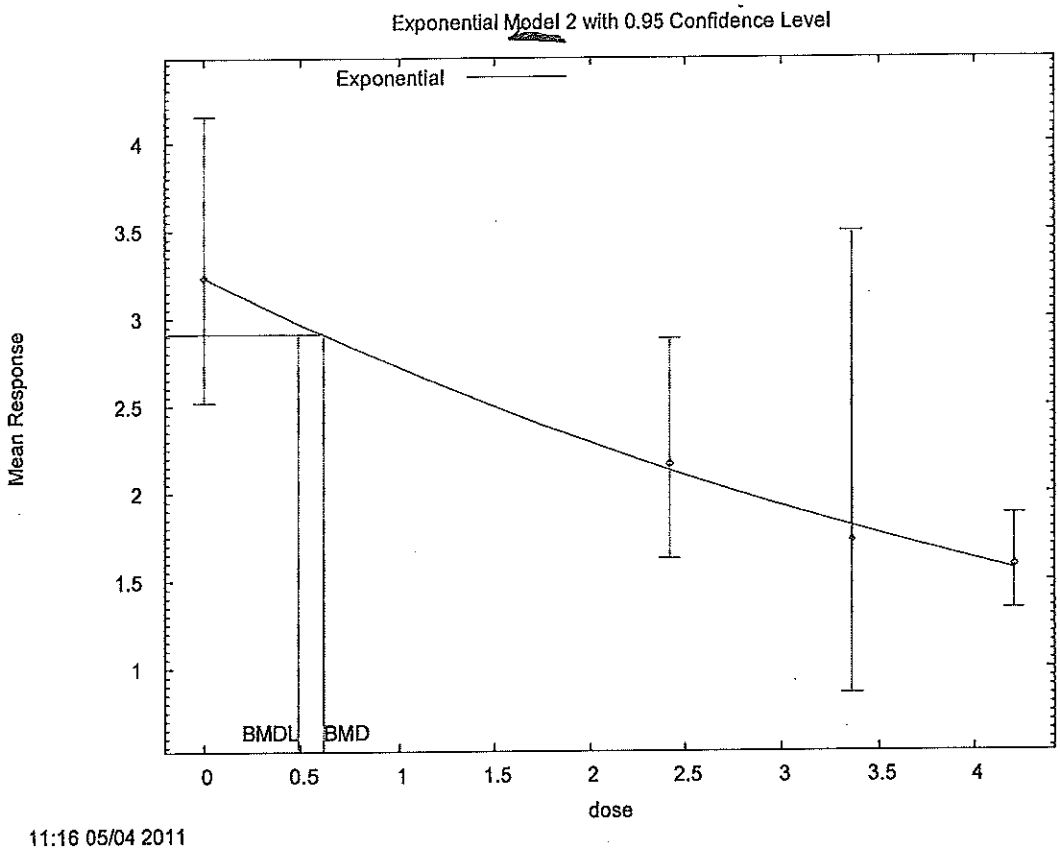
Risk Type = Relative risk

Confidence level = 0.95

```
BMD =        0.633144

BMDL =       0.387553

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




```
================================================================
        Exponential Model. (Version: 1.7;  Date: 12/10/2009)
        Input Data File: C:/USEPA/BMDS212/Data/exp_pfoa mammary_Setting.(d)
        Gnuplot Plotting File:
                                                Wed May 04 11:16:37 2011
================================================================

BMDS Model Run
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  The form of the response function by Model:
     Model 2:     Y[dose] = a * exp{sign * b * dose}
     Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
     Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
     Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

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

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


  Dependent variable = Calculated Median
```

Independent variable = zeroed_log_serum
Data are assumed to be distributed: lognormally
Variance Model: Log-scale variance = exp(lnalpha)
rho is set to 0.
A constant log-scale variance model is fit.

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Approximate

Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-3.53767	-3.53767	-3.53767	-3.53767
rho(S)	0	0	0	0
a	1.41095	1.56697	3.39572	3.39572
b	0.172782	-0.0108424	0.286517	0.286517
c	--	--	0.233395	0.233395
d	--	2	--	1

(S) = Specified

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-3.52095	-3.52095	-3.52146	-3.53767
rho	0	0	0	0
a	3.23432	3.23432	3.24152	3.23402
b	0.172782	0.172782	0.195617	0.414661
c	--	--	0.0802623	0.479395
d	--	1	--	2.43417

Table of Stats From Input Data

Dose	N	Calc'd Median	Calc'd GSD
0	5	3.234	1.223

2.42	4	2.165	1.198
3.36	3	1.729	1.327
4.21	5	1.585	1.147

Estimated Values of Interest

Model	Dose	Est Median	Est GSD	Scaled Residual
2	0	3.234	1.188	-0.0005776
	2.42	2.129	1.188	0.05968
	3.36	1.81	1.188	-0.1176
	4.21	1.563	1.188	0.04216
3	0	3.234	1.188	-0.0005775
	2.42	2.129	1.188	0.05968
	3.36	1.81	1.188	-0.1176
	4.21	1.563	1.188	0.04216
4	0	3.242	1.188	-0.01413
	2.42	2.117	1.188	0.07966
	3.36	1.805	1.188	-0.1109
	4.21	1.569	1.188	0.03103
5	0	3.234	1.186	-4.18e-008
	2.42	2.165	1.186	-1.771e-008
	3.36	1.729	1.186	-1.37e-008
	4.21	1.585	1.186	-2.044e-009

Other models for which likelihoods are calculated:

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

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

Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)
Model  R:        Yij = Mu + e(i)
           Var{e(ij)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	21.57016	5	-33.14031
A2	22.40331	8	-28.80662
A3	21.57016	5	-33.14031
R	9.975396	2	-15.95079
2	21.42811	3	-36.85623
3	21.42811	3	-36.85623
4	21.43241	4	-34.86481
5	21.57016	5	-33.14031

Additive constant for all log-likelihoods = -15.62. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

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

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

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

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

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	24.86	6	0.0003631
Test 2	1.666	3	0.6445
Test 3	1.666	3	0.6445
Test 4	0.2841	2	0.8676
Test 5a	0.2841	2	0.8676
Test 5b	7.105e-014	0	N/A
Test 6a	0.2755	1	0.5997
Test 6b	0.008586	1	0.9262
Test 7a	1.421e-014	0	N/A
Test 7b	0.2841	2	0.8676
Test 7c	0.2755	1	0.5997

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

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

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

The p-value for Test 4 is greater than .1. Model 2 seems to adequately describe the data.

The p-value for Test 5a is greater than .1. Model 3 seems to adequately describe the data.

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

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

The p-value for Test 6b is greater than .05. Model 4 does not seem to fit the data better than Model 2.

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

The p-value for Test 7b is greater than .05. Model 5 does

not seem to fit the data better than Model 3.

The p-value for Test 7c is greater than .05. Model 5 does not seem to fit the data better than Model 4.

Benchmark Dose Computations:

Specified Effect = 0.100000

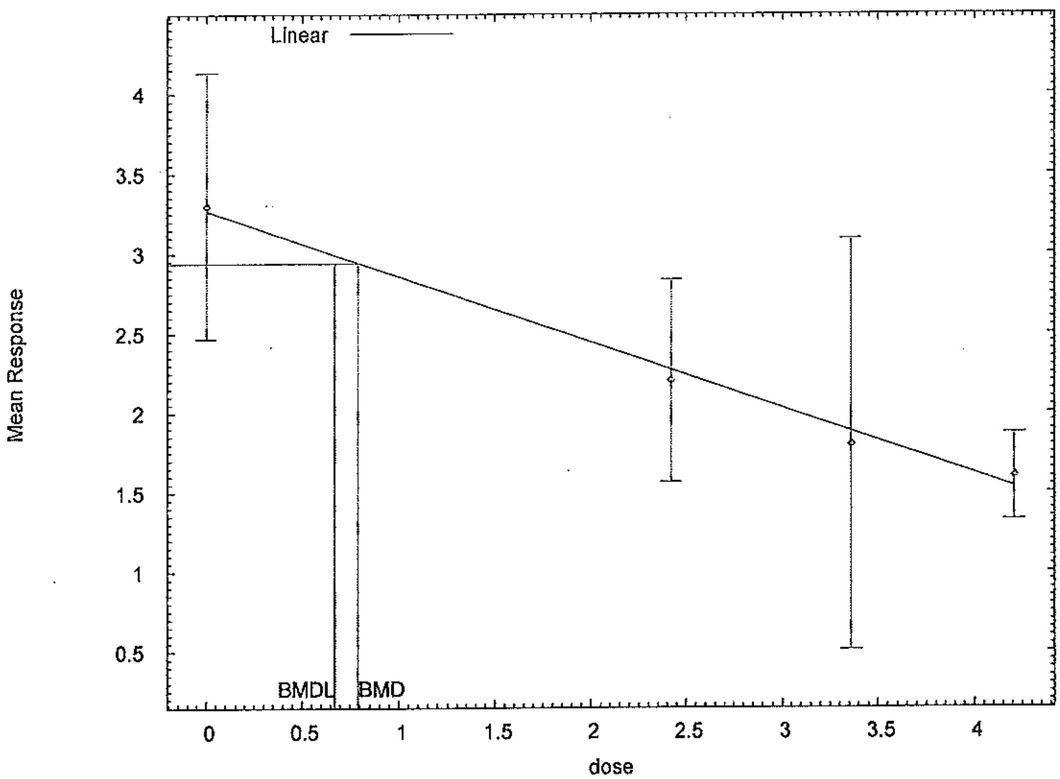
Risk Type = Relative deviation

Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL
2	0.60979	0.489341
3	0.60979	0.489341
4	0.588417	0.286515
5	1.27833	0.299899

Linear Model with 0.95 Confidence Level



11:32 05/04 2011

```
====================================================================
       Polynomial Model. (Version: 2.16;  Date: 05/26/2010)
       Input Data File: C:/USEPA/BMDS212/Data/lin_pfoa mammary_Opt.(d)
       Gnuplot Plotting File:  C:/USEPA/BMDS212/Data/lin_pfoa mammary_Opt.plt
                                              Wed May 04 11:31:56 2011
====================================================================

BMDS Model Run
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  The form of the response function is:

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


  Dependent variable = dev_score
  Independent variable = zeroed_log_serum
  rho is set to 0
  Signs of the polynomial coefficients are not restricted
  A constant variance model is fit

  Total number of dose groups = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008


                Default Initial Parameter Values
                        alpha =      0.231538
```

```
                    rho =            0   Specified
                beta_0 =       3.2622
                beta_1 =    -0.415294
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	beta_0	beta_1
alpha	1	4.6e-010	7.7e-010
beta_0	4.6e-010	1	-0.82
beta_1	7.7e-010	-0.82	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	0.181075	0.0621081	0.0593451	0.302804
beta_0	3.26695	0.180042	2.91407	3.61982
beta_1	-0.412597	0.0614538	-0.533045	-0.29215

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	5	3.3	3.27	0.67	0.426	0.174
2.42	4	2.2	2.27	0.4	0.426	-0.322
3.36	3	1.8	1.88	0.52	0.426	-0.328
4.21	5	1.6	1.53	0.22	0.426	0.368

Model Descriptions for likelihoods calculated

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

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

Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
    Model A3 uses any fixed variance parameters that
    were specified by the user
```

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

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	6.215823	5	-2.431646
A2	8.999248	8	-1.998497
A3	6.215823	5	-2.431646
fitted	6.025186	3	-6.050372
R	-4.983702	2	13.967403

Explanation of Tests

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

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	27.9659	6	<.0001
Test 2	5.56685	3	0.1347
Test 3	5.56685	3	0.1347
Test 4	0.381274	2	0.8264

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

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

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

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Relative risk

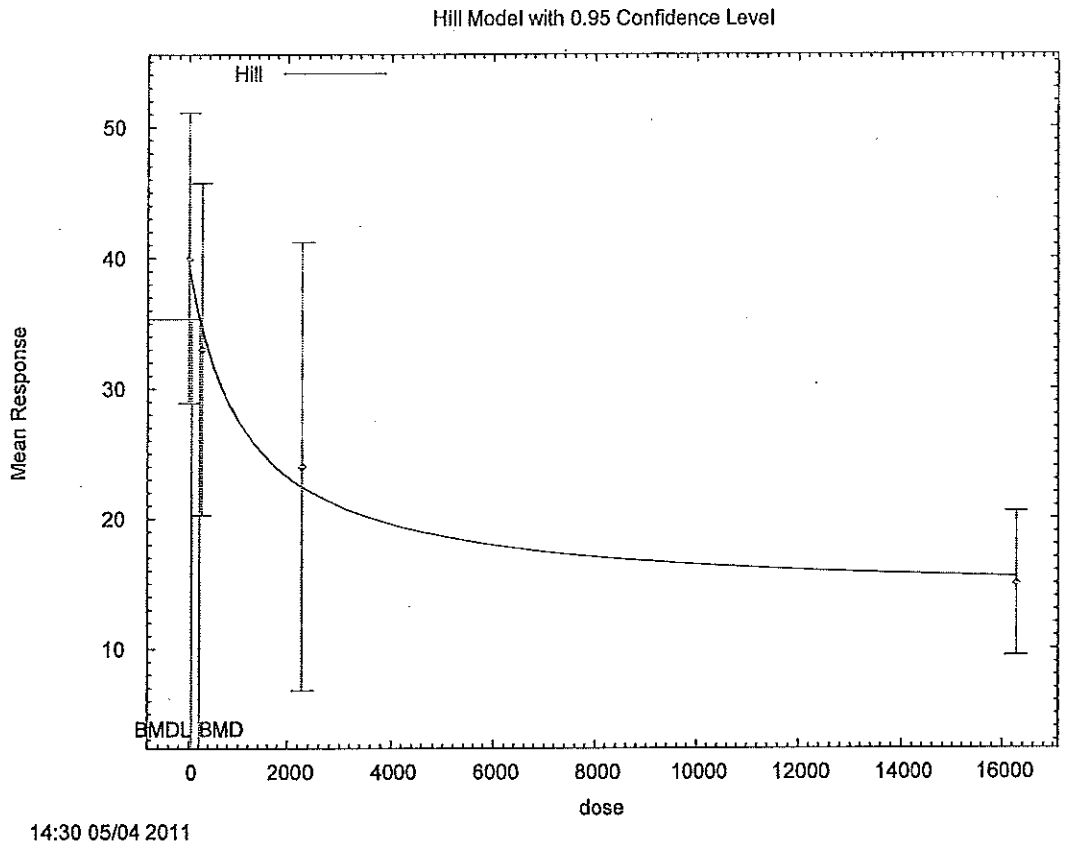
Confidence level = 0.95

BMD = 0.7918

BMDL = 0.673285

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

Appendix 6B: Benchmark Dose Modeling of Number of Terminal Endbud in Response to PFOA Using BMR of 10% Decrease Relative to Controls (Macon et al., 2011)




```
=================================================================
        Hill Model. (Version: 2.15;  Date: 10/28/2009)
        Input Data File: C:/USEPA/BMDS212/Data/hil_pfoa mammary_Opt.(d)
        Gnuplot Plotting File:  C:/USEPA/BMDS212/Data/hil_pfoa mammary_Opt.plt
                                                Wed May 04 14:30:47 2011
=================================================================

BMDS Model Run
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  The form of the response function is:

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


  Dependent variable = buds
  Independent variable = zeroed_serum
  rho is set to 0
  Power parameter restricted to be greater than 1
  A constant variance model is fit

  Total number of dose groups = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008


                Default Initial Parameter Values
```

alpha =	52.8975	
rho =	0	Specified
intercept =	40	
v =	-25	
n =	0.952636	
k =	1495.53	

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho -n
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	intercept	v	k
alpha	1	-3.2e-008	-4.5e-008	4.8e-008
intercept	-3.2e-008	1	-0.37	-0.57
v	-4.5e-008	-0.37	1	-0.35
k	4.8e-008	-0.57	-0.35	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	41.7264	14.312	13.6753	69.7774
intercept	39.2599	2.85248	33.6692	44.8507
v	-25.532	4.27964	-33.9199	-17.144
n	1	NA		
k	1169.31	1119.77	-1025.41	3364.03

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	5	40	39.3	8.94	6.46	0.256
261.7	4	33	34.6	8	6.46	-0.493
2281	3	24	22.4	6.93	6.46	0.434
1.628e+004	5	15	15.4	4.47	6.46	-0.152

Model Descriptions for likelihoods calculated

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

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

Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
    Model A3 uses any fixed variance parameters that
    were specified by the user

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

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-39.950786	5	89.901573
A2	-38.765686	8	93.531372
A3	-39.950786	5	89.901573
fitted	-40.214633	4	88.429266
R	-50.575581	2	105.151162

Explanation of Tests

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

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	23.6198	6	0.0006134
Test 2	2.3702	3	0.4992
Test 3	2.3702	3	0.4992
Test 4	0.527693	1	0.4676

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

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

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

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

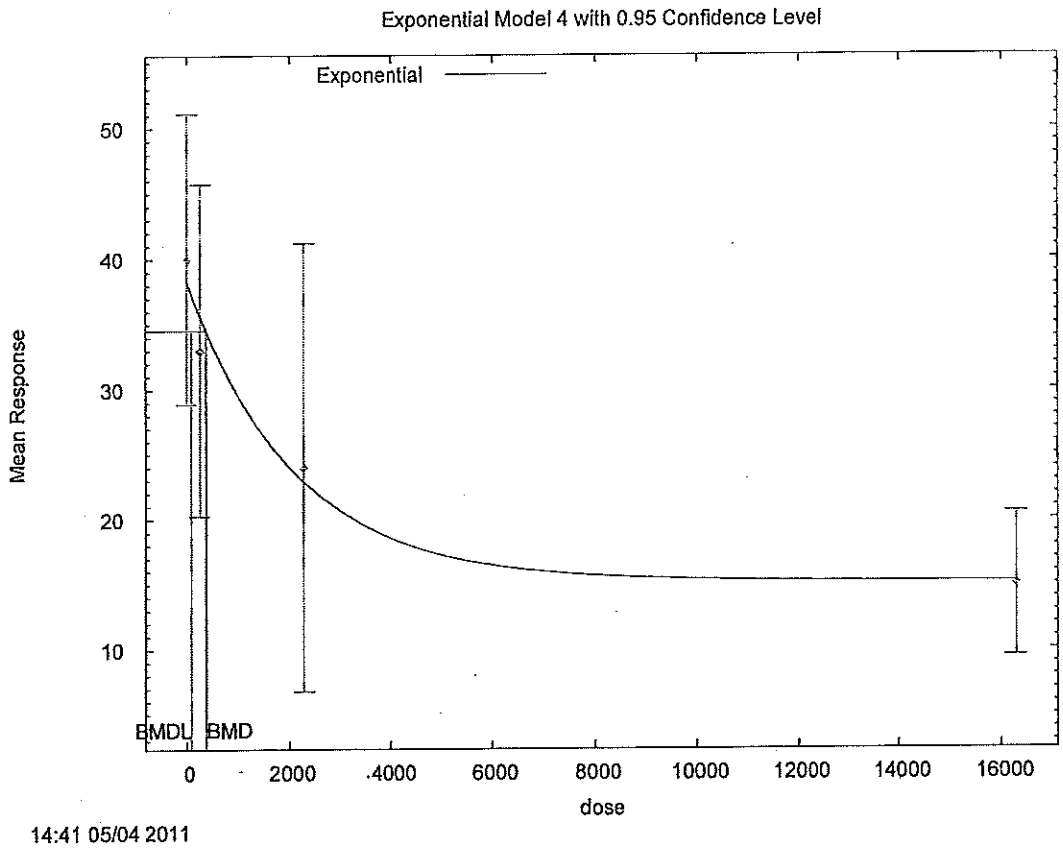
Specified effect = 0.1

```
Risk Type        =     Relative risk

Confidence level =          0.95

             BMD =        212.474

            BMDL =       55.9134
```




```
================================================================
        Exponential Model. (Version: 1.7;  Date: 12/10/2009)
        Input Data File: C:/USEPA/BMDS212/Data/exp_pfoa mammary_Setting.(d)
        Gnuplot Plotting File:
                                                Wed May 04 14:41:27 2011
================================================================

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

  The form of the response function by Model:
     Model 2:     Y[dose] = a * exp{sign * b * dose}
     Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
     Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
     Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

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

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


  Dependent variable = buds
  Independent variable = zeroed_serum
  Data are assumed to be distributed: normally
```

```
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
rho is set to 0.
A constant variance model is fit.

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact
```

Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	3.70009	3.70009	3.70009	3.70009
rho(S)	0	0	0	0
a	19.8825	19.8825	42	42
b	5.23786e-005	5.23786e-005	0.000229525	0.000229525
c	--	--	0.340136	0.340136
d	--	1	--	1

(S) = Specified

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	4.03627	4.03627	3.76287	3.76287
rho	0	0	0	0
a	35.4078	35.4078	38.36	38.36
b	5.87408e-005	5.87408e-005	0.000479266	0.000479266
c	--	--	0.395353	0.395353
d	--	1	--	1

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	5	40	8.94
261.7	4	33	8
2281	3	24	6.93

1.628e+004	5	15	4.47

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
2	0	35.41	7.524	1.365
	261.7	34.87	7.524	-0.4964
	2281	30.97	7.524	-1.604
	1.628e+004	13.61	7.524	0.4144
3	0	35.41	7.524	1.365
	261.7	34.87	7.524	-0.4964
	2281	30.97	7.524	-1.604
	1.628e+004	13.61	7.524	0.4144
4	0	38.36	6.563	0.5588
	261.7	35.63	6.563	-0.8002
	2281	22.94	6.563	0.2797
	1.628e+004	15.18	6.563	-0.05969
5	0	38.36	6.563	0.5588
	261.7	35.63	6.563	-0.8002
	2281	22.94	6.563	0.2797
	1.628e+004	15.18	6.563	-0.05969

Other models for which likelihoods are calculated:

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

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

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

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

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-39.95079	5	89.90157
A2	-38.76569	8	93.53137
A3	-39.95079	5	89.90157
R	-50.57558	2	105.1512
2	-42.80832	3	91.61664
3	-42.80832	3	91.61664
4	-40.48439	4	88.96877
5	-40.48439	4	88.96877

Additive constant for all log-likelihoods = -15.62. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

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

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

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

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

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	23.62	6	0.0006134
Test 2	2.37	3	0.4992
Test 3	2.37	3	0.4992
Test 4	5.715	2	0.05741
Test 5a	5.715	2	0.05741
Test 5b	-1.077e-011	0	N/A
Test 6a	1.067	1	0.3016
Test 6b	4.648	1	0.03109
Test 7a	1.067	1	0.3016
Test 7b	4.648	1	0.03109
Test 7c	1.421e-014	0	N/A

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

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

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

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

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

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

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

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

The p-value for Test 7a is greater than .1. Model 5 seems to adequately describe the data.

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

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

Benchmark Dose Computations:

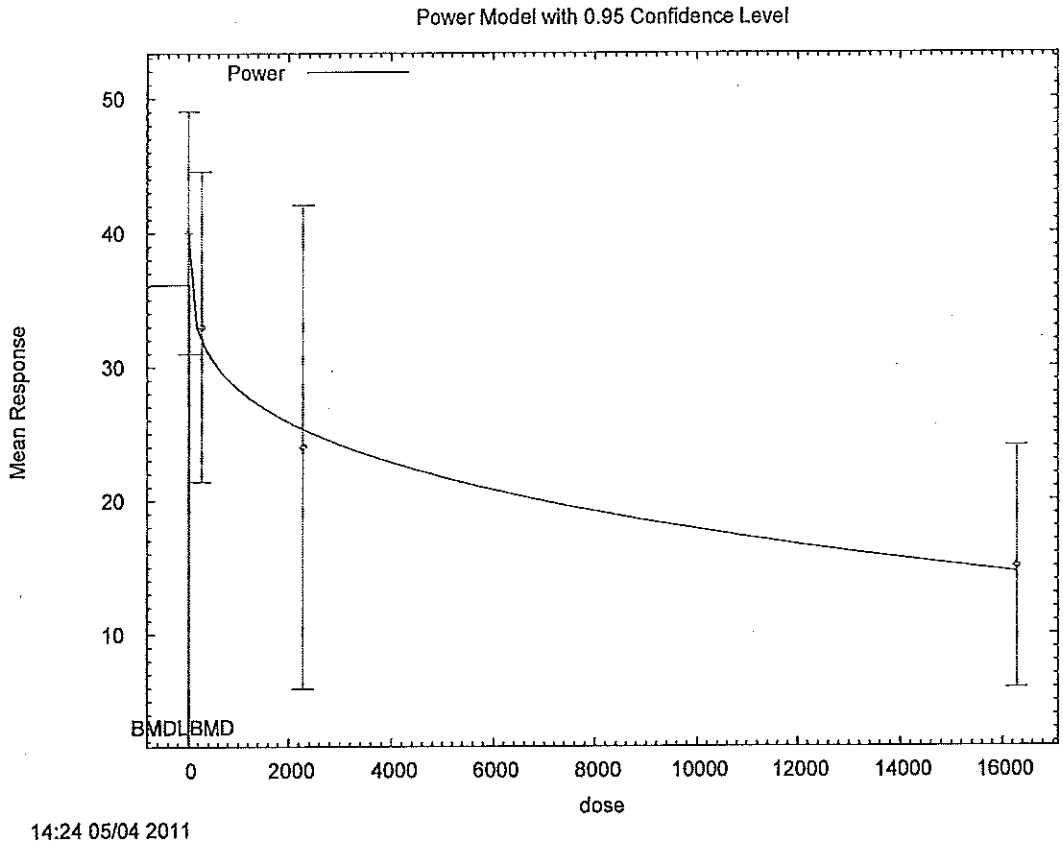
Specified Effect = 0.100000

Risk Type = Relative deviation

Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL
2	1793.65	1101.42
3	1793.65	1101.42
4	377.213	87.861
5	377.213	87.861




```
================================================================
        Power Model. (Version: 2.16;  Date: 10/28/2009)
        Input Data File: C:/USEPA/BMDS212/Data/pow_pfoa mammary_Opt.(d)
        Gnuplot Plotting File:  C:/USEPA/BMDS212/Data/pow_pfoa mammary_Opt.plt
                                          Wed May 04 14:24:01 2011
================================================================

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

  The form of the response function is:

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


  Dependent variable = buds
  Independent variable = zeroed_serum
  rho is set to 0
  The power is not restricted
  A constant variance model is fit

  Total number of dose groups = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008
```

Default Initial Parameter Values

alpha =	52.8975	
rho =	0	Specified
control =	40	
slope =	-1.24383	
power =	0.309416	

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	control	slope	power
alpha	1	1.1e-009	-1.2e-009	-1.4e-009
control	1.1e-009	1	-0.61	-0.51
slope	-1.2e-009	-0.61	1	0.99
power	-1.4e-009	-0.51	0.99	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	41.0366	14.0754	13.4492	68.6239
control	40.1466	2.82483	34.61	45.6831
slope	-1.72177	1.67474	-5.0042	1.56067
power	0.277549	0.0973577	0.0867315	0.468367

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	5	40	40.1	8.94	6.41	-0.0512
261.7	4	33	32.1	8	6.41	0.289
2281	3	24	25.4	6.93	6.41	-0.385
1.628e+004	5	15	14.7	4.47	6.41	0.0905

Model Descriptions for likelihoods calculated

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

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

Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
    Model A3 uses any fixed variance parameters that
    were specified by the user

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

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-39.950786	5	89.901573
A2	-38.765686	8	93.531372
A3	-39.950786	5	89.901573
fitted	-40.072946	4	88.145893
R	-50.575581	2	105.151162

Explanation of Tests

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

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	23.6198	6	0.0006134
Test 2	2.3702	3	0.4992
Test 3	2.3702	3	0.4992
Test 4	0.24432	1	0.6211

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

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

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

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

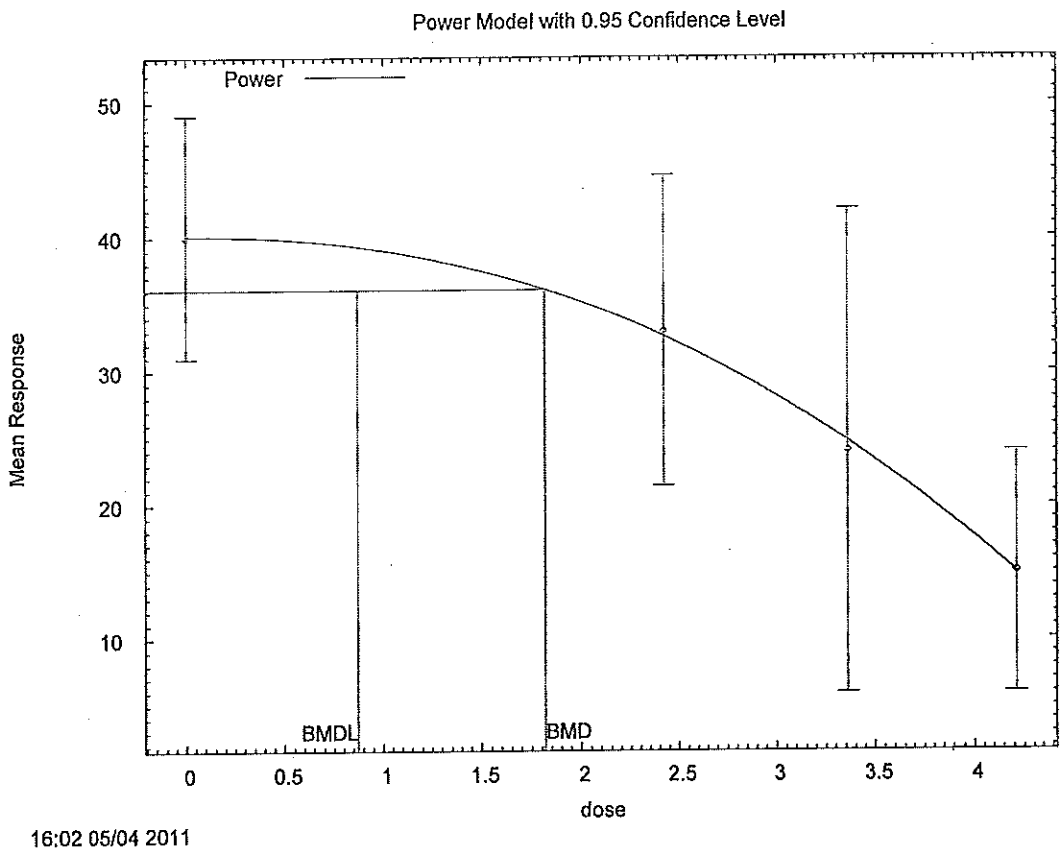
```
Specified effect =          0.1

Risk Type        =     Relative risk

Confidence level =         0.95
```

BMD = 21.1211

BMDL = 0.0759616




```
==================================================================
        Power Model. (Version: 2.16;  Date: 10/28/2009)
        Input Data File: C:/USEPA/BMDS212/Data/pow_pfoa mammary_Opt.(d)
        Gnuplot Plotting File:  C:/USEPA/BMDS212/Data/pow_pfoa mammary_Opt.plt
                                          Wed May 04 16:02:47 2011
==================================================================

BMDS Model Run
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  The form of the response function is:

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


  Dependent variable = buds
  Independent variable = zeroed_log_serum
  rho is set to 0
  The power is restricted to be greater than or equal to 1
  A constant variance model is fit

  Total number of dose groups = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008
```

Default Initial Parameter Values

alpha =	52.8975	
rho =	0	Specified
control =	40	
slope =	-520.606	
power =	-2.11214	

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	control	slope	power
alpha	1	4e-008	3.1e-008	4e-008
control	4e-008	1	-0.6	-0.52
slope	3.1e-008	-0.6	1	0.99
power	4e-008	-0.52	0.99	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	40.5843	13.9203	13.301	67.8676
control	40.0806	2.81393	34.5654	45.5958
slope	-1.08474	1.27305	-3.57987	1.41038
power	2.18902	0.797466	0.626014	3.75202

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	5	40	40.1	8.94	6.37	-0.0283
2.42	4	33	32.6	8	6.37	0.134
3.36	3	24	24.7	6.93	6.37	-0.185
4.21	5	15	14.9	4.47	6.37	0.0519

Model Descriptions for likelihoods calculated

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

Model A2: Yij = Mu(i) + e(ij)

```
          Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
    Model A3 uses any fixed variance parameters that
    were specified by the user

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

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-39.950786	5	89.901573
A2	-38.765686	8	93.531372
A3	-39.950786	5	89.901573
fitted	-39.978739	4	87.957477
R	-50.575581	2	105.151162

Explanation of Tests

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

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	23.6198	6	0.0006134
Test 2	2.3702	3	0.4992
Test 3	2.3702	3	0.4992
Test 4	0.0559044	1	0.8131

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

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

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

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

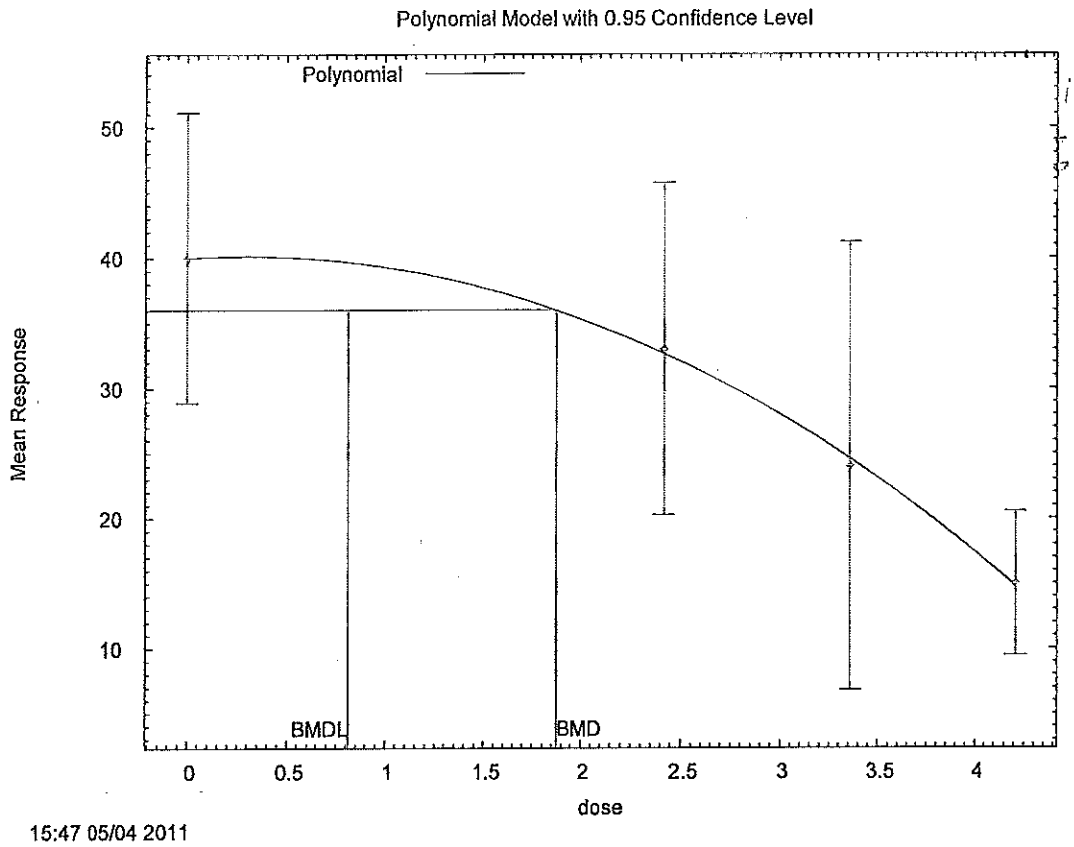
Specified effect = 0.1

Risk Type = Relative risk

Confidence level = 0.95

BMD = 1.81676

BMDL = 0.870604




```
=================================================================
        Polynomial Model. (Version: 2.16;  Date: 05/26/2010)
        Input Data File: C:/USEPA/BMDS212/Data/ply_pfoa mammary_Opt.(d)
        Gnuplot Plotting File:  C:/USEPA/BMDS212/Data/ply_pfoa mammary_Opt.plt
                                          Wed May 04 15:47:37 2011
=================================================================

BMDS Model Run
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  The form of the response function is:

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


  Dependent variable = buds
  Independent variable = zeroed_log_serum
  rho is set to 0
  Signs of the polynomial coefficients are not restricted
  A constant variance model is fit

  Total number of dose groups = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008


                Default Initial Parameter Values
```
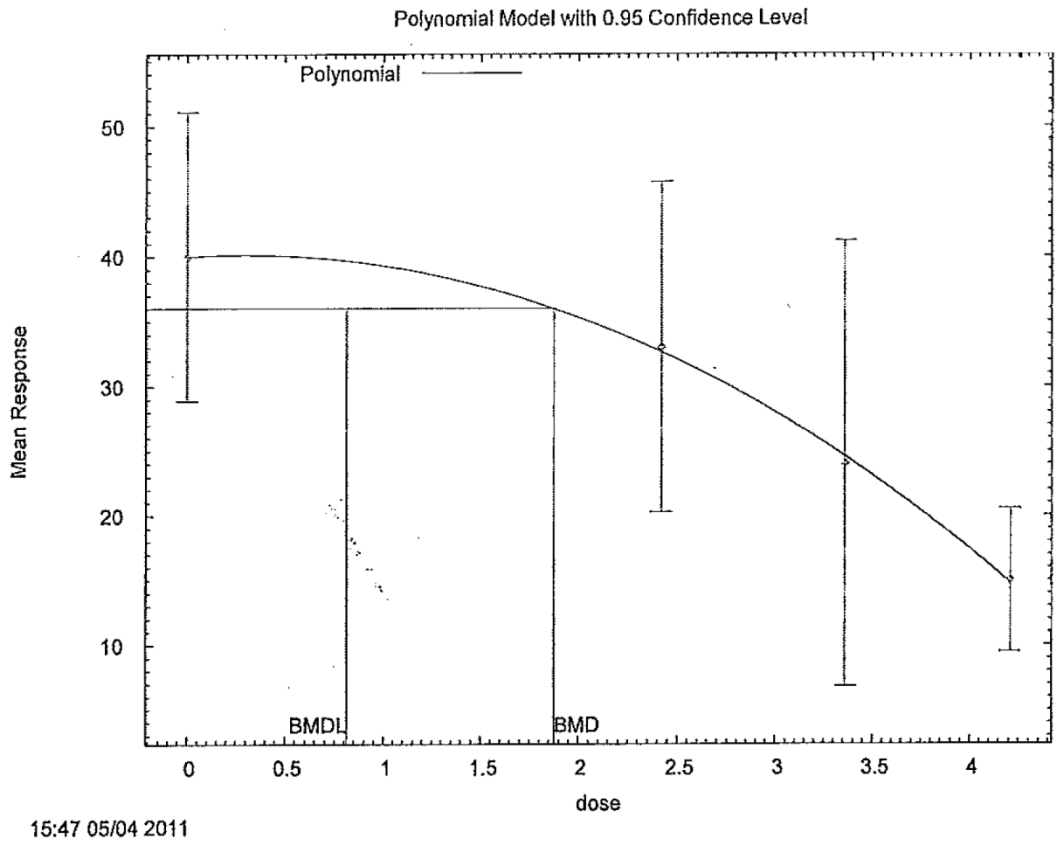




```
=====================================================================
        Polynomial Model. (Version: 2.16;  Date: 05/26/2010)
        Input Data File: C:/USEPA/BMDS212/Data/ply_pfoa mammary_Opt.(d)
        Gnuplot Plotting File:  C:/USEPA/BMDS212/Data/ply_pfoa mammary_Opt.plt
                                          Wed May 04 15:47:37 2011
=====================================================================

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

  The form of the response function is:

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


  Dependent variable = buds
  Independent variable = zeroed_log_serum
  rho is set to 0
  Signs of the polynomial coefficients are not restricted
  A constant variance model is fit

  Total number of dose groups = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008


                Default Initial Parameter Values
```

```
alpha =      52.8975
  rho =            0   Specified
beta_0 =      40.044
beta_1 =    0.912419
beta_2 =    -1.64299
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	beta_0	beta_1	beta_2
alpha	1	-1.5e-007	3.6e-007	-3.7e-007
beta_0	-1.5e-007	1	-0.51	0.32
beta_1	3.6e-007	-0.51	1	-0.96
beta_2	-3.7e-007	0.32	-0.96	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	40.5522	13.9093	13.2905	67.8139
beta_0	40.0297	2.84422	34.4552	45.6043
beta_1	0.953024	3.47875	-5.86521	7.77126
beta_2	-1.64752	0.832185	-3.27858	-0.0164714

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	5	40	40	8.94	6.37	-0.0104
2.42	4	33	32.7	8	6.37	0.0981
3.36	3	24	24.6	6.93	6.37	-0.172
4.21	5	15	14.8	4.47	6.37	0.0558

Model Descriptions for likelihoods calculated

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

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

```
Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
    Model A3 uses any fixed variance parameters that
    were specified by the user

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

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-39.950786	5	89.901573
A2	-38.765686	8	93.531372
A3	-39.950786	5	89.901573
fitted	-39.972016	4	87.944031
R	-50.575581	2	105.151162

Explanation of Tests

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

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	23.6198	6	0.0006134
Test 2	2.3702	3	0.4992
Test 3	2.3702	3	0.4992
Test 4	0.0424585	1	0.8367

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

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

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

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

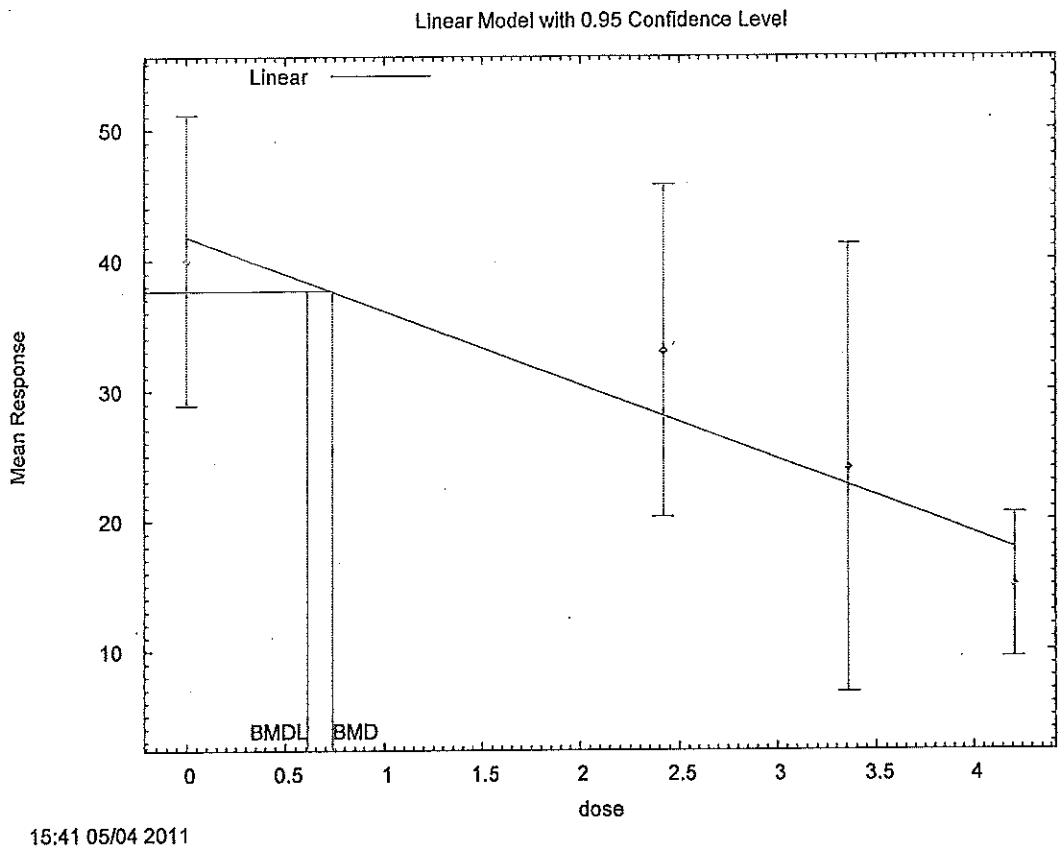
Specified effect = 0.1

Risk Type = Relative risk

Confidence level = 0.95

BMD = 1.87458

```
BMDL =        0.811908

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




```
=================================================================
        Polynomial Model. (Version: 2.16;  Date: 05/26/2010)
        Input Data File: C:/USEPA/BMDS212/Data/lin_pfoa mammary_Opt.(d)
        Gnuplot Plotting File:  C:/USEPA/BMDS212/Data/lin_pfoa mammary_Opt.plt
                                          Wed May 04 15:40:48 2011
=================================================================

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

  The form of the response function is:

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


  Dependent variable = buds
  Independent variable = zeroed_log_serum
  rho is set to 0
  Signs of the polynomial coefficients are not restricted
  A constant variance model is fit

  Total number of dose groups = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008


                 Default Initial Parameter Values
```

```
                    alpha =      52.8975
                      rho =            0   Specified
                   beta_0 =      42.1163
                   beta_1 =     -5.65218
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	beta_0	beta_1
alpha	1	-3.6e-008	-5.5e-010
beta_0	-3.6e-008	1	-0.82
beta_1	-5.5e-010	-0.82	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	49.9017	17.1161	16.3547	83.4487
beta_0	41.8335	2.98884	35.9755	47.6915
beta_1	-5.68904	1.02018	-7.68856	-3.68952

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	5	40	41.8	8.94	7.06	-0.58
2.42	4	33	28.1	8	7.06	1.4
3.36	3	24	22.7	6.93	7.06	0.314
4.21	5	15	17.9	4.47	7.06	-0.912

Model Descriptions for likelihoods calculated

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

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

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that were specified by the user

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

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-39.950786	5	89.901573
A2	-38.765686	8	93.531372
A3	-39.950786	5	89.901573
fitted	-41.735470	3	89.470940
R	-50.575581	2	105.151162

Explanation of Tests

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

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	23.6198	6	0.0006134
Test 2	2.3702	3	0.4992
Test 3	2.3702	3	0.4992
Test 4	3.56937	2	0.1679

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

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

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

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Relative risk

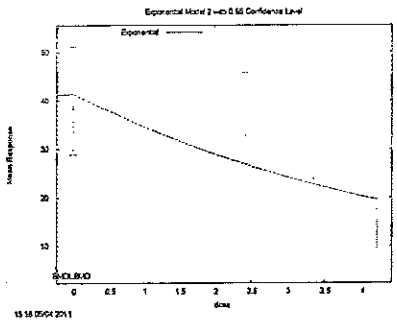
Confidence level = 0.95

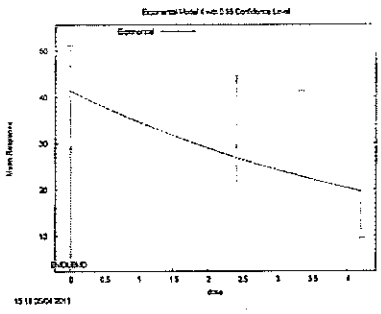
BMD = 0.735335

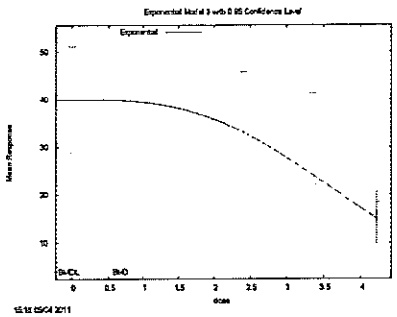
BMDL = 0.610445

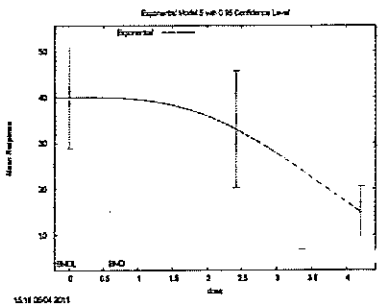
BMDL computation failed for one or more point on the BMDL curve.

The BMDL curve will not be plotted









```
=================================================================
        Exponential Model. (Version: 1.7;  Date: 12/10/2009)
        Input Data File: C:/USEPA/BMDS212/Data/exp_pfoa mammary_Setting.(d)
        Gnuplot Plotting File:
                                                Wed May 04 15:16:09 2011
=================================================================

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

  The form of the response function by Model:
     Model 2:     Y[dose] = a * exp{sign * b * dose}
     Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
     Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
     Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

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

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


  Dependent variable = buds
  Independent variable = zeroed_log_serum
  Data are assumed to be distributed: normally
  Variance Model: exp(lnalpha +rho *ln(Y[dose]))
  rho is set to 0.
  A constant variance model is fit.
```

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	3.70009	3.70009	3.70009	3.70009
rho(S)	0	0	0	0
a	15.5856	15.5856	42	42
b	0.216048	0.216048	0.19508	0.19508
c	--	--	0.000357143	0.000357143
d	--	1	--	1

(S) = Specified

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	4.02818	3.70014	4.02818	3.70009
rho	0	0	0	0
a	41.4292	40.0112	41.4292	40
b	0.178366	0.236123	0.178366	0.245098
c	--	--	0	0.0628183
d	--	2.92913	--	3.01825

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	5	40	8.94
2.42	4	33	8
3.36	3	24	6.93
4.21	5	15	4.47

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
2	0	41.43	7.494	-0.4265
	2.42	26.91	7.494	1.626
	3.36	22.75	7.494	0.2883
	4.21	19.55	7.494	-1.358
3	0	40.01	6.36	-0.003927
	2.42	32.95	6.36	0.01529
	3.36	24.08	6.36	-0.02269
	4.21	14.98	6.36	0.008652
4	0	41.43	7.494	-0.4265
	2.42	26.91	7.494	1.626
	3.36	22.75	7.494	0.2883
	4.21	19.55	7.494	-1.358
5	0	40	6.36	-1.514e-008
	2.42	33	6.36	8.575e-009
	3.36	24	6.36	3.174e-008
	4.21	15	6.36	-4.441e-008

Other models for which likelihoods are calculated:

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

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

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

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

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-39.95079	5	89.90157
A2	-38.76569	8	93.53137
A3	-39.95079	5	89.90157
R	-50.57558	2	105.1512
2	-42.73957	3	91.47914
3	-39.95121	4	87.90241
4	-42.73957	3	91.47914
5	-39.95079	5	89.90157

Additive constant for all log-likelihoods = -15.62. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

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

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

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

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

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	23.62	6	0.0006134
Test 2	2.37	3	0.4992
Test 3	2.37	3	0.4992
Test 4	5.578	2	0.0615
Test 5a	0.0008389	1	0.9769
Test 5b	5.577	1	0.0182
Test 6a	5.578	2	0.0615
Test 6b	0	0	N/A
Test 7a	0	0	N/A
Test 7b	0.0008389	1	0.9769
Test 7c	5.578	2	0.0615

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

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

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

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

The p-value for Test 5a is greater than .1. Model 3 seems to adequately describe the data.

The p-value for Test 5b is less than .05. Model 3 appears to fit the data better than Model 2.

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

Degrees of freedom for Test 6b are less than or equal to 0. The Chi-Square test for fit is not valid.

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

The p-value for Test 7b is greater than .05. Model 5 does not seem to fit the data better than Model 3.

The p-value for Test 7c is greater than .05. Model 5 does not seem to fit the data better than Model 4.

Benchmark Dose Computations:

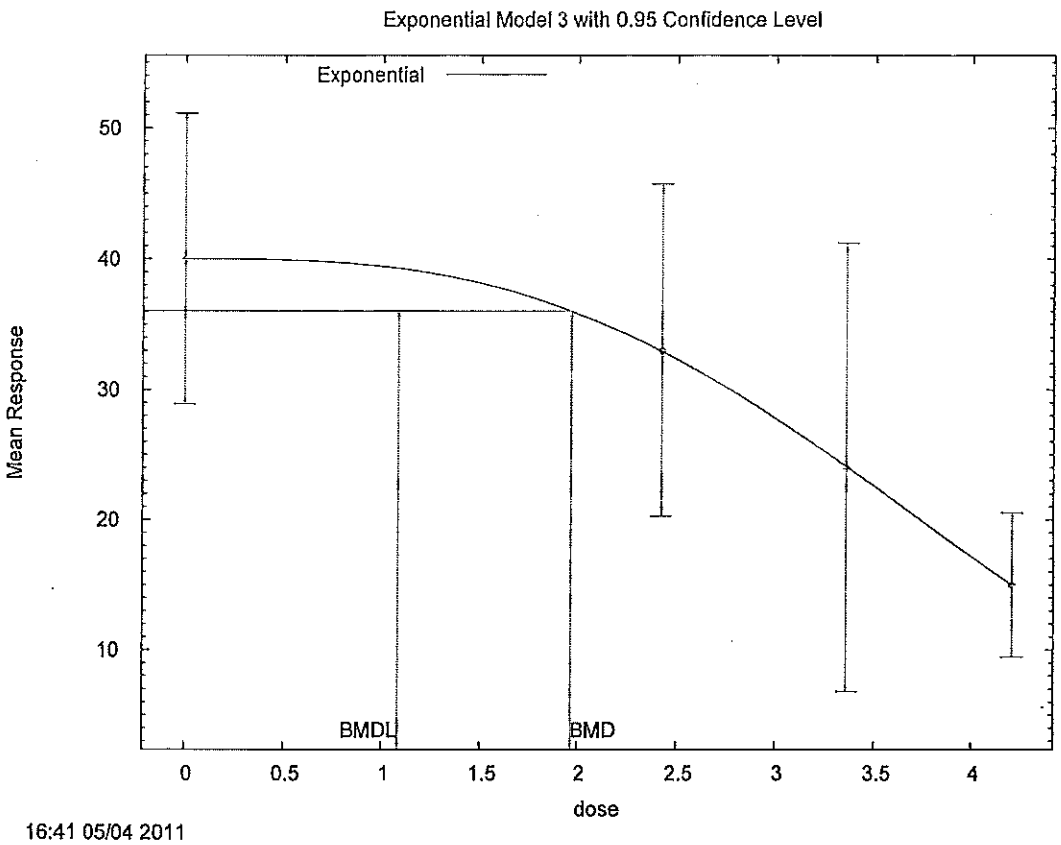
Specified Effect = 0.100000

Risk Type = Absolute deviation

Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL
2	0.0135489	0.00916547
3	0.547859	0.0955696
4	0.0135489	0.00827023
5	0.572901	0.095613


Exponential Model 3 with 0.95 Confidence Level
Exponential
Mean Response
50
40
30
20
10
BMDL
BMD
0
0.5
1
1.5
2
2.5
3
3.5
4
dose
16:41 05/04 2011

APPENDIX 7: Benchmark dose analysis of relative liver weight in response to PFOA (linear/branched) using BMR of 10% increase relative to controls (Loveless et al., 2006).

Model	Chi-sq p-value	AIC	BMD (µg/ml in serum)	BMDL (µg/ml in serum)
Exponential (Models 4 and 5)	**0.2636**	**2.12782**	**4.90419**	**4.46612**
Hill [a]	-	-	-	-
Linear [b]	-	-	-	-
Polynomial (2nd deg)	0.03245 [c]	6.92134	5.31705	4.89558
Polynomial (3rd deg) [d]	**0.4678**	**1.66669**	**4.68238**	**4.23604**

[a] The individual observations in these data have unequal variance in response. When modeled to account for the unequal variance, the Hill model fails (with or without restriction of the exponential function to > 1)

[b] Scaled residuals for one or more doses/serum concentrations for each of the four exponential models were > │2│. The fit of these models is considered inadequate for benchmark dose modeling.

[c] Note that the p-value is less than the value of 0.1 used by the BMDS software as a cut-off for determining goodness of fit by this test. However, the AIC value indicates a reasonable fit.

[d] The 3rd degree polynomial model over fits the data at the high doses (see graphic description). However, the fit at the lower doses (including the doses contributing most to the BMD and BMDL) appears appropriate

Summary

Both the exponential models (models 4 and 5 give identical fits) and the 3rd degree polynomial model give acceptable fits to these data. The 3rd degree polynomial model over fits the data at the high dose, forcing a fit and resulting in a biologically unlikely fit in this area of the dose-response. However, the fit of the model at the lower doses (i.e., in the range of the BMD) is regular and biologically appropriate. It is unlikely that the forced fit at the high dose has any significant influence on the fit of the model at the BMD. Although this model gives a slightly better fit than the exponential models and also yields a slightly lower BMDL, the exponential models give a highly comparable fit and a similar BMDL. As neither model appears to have a claim to greater biological significance, it is recommended that the point-of-departure be derived as the average of the BMDLs for both of these models. **This yields an average BMDL of 4.35 µg/ml.**

```
 ====================================================================
         Exponential Model. (Version: 1.10;  Date: 01/12/2015)
         Input Data File: C:/BMDS2601_20150629/BMDS2601/Data/exp_Loveless 2006 - Mouose- rel
liver wt - linear-branched_Opt.(d)
         Gnuplot Plotting File:
                                                    Thu Mar 24 11:27:03 2016
 ====================================================================

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

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

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

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


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

   Total number of dose groups = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 500
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

   MLE solution provided: Exact


                              Initial Parameter Values

     Variable          Model 2             Model 3             Model 4             Model 5
     --------          -------             -------             -------             -------
     lnalpha           -7.4076             -7.4076             -7.4076             -7.4076
         rho           2.77531             2.77531             2.77531             2.77531
           a           6.24624             6.24624               4.883               4.883
           b        0.00485613          0.00485613           0.0101307           0.0101307
           c                 0 *                 0 *           3.93078             3.93078
           d                 1 *                 1                   1 *                 1

     * Indicates that this parameter has been specified


                             Parameter Estimates by Model

     Variable          Model 2             Model 3            Model 4             Model 5
     --------          -------             -------            -------             -------
     lnalpha         -0.912445           -0.912444            -7.45681            -7.45681
         rho          0.694024            0.694024             2.79344             2.79344
           a           6.58236             6.58236             5.13807             5.13807
           b        0.00447289          0.00447289          0.00664742          0.00664742
           c              --                  --               4.11774             4.11774
           d              --                    1                 --                    1

    -- Indicates that this parameter does not appear in model
```

Std. Err. Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	0.192224	1.23391	0.893622	0.893622
rho	0.536418	0.536418	0.386543	0.386543
a	0.227146	0.227146	0.0668152	0.0668152
b	0.000208654	0.000208654	0.00066635	0.00066635
c	NA	NA	0.180148	0.180147
d	NA	NA	NA	NA

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

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0.04	10	5.14	0.27
10	10	6.12	0.25
27	10	7.92	0.49
66	10	10.72	0.63
190	10	16.27	1.05
241	10	18.28	1.57

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
2	0.04	6.584	1.219	-3.746
	10	6.883	1.238	-1.951
	27	7.427	1.271	1.226
	66	8.843	1.35	4.397
	190	15.4	1.637	1.685
	241	19.34	1.771	-1.899
3	0.04	6.584	1.219	-3.746
	10	6.883	1.238	-1.951
	27	7.427	1.271	1.226
	66	8.843	1.35	4.397
	190	15.4	1.637	1.685
	241	19.34	1.771	-1.899
4	0.04	5.142	0.2366	-0.03107
	10	6.168	0.3051	-0.5007
	27	7.77	0.4211	1.127
	66	10.83	0.6694	-0.5063
	190	16.63	1.219	-0.9262
	241	17.93	1.354	0.8184
5	0.04	5.142	0.2366	-0.03107
	10	6.168	0.3051	-0.5007
	27	7.77	0.4211	1.127
	66	10.83	0.6694	-0.5063
	190	16.63	1.219	-0.9262
	241	17.93	1.354	0.8184

Other models for which likelihoods are calculated:

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

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

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

Model  R:        Yij = Mu + e(i)
```

Var{e(ij)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-17.12125	7	48.2425
A2	6.872288	12	10.25542
A3	5.926418	8	4.147163
R	-126.9627	2	257.9255
2	-50.15017	4	108.3003
3	-50.15017	4	108.3003
4	3.936092	5	2.127815
5	3.936092	5	2.127815

Additive constant for all log-likelihoods = -55.14. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

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

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

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

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

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	267.7	10	< 0.0001
Test 2	47.99	5	< 0.0001
Test 3	1.892	4	0.7557
Test 4	112.2	4	< 0.0001
Test 5a	112.2	4	< 0.0001
Test 5b	-6.736e-012	0	N/A
Test 6a	3.981	3	0.2636
Test 6b	108.2	1	< 0.0001
Test 7a	3.981	3	0.2636
Test 7b	108.2	1	< 0.0001
Test 7c	-3.023e-012	0	N/A

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

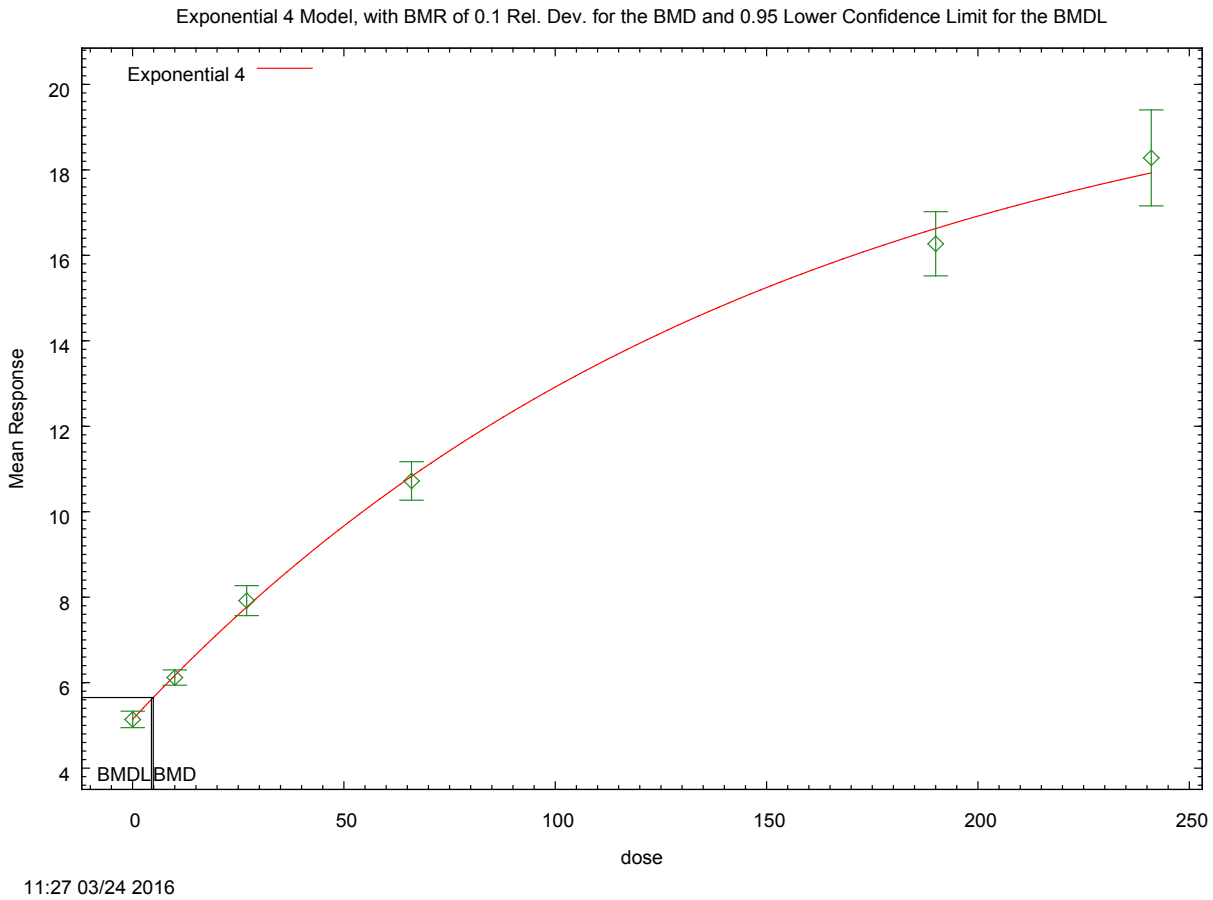
The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

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

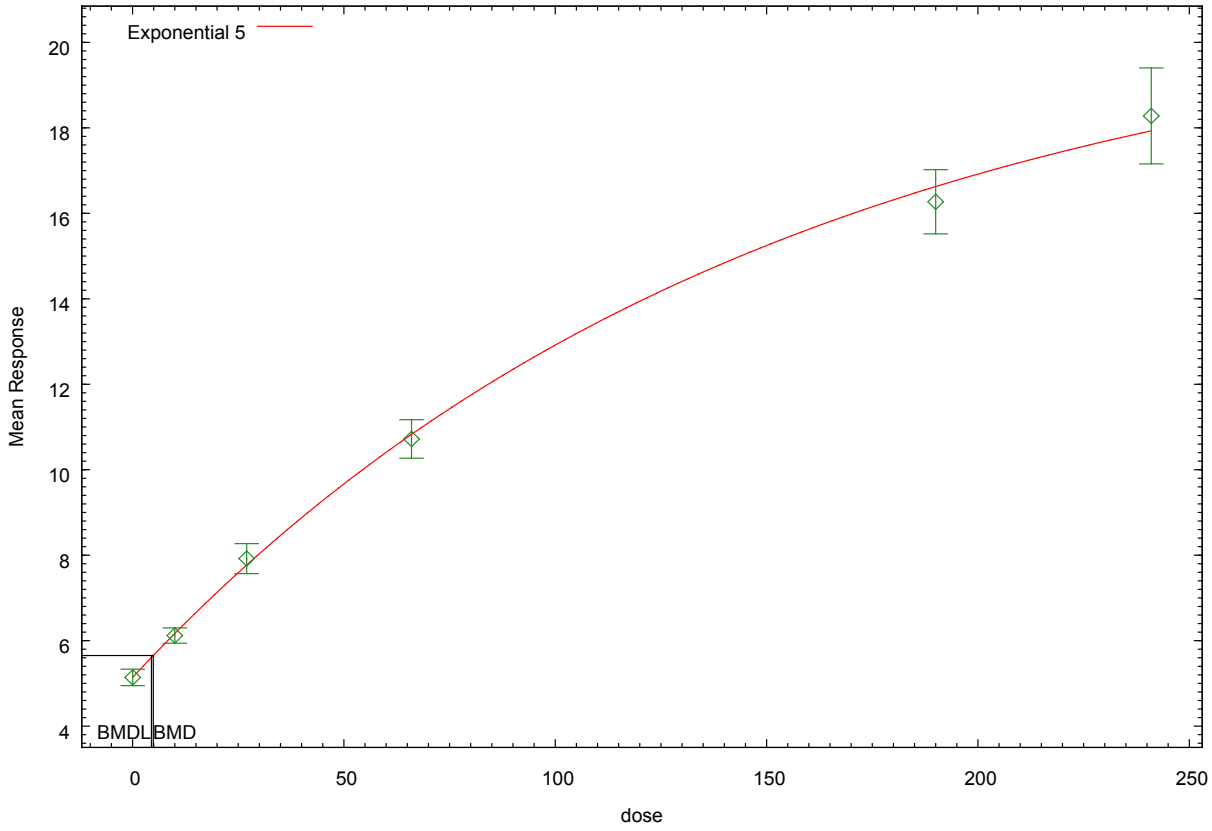
The p-value for Test 4 is less than .1. Model 2 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5a is less than .1. Model 3 may not adequately

```
  describe the data; you may want to consider another model.

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

  The p-value for Test 6a is greater than .1.  Model 4 seems
  to adequately describe the data.

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

  The p-value for Test 7a is greater than .1.  Model 5 seems
  to adequately describe the data.

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

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


Benchmark Dose Computations:

  Specified Effect = 0.100000

         Risk Type = Relative deviation

  Confidence Level = 0.950000


             BMD and BMDL by Model

   Model             BMD                BMDL
  -------        ------------        ----------
     2             21.3084            19.7104
     3             21.3084            19.7104
     4             4.90419            4.46612
     5             4.90419            4.46612
```


Exponential 4 Model, with BMR of 0.1 Rel. Dev. for the BMD and 0.95 Lower Confidence Limit for the BMDL
Exponential 4
Mean Response
20
18
16
14
12
10
8
6
4
BMDL
BMD
0
50
100
150
200
250
dose
11:27 03/24 2016

Exponential 5 Model, with BMR of 0.1 Rel. Dev. for the BMD and 0.95 Lower Confidence Limit for the BMDL



11:26 03/24 2016

```
=====================================================================
         Polynomial Model. (Version: 2.20;  Date: 10/22/2014)
         Input Data File: C:/BMDS2601_20150629/BMDS2601/Data/ply_Loveless 2006 - Mouose- rel
liver wt - linear-branched_Opt.(d)
         Gnuplot Plotting File:  C:/BMDS2601_20150629/BMDS2601/Data/ply_Loveless 2006 - Mouose-
rel liver wt - linear-branched_Opt.plt
                                                    Thu Mar 24 11:40:48 2016
 ====================================================================

 BMDS Model Run
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   The form of the response function is:

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


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

   Total number of dose groups = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 500
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                         lalpha =    -0.323931
                            rho =            0
                         beta_0 =      5.38261
                         beta_1 =    0.0864747
                         beta_2 = -0.000141161


           Asymptotic Correlation Matrix of Parameter Estimates

                 lalpha          rho       beta_0       beta_1       beta_2

    lalpha            1        -0.98         0.17        -0.25         0.24

       rho        -0.98            1        -0.17         0.25        -0.24

    beta_0         0.17        -0.17            1        -0.59          0.5

    beta_1        -0.25         0.25        -0.59            1        -0.94

    beta_2         0.24        -0.24          0.5        -0.94            1


                                  Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         lalpha         -7.55129         0.915261            -9.34516            -5.75741
            rho          2.86927          0.39557             2.09396             3.64457
         beta_0            5.175        0.0667518             5.04417             5.30584
         beta_1        0.0983433        0.0042131           0.0900857            0.106601
         beta_2     -0.000190851     1.99132e-005         -0.00022988        -0.000151822


     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------
```

0.04	10	5.14	5.18	0.27	0.243	-0.508
10	10	6.12	6.14	0.25	0.31	-0.198
27	10	7.92	7.69	0.49	0.428	1.69
66	10	10.7	10.8	0.63	0.7	-0.517
190	10	16.3	17	1.05	1.33	-1.66
241	10	18.3	17.8	1.57	1.43	1.09

Model Descriptions for likelihoods calculated

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

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

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

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

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-17.121248	7	48.242497
A2	6.872288	12	10.255424
A3	5.926418	8	4.147163
fitted	1.539332	5	6.921336
R	-126.962744	2	257.925488

Explanation of Tests

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

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	267.67	10	<.0001
Test 2	47.9871	5	<.0001
Test 3	1.89174	4	0.7557
Test 4	8.77417	3	0.03245

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

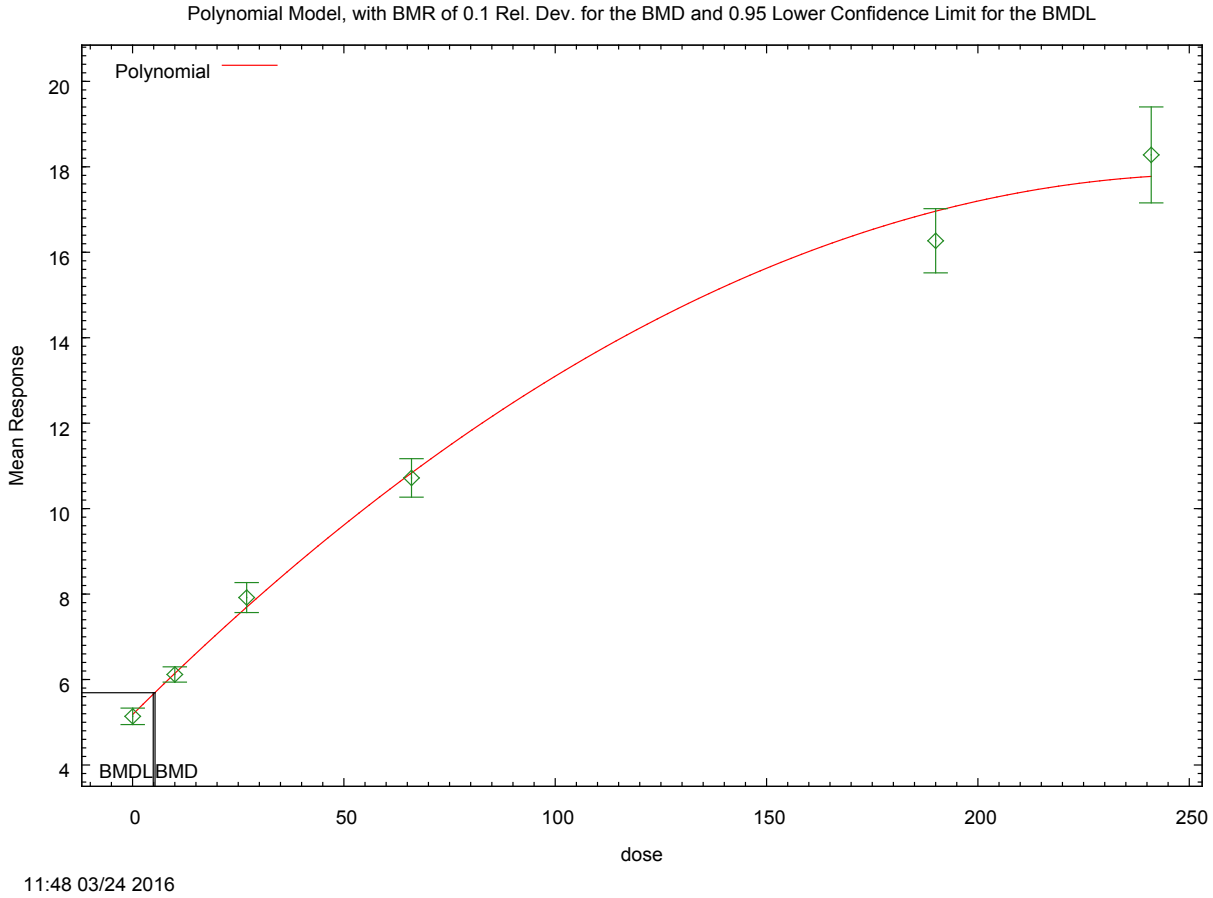
The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

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

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

Benchmark Dose Computation

```
Specified effect =          0.1

Risk Type        =     Relative deviation

Confidence level =          0.95

             BMD =        5.31705

            BMDL =        4.89558
```



```
=====================================================================
         Polynomial Model. (Version: 2.20;  Date: 10/22/2014)
         Input Data File: C:/BMDS2601_20150629/BMDS2601/Data/ply_Loveless 2006 -
Mouose- rel liver wt - linear-branched_Opt.(d)
         Gnuplot Plotting File:  C:/BMDS2601_20150629/BMDS2601/Data/ply_Loveless 2006
- Mouose- rel liver wt - linear-branched_Opt.plt
                                                Thu Mar 24 11:45:36 2016
 ====================================================================

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

   The form of the response function is:

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


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

   Total number of dose groups = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 500
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                         lalpha =    -0.323931
                            rho =            0
                         beta_0 =      5.13303
                         beta_1 =     0.109803
                         beta_2 = -0.000421158
                         beta_3 = 7.96683e-007


           Asymptotic Correlation Matrix of Parameter Estimates

                  lalpha          rho       beta_0       beta_1       beta_2
beta_3

    lalpha            1        -0.98        0.012       -0.028        0.043       -
0.048

       rho        -0.98            1       -0.012        0.031        -0.05
0.056

    beta_0        0.012       -0.012            1        -0.59          0.4        -
0.32

    beta_1       -0.028        0.031        -0.59            1        -0.88
0.78

    beta_2        0.043        -0.05          0.4        -0.88            1        -
0.98

    beta_3       -0.048        0.056        -0.32         0.78        -0.98
1
```

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	-7.33636	0.87882	-9.05882	-5.6139
rho	2.72236	0.379829	1.97791	3.46681
beta_0	5.11878	0.0674735	4.98653	5.25102
beta_1	0.111381	0.00620885	0.0992116	0.12355
beta_2	-0.000444193	9.26512e-005	-0.000625786	-0.0002626
beta_3	8.69446e-007	3.09892e-007	2.62068e-007	1.47682e-006

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0.04	10	5.14	5.12	0.27	0.236	0.225
10	10	6.12	6.19	0.25	0.305	-0.715
27	10	7.92	7.82	0.49	0.419	0.759
66	10	10.7	10.8	0.63	0.65	-0.316
190	10	16.3	16.2	1.05	1.13	0.17
241	10	18.3	18.3	1.57	1.34	-0.124

Model Descriptions for likelihoods calculated

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

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

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

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

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-17.121248	7	48.242497
A2	6.872288	12	10.255424
A3	5.926418	8	4.147163
fitted	5.166654	6	1.666693
R	-126.962744	2	257.925488

Explanation of Tests

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

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	267.67	10	<.0001
Test 2	47.9871	5	<.0001
Test 3	1.89174	4	0.7557
Test 4	1.51953	2	0.4678

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

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

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

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

Specified effect = 0.1

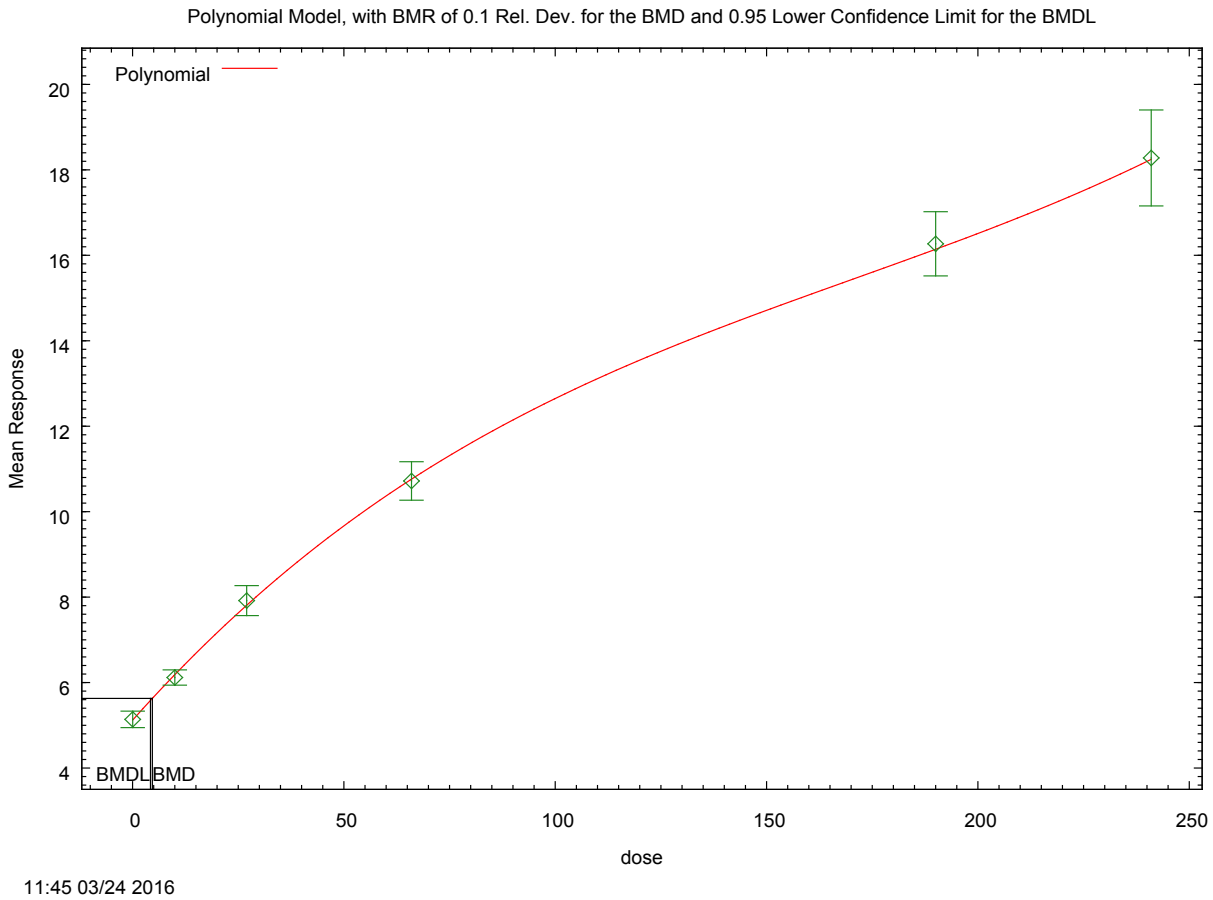
Risk Type = Relative deviation

Confidence level = 0.95

BMD = 4.68238

BMDL = 4.23604

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


Polynomial Model, with BMR of 0.1 Rel. Dev. for the BMD and 0.95 Lower Confidence Limit for the BMDL
Polynomial
Mean Response
20
18
16
14
12
10
8
6
4
BMDL
BMD
0
50
100
150
200
250
dose
11:45 03/24 2016

APPENDIX 8: Benchmark dose analysis of testicular tumor data in response to PFOA using BMR of 5% tumor incidence (Butenhoff et al., 2012)

Model	Chi-sq stat	Chi-sq p-value	AIC	BMD (mg/kg/d)	BMDL (mg/kg/d)	Cancer Potency Slope $(mg/kg/d)^{-1}$	Dose at 1×10^{-6} risk (mg/kg/d)
Gamma (power restricted to ≥ 1) **Multistage (betas ≥ 0)** **Weibull (power restricted to ≥ 1)** **Quantal linear**	**1.45**	**0.2292**	**62.6851**	**4.42913**	**2.50664**	**0.020**	**5.0×10^{-5}**
Gamma (power unrestricted)	0.00	1.00	61.2908	4.42913	1.36483e-6		
Log-logistic (power unrestricted)	0.00	1.00	61.2908	1.95859	2.00091e-6		
Logisitic	1.71	0.1905	63.6843	8.85708	6.49805		
Log-logistic (slope restricted to ≤ 1)	**1.42**	**0.2338**	**62.5526**	**4.02707**	**2.2101**	**0.023**	**4.3×10^{-5}**
Probit	1.68	0.1948	63.625	8.32341	5.95965		
Weibull (power unrestricted)	0.00	1.00	61.2908	1.97407	1.65976e-6		

Summary

The BMR of 0.05 (5%) was selected for consistency with the recommendations for selection of point-of-departure (POD) for cancer potency slope derivation in the USEPA (2005) Guidance for Carcinogen Risk Assessment. This value is close to the 4% response at the lowest dose in this data set. The Gamma model (power restricted to ≥ 1); Multistage model (betas ≥ 0); Weibull

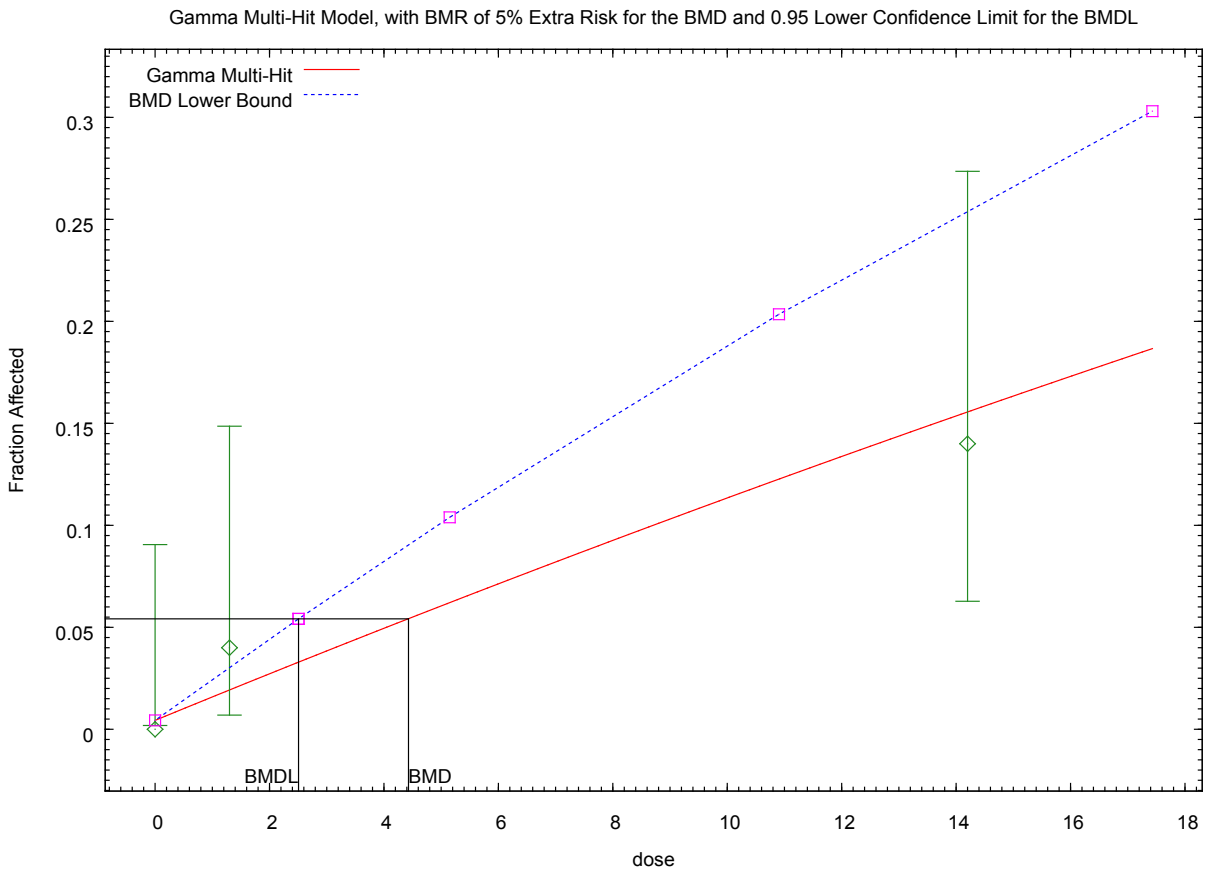
model (power restricted to ≥ 1); and Quantal linear model all give good and essentially identical fits to this dataset and consequently yield identical BMDLs and cancer potency slopes. The Log-logistic model (slope restricted to ≤ 1) gives a closely comparable fit to these data and a similar BMDLs and cancer potency estimate. These models were selected as most appropriate for deriving a cancer potency slope from these data.

The Gamma model with power unrestricted; the Log-logistic model with power unrestricted; and the Weibull model with power unrestricted all fit the data exactly and yield very small BMDLs. However, despite their better fit of the data than the selected models, these models are not considered appropriate for derivation of a cancer potency slope from these data. This is because these models all assume a steep dose-response relationship at low doses which becomes much less steep at higher doses. The steepness of the dose-response at low doses (i.e., doses associated with a less than 5% response) leads to the very small BMDL values associated with these models. The steepness of the dose-response at low doses is a highly specific assumption which is not necessary and which is not dictated by any empirical or theoretical consideration. These models are therefore rejected.

The Gamma, power restricted to ≥ 1, (and the other models giving essentially identical results) and the Log-logistic model (with slope restricted to ≤ 1) fit the data with very similar fits and yield very similar BMDLs. Additionally, none of these models has a form that is obviously more biologically accurate. For these reasons, it is appropriate to average their BMDLs. The average BMDL for these models is 2.36 mg/kg/day. For a 5% BMR, the corresponding linear (no threshold) cancer potency slope is 0.021 $(mg/kg/day)^{-}$, and the dose corresponding to a 1×10^{-6} risk is 4.8×10^{-5} mg/kg/day.

```
 ========================================================================
          Gamma Model. (Version: 2.16;  Date: 2/28/2013)
          Input Data File: C:/BMDS2601_20150629/BMDS2601/Data/gam_butenhoff testicular
tumors_Opt.(d)
          Gnuplot Plotting File:  C:/BMDS2601_20150629/BMDS2601/Data/gam_butenhoff testicular
tumors_Opt.plt
                                                     Tue Mar 01 13:55:34 2016
 ========================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response]= background+(1-background)*CumGamma[slope*dose,power],
   where CumGamma(.) is the cummulative Gamma distribution function


   Dependent variable = Incidence
   Independent variable = Dose
   Power parameter is restricted as power >=1

   Total number of observations = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 500
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial (and Specified) Parameter Values
                     Background =    0.0196078
                          Slope =    0.0294212
                          Power =          1.3


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Power
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

             Background        Slope

Background            1        -0.58

     Slope        -0.58            1



                                 Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background       0.00436511        0.0163662           -0.027712           0.0364423
          Slope        0.0115809       0.00506549           0.0016527           0.0215091
          Power                1               NA

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -28.6454         3
   Fitted model        -29.3426         2       1.39438      1          0.2377
  Reduced model         -33.983         1       10.6753      2        0.004807
```

```
          AIC:         62.6851

                              Goodness  of  Fit
                                                                    Scaled
    Dose      Est._Prob.    Expected    Observed     Size        Residual
  ----------------------------------------------------------------------
    0.0000     0.0044         0.214      0.000      49.000       -0.463
    1.3000     0.0192         0.962      2.000      50.000        1.068
   14.2000     0.1553         7.767      7.000      50.000       -0.299

 Chi^2 = 1.45      d.f. = 1        P-value = 0.2292


   Benchmark Dose Computation

Specified effect =           0.05

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        4.42913

            BMDL =       2.50664
```



13:55 03/01 2016

```
 ======================================================================
          Multistage Model. (Version: 3.4;  Date: 05/02/2014)
          Input Data File: C:/BMDS2601_20150629/BMDS2601/Data/mst_butenhoff testicular
tumors_Opt.(d)
          Gnuplot Plotting File:  C:/BMDS2601_20150629/BMDS2601/Data/mst_butenhoff testicular
tumors_Opt.plt
                                                        Tue Mar 01 14:29:06 2016
 ======================================================================
 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive


   Dependent variable = Incidence
   Independent variable = Dose

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 Maximum number of iterations = 500
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                     Background =    0.0132945
                        Beta(1) =    0.0097738
                        Beta(2) =            0


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Beta(2)
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

             Background      Beta(1)

Background            1        -0.58

   Beta(1)        -0.58            1


                                 Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background       0.00436512        0.0163652          -0.0277101           0.0364403
        Beta(1)        0.0115808       0.00506553          0.00165257           0.0215091
        Beta(2)                0               NA

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.


                         Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
```

Full model	-28.6454	3			
Fitted model	-29.3426	2	1.39438	1	0.2377
Reduced model	-33.983	1	10.6753	2	0.004807

AIC: 62.6851

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0044	0.214	0.000	49.000	-0.463
1.3000	0.0192	0.962	2.000	50.000	1.068
14.2000	0.1553	7.767	7.000	50.000	-0.299

Chi^2 = 1.45 d.f. = 1 P-value = 0.2292

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

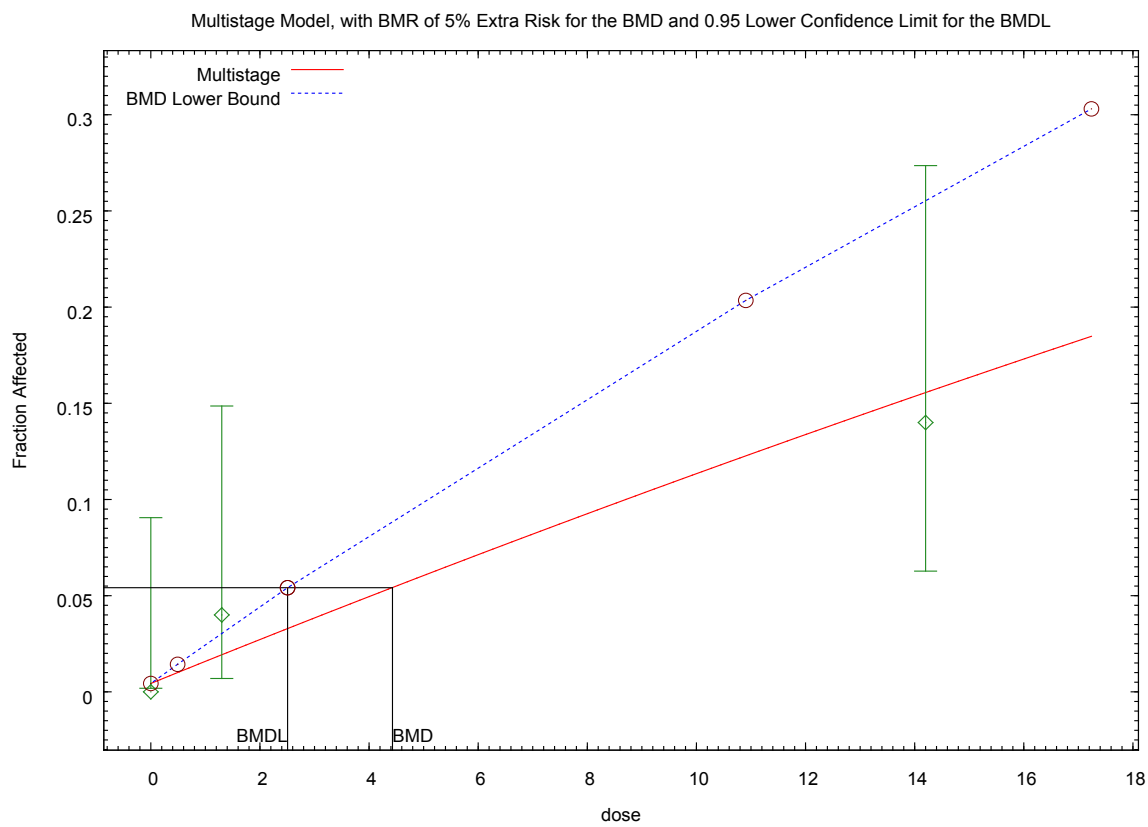
Confidence level = 0.95

BMD = 4.42916

BMDL = 2.50664

BMDU = 12.158

Taken together, (2.50664, 12.158) is a 90 % two-sided confidence interval for the BMD



14:29 03/01 2016

```
=================================================================
          Weibull Model using Weibull Model (Version: 2.16;  Date: 2/28/2013)
          Input Data File: C:/BMDS2601_20150629/BMDS2601/Data/wei_butenhoff testicular
tumors_Opt.(d)
          Gnuplot Plotting File:  C:/BMDS2601_20150629/BMDS2601/Data/wei_butenhoff testicular
tumors_Opt.plt
                                                    Tue Mar 01 14:38:20 2016
 =================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]


   Dependent variable = Incidence
   Independent variable = Dose
   Power parameter is restricted as power >= 1.000000

   Total number of observations = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 500
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial (and Specified) Parameter Values
                     Background =    0.0196078
                          Slope =    0.0103698
                          Power =            1


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Power
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

             Background        Slope

Background            1        -0.58

     Slope        -0.58            1



                                 Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background       0.00436513        0.0163652          -0.0277102           0.0364404
          Slope        0.0115809       0.00506554          0.00165257           0.0215091
          Power                1               NA

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.



                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -28.6454         3
   Fitted model        -29.3426         2       1.39438      1          0.2377
```

```
 Reduced model          -33.983          1        10.6753       2         0.004807

          AIC:          62.6851
```

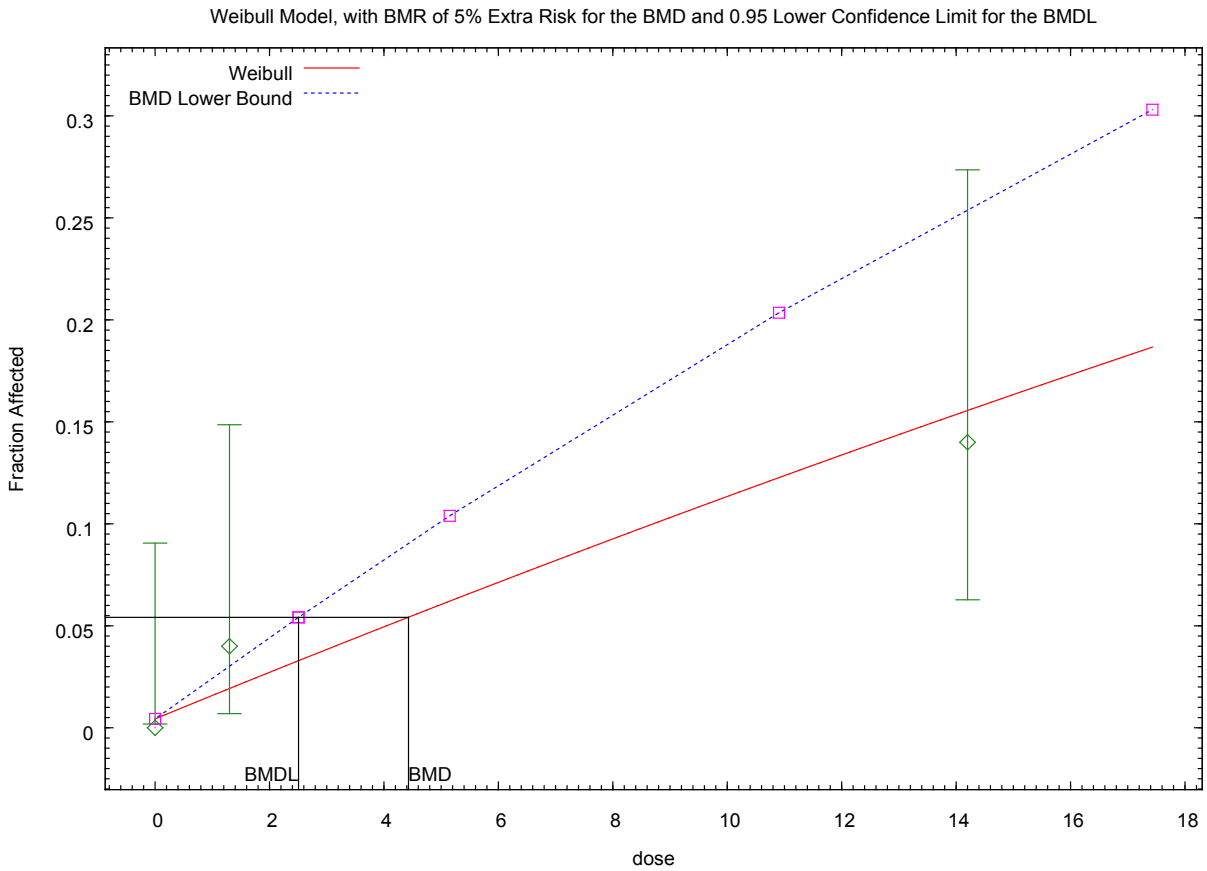
Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0044	0.214	0.000	49.000	-0.463
1.3000	0.0192	0.962	2.000	50.000	1.068
14.2000	0.1553	7.767	7.000	50.000	-0.299

```
Chi^2 = 1.45      d.f. = 1        P-value = 0.2292
```

Benchmark Dose Computation

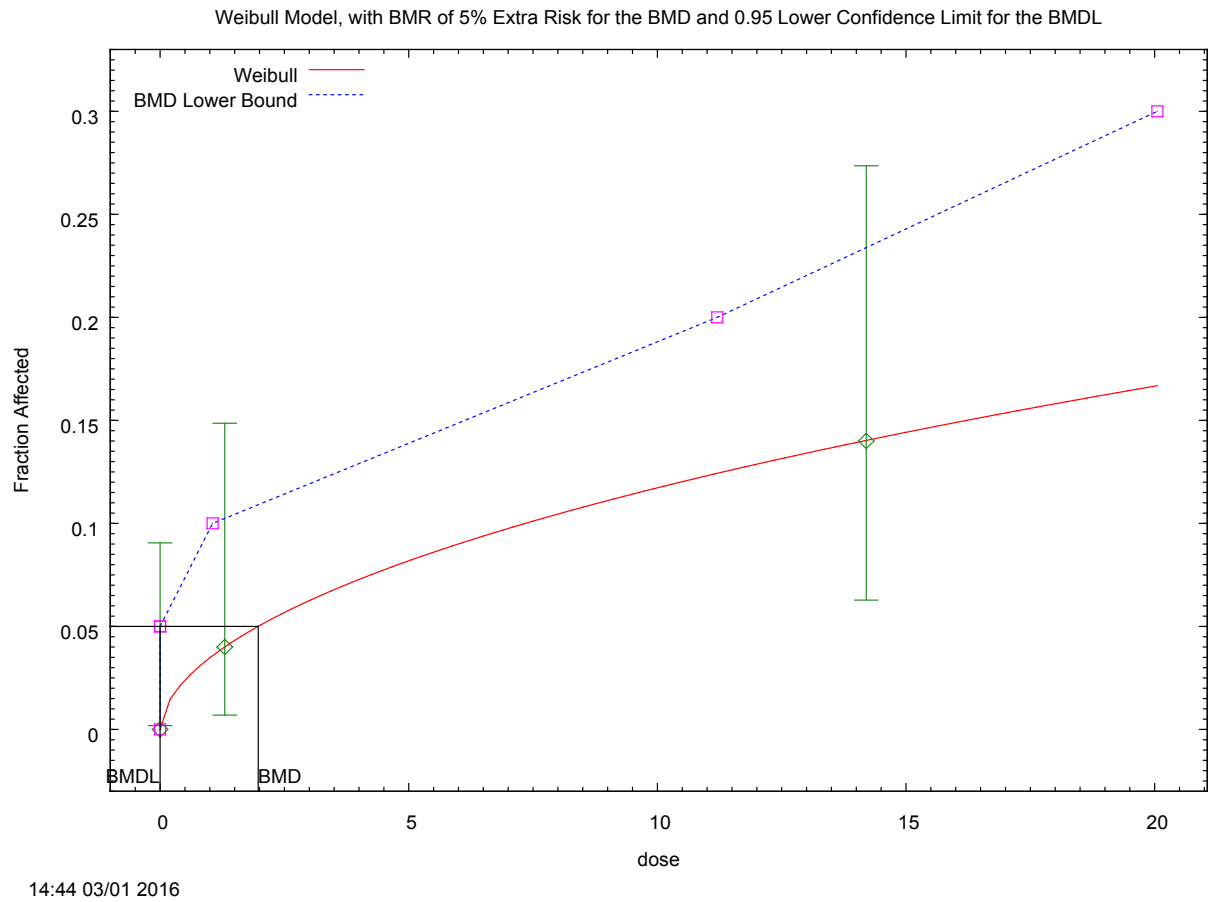
```
Specified effect =           0.05

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        4.42915

            BMDL =       2.50664
```



14:38 03/01 2016

```
=====================================================================
         Weibull Model using Weibull Model (Version: 2.16;  Date: 2/28/2013)
         Input Data File: C:/BMDS2601_20150629/BMDS2601/Data/wei_butenhoff testicular
tumors_Opt.(d)
         Gnuplot Plotting File:  C:/BMDS2601_20150629/BMDS2601/Data/wei_butenhoff testicular
tumors_Opt.plt
                                                    Tue Mar 01 14:44:17 2016
 =====================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]


   Dependent variable = Incidence
   Independent variable = Dose
   Power parameter is not restricted

   Total number of observations = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 500
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial (and Specified) Parameter Values
                     Background =    0.0196078
                          Slope =    0.0214176
                          Power =     0.726632


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                  Slope        Power

     Slope            1        -0.91

     Power        -0.91            1



                                 Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background                0               NA
          Slope        0.0353681        0.0277941          -0.0191074           0.0898435
          Power         0.546613          0.33544           -0.110837             1.20406

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.

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


                       Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -28.6454         3
   Fitted model        -28.6454         2 -7.10543e-015      1                  -1
  Reduced model         -33.983         1       10.6753      2        0.004807
```

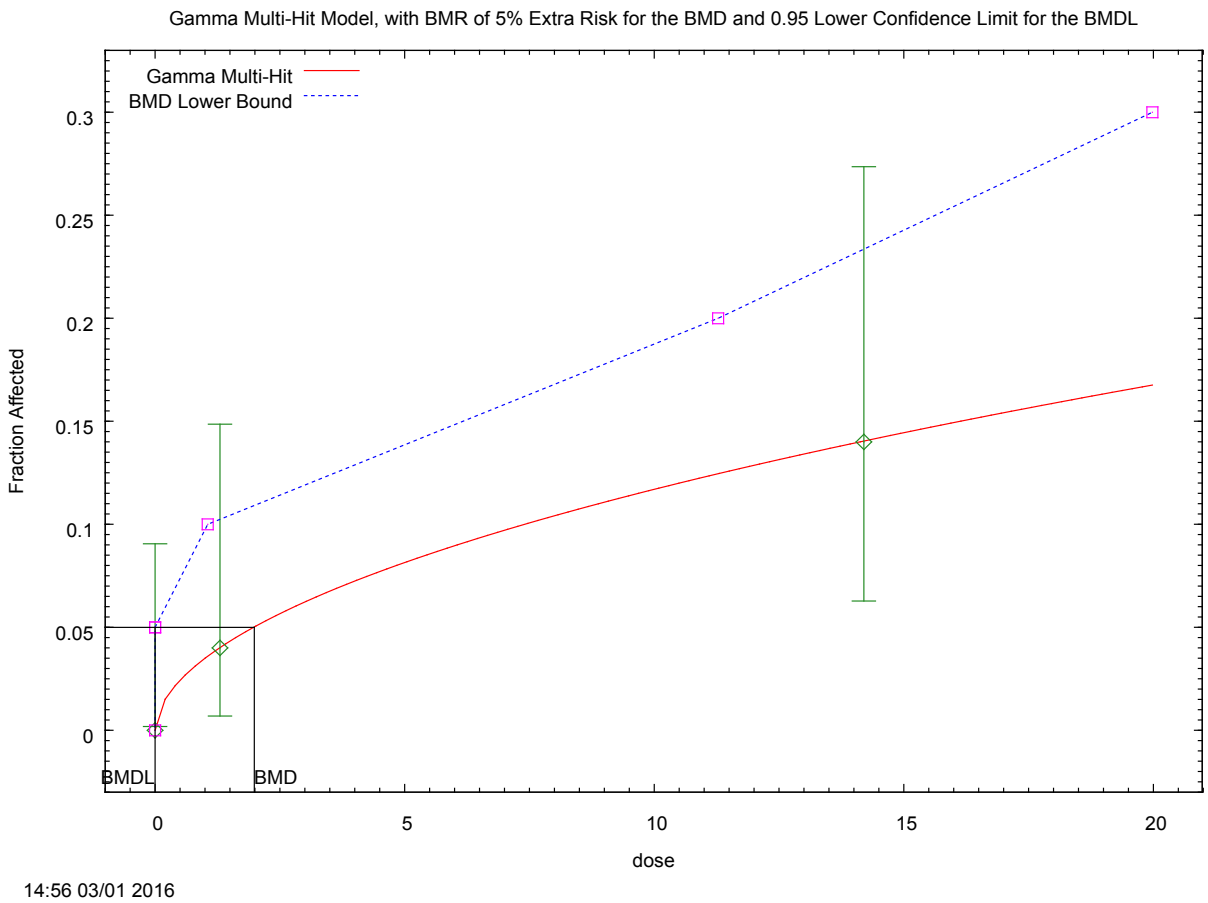
```
AIC:          61.2908

                          Goodness  of  Fit
                                                          Scaled
    Dose      Est._Prob.    Expected    Observed     Size       Residual
 ----------------------------------------------------------------------
   0.0000      0.0000         0.000      0.000      49.000        0.000
   1.3000      0.0400         2.000      2.000      50.000        0.000
  14.2000      0.1400         7.000      7.000      50.000       -0.000

 Chi^2 = 0.00      d.f. = 1        P-value = 1.0000


   Benchmark Dose Computation

Specified effect =           0.05

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        1.97407

            BMDL =  1.65976e-006
```



14:44 03/01 2016

```
=====================================================================
          Gamma Model. (Version: 2.16;  Date: 2/28/2013)
          Input Data File: C:/BMDS2601_20150629/BMDS2601/Data/gam_butenhoff testicular
tumors_Opt.(d)
          Gnuplot Plotting File:  C:/BMDS2601_20150629/BMDS2601/Data/gam_butenhoff testicular
tumors_Opt.plt
                                                     Tue Mar 01 14:56:54 2016
 =====================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response]= background+(1-background)*CumGamma[slope*dose,power],
   where CumGamma(.) is the cummulative Gamma distribution function


   Dependent variable = Incidence
   Independent variable = Dose
   Power parameter is not restricted

   Total number of observations = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 500
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial (and Specified) Parameter Values
                     Background =    0.0196078
                          Slope =    0.0294212
                          Power =          1.3


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                  Slope        Power

     Slope            1         0.98

     Power         0.98            1



                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background                0              NA
          Slope       0.00135774       0.00399687         -0.00647598          0.00919146
          Power         0.526497         0.333129           -0.126424             1.17942

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.



                         Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -28.6454         3
   Fitted model        -28.6454         2  1.66125e-010      1               1
  Reduced model         -33.983         1       10.6753      2        0.004807
```

```
          AIC:         61.2908

                                Goodness  of  Fit
                                                                      Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ----------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000      49.000        0.000
    1.3000     0.0400         2.000     2.000      50.000        0.000
   14.2000     0.1400         7.000     7.000      50.000        0.000

 Chi^2 = 0.00      d.f. = 1        P-value = 1.0000


   Benchmark Dose Computation

Specified effect =           0.05

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        1.98737

            BMDL =  1.36483e-006
```




```
=====================================================================
          Logistic Model. (Version: 2.14; Date: 2/28/2013)
          Input Data File: C:/BMDS2601_20150629/BMDS2601/Data/lnl_butenhoff testicular
tumors_Opt.(d)
          Gnuplot Plotting File:  C:/BMDS2601_20150629/BMDS2601/Data/lnl_butenhoff testicular
tumors_Opt.plt
                                                    Tue Mar 01 15:00:41 2016
 ====================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = Incidence
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 500
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
```

User has chosen the log transformed model

Default Initial Parameter Values
background = 0
intercept = -4.17047
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -slope
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	background	intercept
background	1	-0.63
intercept	-0.63	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0.00240787	0.0171729	-0.0312504	0.0360661
intercept	-4.33748	0.484279	-5.28665	-3.38831
slope	1	NA		

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-28.6454	3			
Fitted model	-29.2763	2	1.26188	1	0.2613
Reduced model	-33.983	1	10.6753	2	0.004807

AIC: 62.5526

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0024	0.118	0.000	49.000	-0.344
1.3000	0.0191	0.954	2.000	50.000	1.082
14.2000	0.1586	7.928	7.000	50.000	-0.359

Chi^2 = 1.42 d.f. = 1 P-value = 0.2338

Benchmark Dose Computation

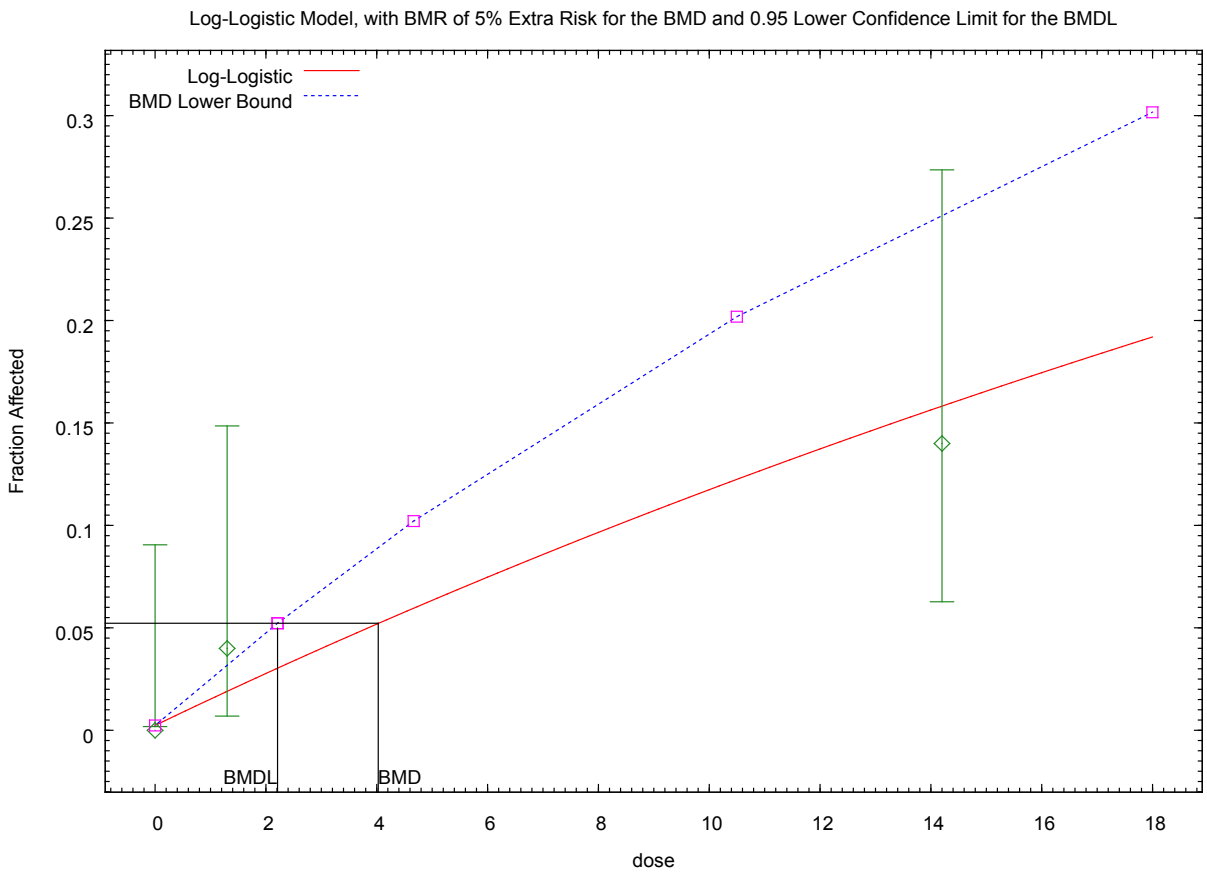
Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

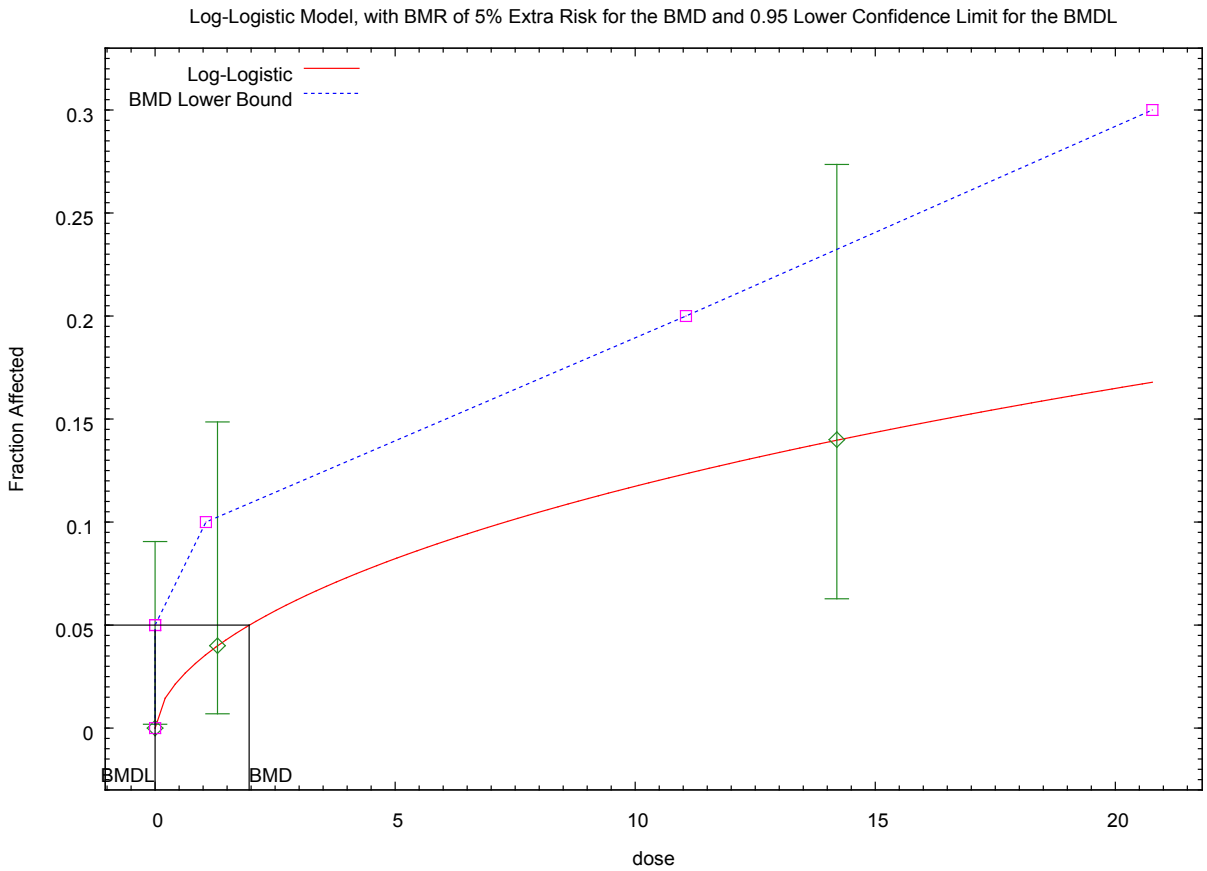
BMD = 4.02707

BMDL = 2.2101




```
=====================================================================
          Logistic Model. (Version: 2.14; Date: 2/28/2013)
          Input Data File: C:/BMDS2601_20150629/BMDS2601/Data/lnl_butenhoff testicular
tumors_Opt.(d)
          Gnuplot Plotting File:  C:/BMDS2601_20150629/BMDS2601/Data/lnl_butenhoff testicular
tumors_Opt.plt
                                                    Tue Mar 01 15:07:36 2016
 =====================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = Incidence
   Independent variable = Dose
   Slope parameter is not restricted

   Total number of observations = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 500
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model
```

```
                 Default Initial Parameter Values
                    background =            0
                     intercept =      -3.3276
                         slope =     0.569985


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -background
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

             intercept        slope

 intercept            1         -0.9

     slope         -0.9            1



                              Parameter Estimates

                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    background                0               NA
     intercept          -3.3276         0.802128            -4.89974            -1.75546
         slope         0.569985         0.346658           -0.109453             1.24942

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.

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


                       Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
    Full model        -28.6454         3
  Fitted model        -28.6454         2 -7.10543e-015      1                  -1
 Reduced model         -33.983         1       10.6753      2        0.004807

          AIC:         61.2908


                                 Goodness  of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ----------------------------------------------------------------------
   0.0000     0.0000         0.000     0.000      49.000        0.000
   1.3000     0.0400         2.000     2.000      50.000       -0.000
  14.2000     0.1400         7.000     7.000      50.000       -0.000

 Chi^2 = 0.00      d.f. = 1        P-value = 1.0000


   Benchmark Dose Computation

Specified effect =           0.05

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        1.95859

            BMDL =   2.00091e-006
```



15:07 03/01 2016

```
=====================================================================
          Logistic Model. (Version: 2.14; Date: 2/28/2013)
          Input Data File: C:/BMDS2601_20150629/BMDS2601/Data/log_butenhoff testicular
tumors_Opt.(d)
          Gnuplot Plotting File:  C:/BMDS2601_20150629/BMDS2601/Data/log_butenhoff testicular
tumors_Opt.plt
                                                    Tue Mar 01 15:17:50 2016
 ====================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = 1/[1+EXP(-intercept-slope*dose)]


   Dependent variable = Incidence
   Independent variable = Dose
   Slope parameter is not restricted

   Total number of observations = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 500
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                    Default Initial Parameter Values
                       background =            0   Specified
                        intercept =     -3.91633
```

slope = 0.156823

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	intercept	slope
intercept	1	-0.89
slope	-0.89	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
intercept	-4.0346	0.768353	-5.54055	-2.52866
slope	0.157289	0.0618536	0.0360579	0.27852

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-28.6454	3			
Fitted model	-29.8421	2	2.3935	1	0.1218
Reduced model	-33.983	1	10.6753	2	0.004807

AIC: 63.6843

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0174	0.852	0.000	49.000	-0.931
1.3000	0.0212	1.062	2.000	50.000	0.920
14.2000	0.1417	7.086	7.000	50.000	-0.035

Chi^2 = 1.71 d.f. = 1 P-value = 0.1905

Benchmark Dose Computation

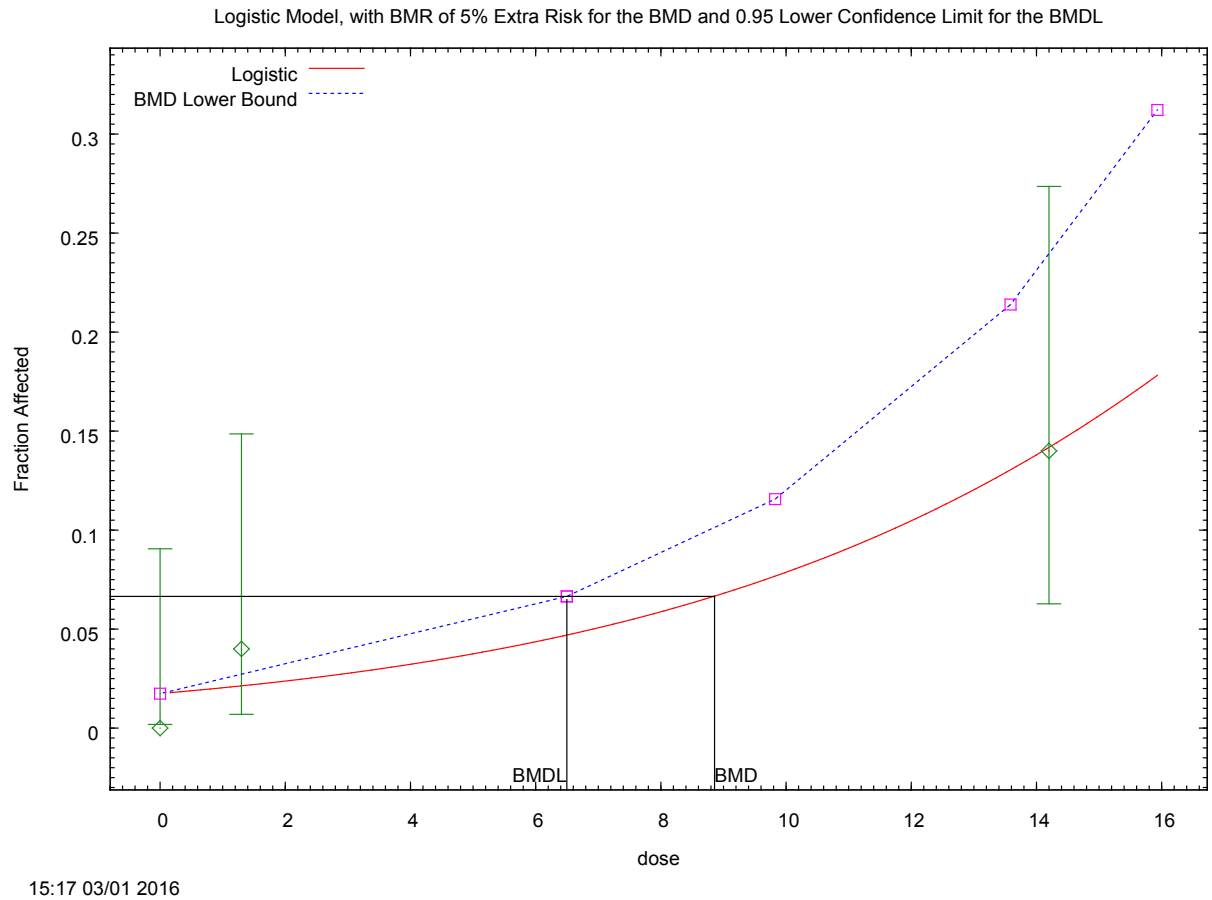
Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

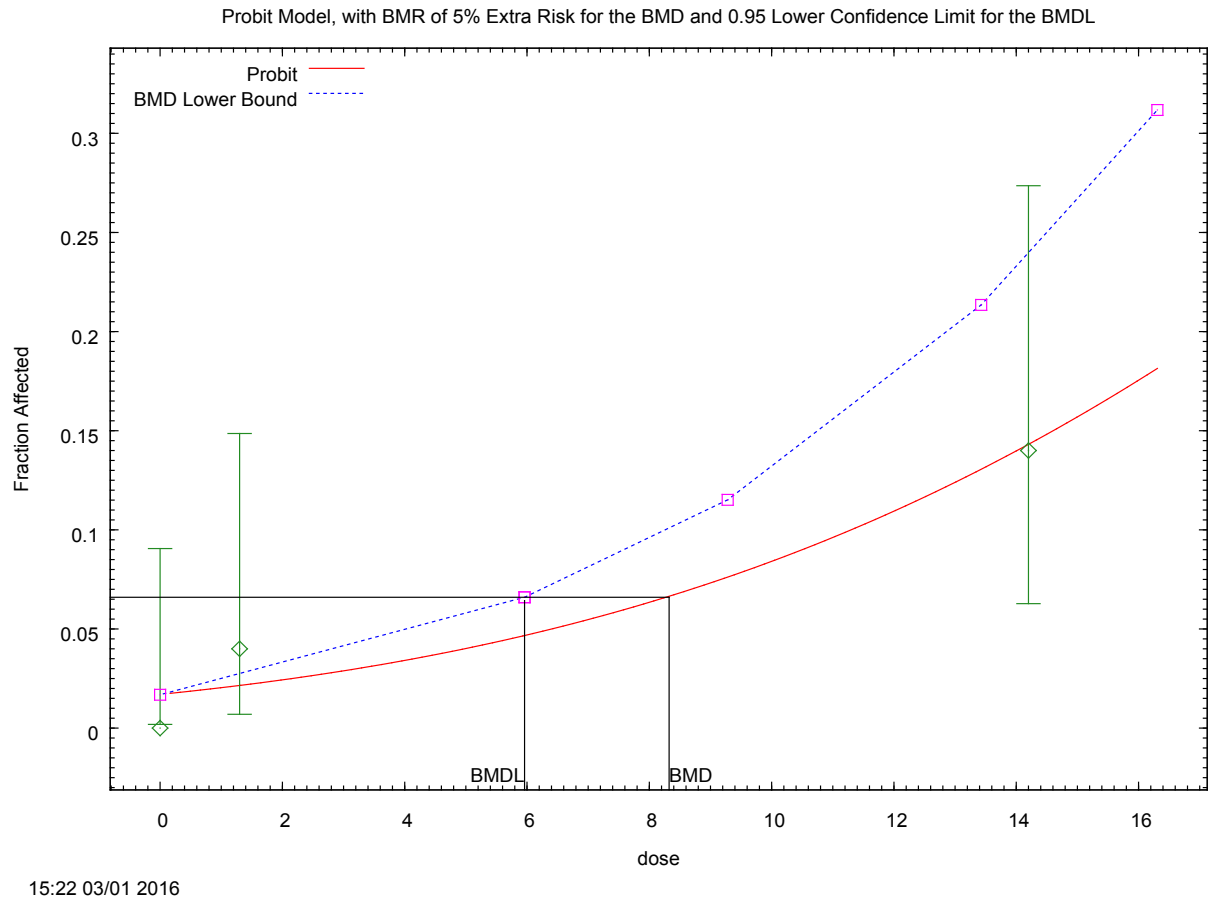
BMD = 8.85708

BMDL = 6.49805




```
=====================================================================
          Probit Model. (Version: 3.3;  Date: 2/28/2013)
          Input Data File: C:/BMDS2601_20150629/BMDS2601/Data/pro_butenhoff testicular
tumors_Opt.(d)
          Gnuplot Plotting File:  C:/BMDS2601_20150629/BMDS2601/Data/pro_butenhoff testicular
tumors_Opt.plt
                                                    Tue Mar 01 15:22:19 2016
 ====================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = CumNorm(Intercept+Slope*Dose),

   where CumNorm(.) is the cumulative normal distribution function


   Dependent variable = Incidence
   Independent variable = Dose
   Slope parameter is not restricted

   Total number of observations = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 500
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
```

```
Default Initial (and Specified) Parameter Values
   background =            0   Specified
    intercept =     -2.30551
        slope =     0.089861


Asymptotic Correlation Matrix of Parameter Estimates

( *** The model parameter(s)  -background
      have been estimated at a boundary point, or have been specified by the user,
      and do not appear in the correlation matrix )

             intercept        slope

 intercept           1        -0.83

     slope       -0.83            1


                          Parameter Estimates

                                                     95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     intercept         -2.12433         0.312226            -2.73628            -1.51238
         slope        0.0742381        0.0273837            0.020567            0.127909


                     Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
    Full model        -28.6454         3
   Fitted model       -29.8125         2       2.33427      1          0.1266
  Reduced model        -33.983         1       10.6753      2        0.004807

          AIC:          63.625


                          Goodness  of  Fit
                                                                  Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ----------------------------------------------------------------------
   0.0000     0.0168         0.824     0.000      49.000       -0.916
   1.3000     0.0213         1.064     2.000      50.000        0.917
  14.2000     0.1423         7.114     7.000      50.000       -0.046

 Chi^2 = 1.68      d.f. = 1        P-value = 0.1948


   Benchmark Dose Computation

Specified effect =           0.05

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        8.32341

            BMDL =        5.95965
```




```
=====================================================================
          Quantal Linear Model using Weibull Model (Version: 2.16;  Date: 2/28/2013)
          Input Data File: C:/BMDS2601_20150629/BMDS2601/Data/qln_butenhoff testicular
tumors_Opt.(d)
          Gnuplot Plotting File:  C:/BMDS2601_20150629/BMDS2601/Data/qln_butenhoff testicular
tumors_Opt.plt
                                                    Tue Mar 01 15:30:52 2016
 ====================================================================

 BMDS_Model_Run
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose)]


   Dependent variable = Incidence
   Independent variable = Dose

   Total number of observations = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 500
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                  Default Initial (and Specified) Parameter Values
                     Background =    0.0196078
                          Slope =    0.0103698
```

Power = 1 Specified

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Power
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Background	Slope
Background	1	-0.58
Slope	-0.58	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.00436513	0.0163652	-0.0277102	0.0364404
Slope	0.0115809	0.00506554	0.00165257	0.0215091

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-28.6454	3			
Fitted model	-29.3426	2	1.39438	1	0.2377
Reduced model	-33.983	1	10.6753	2	0.004807

AIC: 62.6851

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0044	0.214	0.000	49.000	-0.463
1.3000	0.0192	0.962	2.000	50.000	1.068
14.2000	0.1553	7.767	7.000	50.000	-0.299

Chi^2 = 1.45 d.f. = 1 P-value = 0.2292

Benchmark Dose Computation

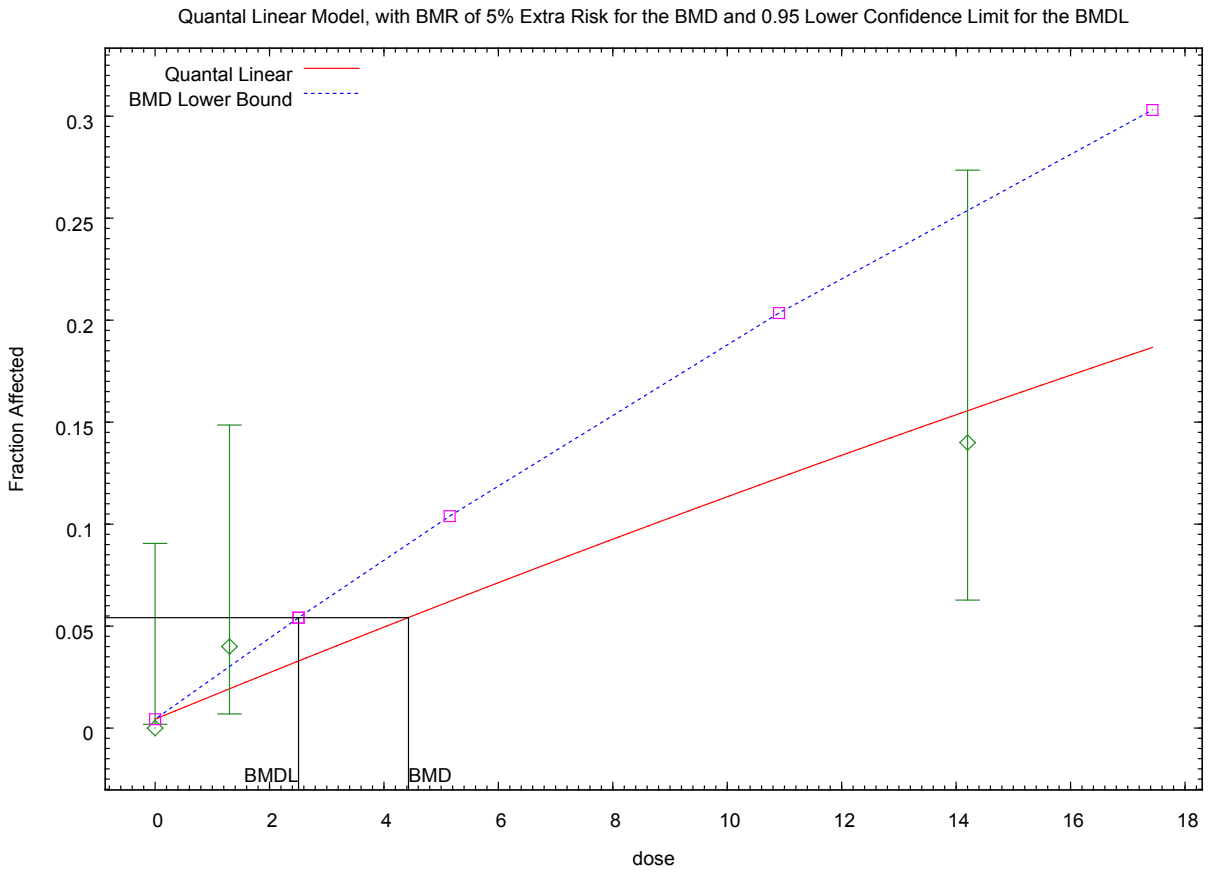
Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMD = 4.42915

BMDL = 2.50664



15:30 03/01 2016

November 19, 2016

watersupply@dep.nj.gov
New Jersey Department of Environmental Protection
Trenton, New Jersey

Re: Health-Based Maximum Contaminant Level Support Document: Perfluorooctanoic Acid (PFOA)

Please find enclosed a technical analysis prepared by Fardin Oliaei, MPA, PhD, and Don Kriens, Sc.D., P.E. of Cambridge Environmental Consulting commissioned by Delaware Riverkeeper Network and submitted on behalf of the organization and its membership regarding the Support Document and recommendation by the Drinking Water Quality Institute for a **Health-Based Maximum Contaminant Level for Perfluorooctanoic Acid (PFOA)**. Also attached are two PDFs containing the Curriculum Vitae for Dr. Oliaei and for Don Kriens, Sc.D., P.E.

Delaware Riverkeeper Network submits these comments advocating that the public be protected from PFOA contamination and that New Jersey's drinking water be required to be treated to a safe level based on the best available scientific evidence.

We support all the recommendations and findings made by Dr. Oliaei and Cambridge Environmental Consulting in this technical analysis. We advocate that an appropriately protective MCL be recommended to and acted upon by the New Jersey Department of Environmental Protection and agree with Dr. Oliaei's finding that that the proposed drinking water MCL of 14 ng/L for PFOA based on increased relative liver weight is not adequately protective of all population segments. We support Dr. Oliaei's position that the standard may be developed based on an immunotoxic association in children or, alternatively, evidence of developmental effects shown in rodent studies. Both of these approaches provide more sensitive endpoints with quantitative data to develop an MCL, providing greater protection. We support Dr. Oliaei's analysis and final conclusion that the recommended MCL should be lowered to 1 ng/L, or alternatively, should be no higher than 6 ng/L.

Thank you for proposing a recommended MCL for PFOA, an action that is critically needed to remove this toxic compound from New Jersey's drinking water supplies.

DELAWARE RIVERKEEPER NETWORK
925 Canal Street, Suite 3701
Bristol, PA 19007
Office: (215) 369-1188
fax: (215)369-1181
drn@delawareriverkeeper.org
www.delawareriverkeeper.org

Sincerely,

Maya van Rossum
the Delaware Riverkeeper

Tracy Carluccio
Deputy Director

Attached: Technical Analyses of New Jersey Drinking Water Quality Institute Proposed Health-Based Maximum Contaminant Level for Perfluorooctanoic Acid (PFOA) in Drinking Water, Fardin Z. Oliaei, Don Kriens, Cambridge Environmental Consulting, Nov. 18, 2016

Technical Analyses of New Jersey Drinking Water Quality Institute

Proposed Health-Based Maximum Contaminant Level (MCL) for Perfluorooctanoic Acid (PFOA) in Drinking Water

prepared by

Fardin Z. Oliaei MPA, Ph.D.
Don L. Kriens* Sc.D., P.E.

Cambridge Environmental Consulting

November 18, 2016

PREFACE

The opinions in this report are stated to a reasonable degree of scientific probability. The methods and principals used in forming these opinions are generally accepted within the scientific community and are consistent with their regular application within the scientific community. Qualifications of the authors, including publications where applicable, are summarized in the attached resumes. We reserve the right to modify or supplement opinions stated in this report.

** The views expressed in this report do not necessarily reflect those of the Harvard T.H. Chan School of Public Health, Harvard University, of which the author is affiliated as a Research Fellow.*

Technical Analysis of NJDWQI Proposed Health-Based Maximum Contaminant Level (MCL) for Perfluorooctanoic Acid (PFOA)

by

Cambridge Environmental Consulting

Executive Summary

We conclude that the proposed drinking water MCL of 14 ng/L for PFOA based on increased relative liver weight is not adequately protective of all population segments. The criterion may be developed on the basis of epidemiologic evidence of a significant immunotoxic association in children or, alternatively, evidence of significant adverse developmental effects shown in rodent studies. Both of these offer more sensitive endpoints with quantitative data to develop an MCL to assure greater health protection. We calculate an approximate MCL of 0.5 ng/L based on the BMDL determined and the association found between immune suppression and serum PFOA levels in children as reported by Grandjean and Budtz-Jørgensen, or an approximate MCL of 1.0 ng/L based on the BMDL determined in the delayed mammary gland developmental effects in mice studies. Alternatively, we calculate a MCL of 6 ng/L for children group ages 1-6 using the increased liver weight endpoint, with exposure values we determined for mean weight and 90th percentile water intake in that group. We propose that NJDWQI lower the proposed MCL to 1.0 ng/L, consistent with the values found pursuant to the immunotoxic epidemiologic study and/or animal studies showing adverse developmental effects. Excluding use of these values the MCL should be no greater than 6 ng/L to assure protection of children.

Introduction

This is a summary of our analysis and evaluation of the proposed health based maximum contaminant level (MCL) for PFOA in drinking water developed by the New Jersey Drinking Water Quality Institute (NJDWQI), as described in its report Health-Based Maximum Contaminant Level Support Document: Perfluorooctanoic Acid (PFOA), dated June 27, 2016, hereinafter referred to as the NJDWQI Report.

The presence of PFOA in New Jersey water supplies is of great concern because high concentrations are found in groundwater and surface water within the Delaware River Watershed and other locations in New Jersey. According to NJDEP database as of January 2016, of 72 public water supplies (PWS) tested 47% or 66 PWS were found contaminated with PFOA at levels equal to or exceeding the reporting limit (5 ng/l). Thirty-two PWS or 45% had levels exceeding 10 ng/l, and 12 PWS or 17% had levels exceeding 40 ng/l (NJDWQI 2016). Water tested at these PWS includes both raw and finished water; negligible to no removal of PFOA is achieved in the conventional water treatment systems used at these PWS. The affected population was not listed although we would expect it to exceed 1 million. NJDEP has not

published studies of PFOA in private wells, however PFOA has been found at levels exceeding 40 ng/L (maximum >400 ng/L), in 59 private wells within 2 miles of a New Jersey industrial source (NJDWQI 2016 Report, DuPont, 2009).

Drinking water represents a significant portion of total human exposure to PFOA. The relative portion depends significantly upon the concentration of PFOA in drinking water. One study found that drinking water (at 9.66 ng/l) represented 24% of total exposure (Thompson et al 2011). Using NHANES 2003/2004 data, Lorber and Egeghy also determined a relative contribution of drinking water to total intake at 24%. They note that this rate is similar among adults and children (Lorber and Egeghy 2011). Others have found that drinking water represents a much higher portion of total exposure (Noorlander et al. 2011). A 20% contribution to total intake is used as a default value for relative source contribution (RSC) in this risk analyses.

PFOA exists predominantly in anionic form in drinking water sources. PFOA is non-volatile and therefore inhalation exposures to PFOA during showering and bathing and other domestic uses are negligible. PFOA does not cross the skin barrier and therefore PFOA is not absorbed into circulation via the skin, based on skin permeability of PFOA (Franko et al. 2012).

PFOA may escape water via aerosolization. In a laboratory study aerosols generated from deionized, fresh, and ocean waters spiked with PFO were found to have significantly higher concentrations of PFO than the parent water body, ≤ 80 times for ocean waters (McMurdo et al. 2008). Aerosols are produced by breaking waves on surface waters to generate air bubble beneath the surface which, when bursting at the surface, eject aerosol droplets into the atmosphere. This study also suggests that gas-phase evolution of PFOA from the aerosol-bound PFO into the atmosphere likely occurs due to the short aerosol-to-gas phase transfer half-life, about 7 seconds. Aerosol generation may also account for long-range air transport of PFOA, in addition to pathways of atmospheric transport of volatile precursors (8:2 FTOH) and transport of PFOA via the ocean.

Localized surface and groundwater PFOA contamination is primarily caused by wastewater discharges, air transport and deposition from PFOA emission sources, and groundwater plume migration. The extent of PFOA-laden aerosols via short-range air transport and potential direct exposure to humans is unknown but may help to explain, in part, PFOA concentrations in ground and surface waters in some locales proximate to factory sources, such as those found in Minnesota (Oliaei et al 2012).

We calculated that exposure to PFOA from drinking water source aerosols produced during typical showering conditions are likely to be negligible at a range of source water concentrations, based on equations we used in inhalation studies of aerosol particulates during showering (Cowen and Ollison, 2006; Zhou et al. 2010).

Calculation of MCL Using Quantitative Epidemiologic Data (Immunotoxicity)

The National Toxicology Program (NTP) supports a conclusion that PFOA alters human immune function (NTP 2016). A number of studies have shown PFOA immunotoxicity in that PFOA suppresses immune response. Four studies assessing associations with antibody concentrations following vaccination had prospective study designs that allowed temporality assessment. Among these, a prospective birth cohort study in Norway found strong evidence of decreased rubella-induced antibodies with increasing PFOA maternal serum concentrations in 99 pregnant women with a subsequent follow-up of 56 children at 3 years of age (Granum et al. 2013). Although no statistically significant associations were found with responses to vaccines for Influenza Type B or Influenza Type A H1N1, a large prospective cohort study of 411 adults in the mid-Ohio valley found decreasing antibody concentrations following Influenza A H3N2 vaccination (Looker et al., 2014). A large prospective cohort of 656 consecutive singleton births in the Faroe Islands with prospective follow-up of 587 cohort members at ages 5 and 7 years, found a strong association between serum PFC concentrations (PFOA and PFOS) and serum antibody concentrations against tetanus and diphtheria toxoids (Grandjean and Budtz-Jørgensen 2013).

The NJDWQI report acknowledged that "data from other human studies and toxicology studies provides support for biological plausibility of decreased immune system response to vaccines in humans" (NJDWQI Report 2016). The Report cites Fletcher et al. (2009), which "reported several statistically significant associations between several markers of immune function (decreased IgA; decreased IgE in females only; increased anti-nuclear antibody; decreased C-reactive protein) and serum PFOA levels in communities with drinking water exposure to PFOA in a C8 Science Panel status report" (NJDWQI 2016).

There is concordance with animal studies showing suppression of immune response. As noted in the NJDWQI report these include (in mice) decreased absolute and relative spleen and thymus weights, decreased thymocyte and splenocyte counts, decreased immunoglobulin response, and changes in total numbers and/or specific populations of lymphocytes in the spleen, thymus, peripheral blood, and bone marrow" (NJDWQI report).

NJDWQI notes that a "review of epidemiologic studies provides evidence of consistent findings among studies of decreased antibody concentrations following vaccination and PFOA. However, while there is epidemiologic evidence of temporality, evidence of an exposure-response is limited" (NJDWQI 2016). We disagree. We believe that where there is strong, significant epidemiologic evidence that includes quantitative data to enable derivation of a BMDL, such data should be taken into account in derivation of the MCL.

The Grandjean and Budtz-Jørgensen study represents the greatest sensitivity to PFOA thus studied, un-confounded by exposure to other chemical contaminants. In this study regression modeling of PFC concentrations (PFOA and PFOS) as independent variables along with potential confounders of sex, age, and booster type at age 5 and 7, with antibody concentrations as outcome, allowed determination of benchmark response (BMR) and benchmark dose (BMD).

The lower one-sided 95% CL (confidence limit) of the BMD, the BMDL (benchmark dose level), was determined in this study to be approximately 0.33 ng/ml for PFOA and 1.3 ng/ml for PFOS, based on the linear slope model of the regression. The study notes strong correlation between PFOS and PFOA, making mutual adjustment in the regression difficult. However, in spite of this the BMDL developed does provide a strong epidemiologic basis to develop a MCL.

Based on the immunotoxic effects shown in this study we propose that a 0.33 ng/ml BMDL for PFOA be used as a target human serum level. Assuming a serum:water ratio of 100:1 and an uncertainty factor (UF) of 10 to account for human variation in susceptibility, we calculate a MCL as follows:

$$\text{MCL} = \frac{0.33\ \text{ng/ml}}{\text{UF } 10 \times 100 \text{ serum:water ratio}} = \frac{330\ \text{ng/L}}{1000} = 0.33\ \text{ng/L (rounded to } 0.5\ \text{ng/L)}$$

Alternatively, the NJDWQI methodology uses a clearance factor of 0.00014 L/kg/day to apply to the Target Human Serum Level. Using that methodology, a BMDL of 0.3 ng/ml as the POD (point of departure) for RfD determination, and a UF of 10 for human variation in susceptibility to determine the Target Human Serum Level, the RfD is:

$$\text{RfD} = \frac{330\ \text{ng/L}}{\text{UF } 10} \times .00014\ \text{L/kg/day} = 0.0046\ \text{ng/kg/day}$$

Using NJDWQI default adult exposure values of 70 kg body weight, 2 L/day water intake, and a relative source contribution of 0.2 the MCL is:

$$\text{MCL} = \frac{0.0046\ \text{ng/kg/day} \times 70\ \text{kg} \times 0.2}{2\ \text{L/day}} = 0.032\ \text{ng/L}$$

Based on the above we propose that the MCL for PFOA be 0.5 ng/L.

Calculation of MCL based on Delayed Mammary Gland Development (Animal Studies)

Delayed mammary gland development in mice resulting from developmental exposures to PFOA is a sensitive endpoint. This toxicity effect has been shown in nine different studies (NJDWQI report 2016). Delayed mammary gland development is especially concerning since adverse effects including histological changes related to delayed mammary gland development persist into adulthood and become permanent. Several researchers indicate that delayed mammary gland growth may result in greater susceptibility to cancer later in life (Fenton 2006; Rudel et al., 2011; Fenton et al., 2012; Osborne et al. 2015). Others note that developmental exposures in sensitive time periods can result in increased risk of later disease or dysfunction (Heindel and Vandenberg, 2015). Mode of action is explained by Osborne: “Anything that changes the timing of mammary development will affect the timing of the presence of TEBs (terminal end buds), and therefore the window of susceptibility to

carcinogens. Late initiation of mammary development causes decreased longitudinal growth of the epithelium and fewer TEBs, and decreased alveolar budding at weaning. As development progresses, these glands may have more TEBs at puberty, because the pace of development is slower. It is hypothesized that factors that lengthen the period when TEBs are present lengthen the period during which the MG is susceptible to carcinogens" (Osborne et al., 2015).

NJDWQI acknowledged these studies, which may result in increased susceptibility to cancer later in life. The NJDWQI states that "The Health Effects Subcommittee chose not to use this (delayed mammary gland development) RfD as the basis for a recommended Health-based MCL, not because of uncertainty about the scientific validity of doing so, but rather because of lack of precedent for use of this endpoint as the primary basis for health-based criteria for environmental contaminants. Instead the Subcommittee arbitrarily applied an additional 10 UF to an unrelated endpoint (increased liver weight that forms the basis for their MCL derivation) to compensate for the more sensitive endpoint (delayed mammary gland development). This is confusing. Why not use the more sensitive endpoint for which adequate toxicity data already exists, including a BMDL, even if that endpoint has not previously been used, versus adding an additional uncertainty factor to an alternate endpoint to compensate for an uncertainty that is, in fact, known?

We propose that the MCL be determined using the sensitive endpoint BMDL for delayed mammary gland development, clearance factor, and default adult exposure values per NJDWQI analyses, as follows:

Summary of variables used and values

BMDL	POD of 22.9 ng/ml (22,900 ng/L)
total UF	30 (10 human variation, 3 animal-to-human extrapolation)
RSC	0.20
clearance factor	0.00014 L/Kg/day
default adult body weight	70 kg per NJDWQI report
default adult intake	2.0 L per NJDWQI report

$$\text{RfD} = \frac{22{,}900 \text{ ng/L} \times 0.00014 \text{ L/kg/day}}{30 \text{ UF}} = 0.107 \text{ ng/kg/day}$$

$$\text{MCL} = \frac{0.107 \text{ ng/kg/day} \times 70 \text{ kg} \times 0.2}{2 \text{ L/day}} = 0.75 \text{ ng/L (rounded to 1 ng/L)}$$

Based on the above we propose that the MCL for PFOA be 1 ng/L.

Children, PFOA Exposure, and Use of Adult Default Exposure Values

There is evidence that young children are exposed to greater levels of PFOA than adults. This

may occur because of age-specific behaviors such as hand-to-mouth behaviors resulting in greater ingestion of house particulates, and more time spent on floors with treated carpets. Using NHANES data, Lorber and Egeghy found that incidental ingestion of dust is far less important among adults than among children (Lorber and Egeghy 2011). Children's dust intakes are highly variable due to the distribution of dust PFOA concentrations in homes. The 95th percentile intake from dust ingestion is about three times the intake from food ingestion (Lorber and Egeghy 2011).

Peak serum PFOA concentrations occur during the first year of life, in part due to "off-loading" from the mother at birth. As noted in NJDWQI report, levels remain elevated for at least several additional years. Blood serum levels have been found to be higher in children. Higher serum levels were observed in children ages 2-5 versus older children and adults in Little Hocking, Ohio residents who have been exposed to PFOA in drinking water (Emmet et al 2006). Toxicity effects to children during this developmental period may persist into adulthood and become permanent.

Children therefore represent a special case. They have greater drinking water and food consumption on a body weight basis. Using adult default exposure values is inappropriate since a priori use of adult default values for body weight and water intake omits protection to children, the population's most vulnerable exposure group. Calculation of a MCL using adult default values results in a RfD to children (age group 1-6) that significantly exceeds that deemed allowable by NJDWQI based on the increased liver weight toxicity endpoint.

Although the MCL should be based on human immunotoxicity and/or the delayed mammary gland development shown in test animals, as calculated above, we believe that at a minimum MCL calculations using increased liver weight as an endpoint should be based on children exposure values for body weight and drinking water intakes. Using children group ages 1-6 we determined the MCL as follows:

<u>Summary of variables used and values</u>

BMDL	POD of 4351 ng/ml (4,351,000 ng/L)
CUF	300 (10 human variation, 3 animal-to-human extrapolation, 10 for delayed mammary gland development
RSC	0.20
Children body weight[a]	16.8 kg
Children intake[b]	0.69 L/day mean, 1.19 L/day 90th percentile

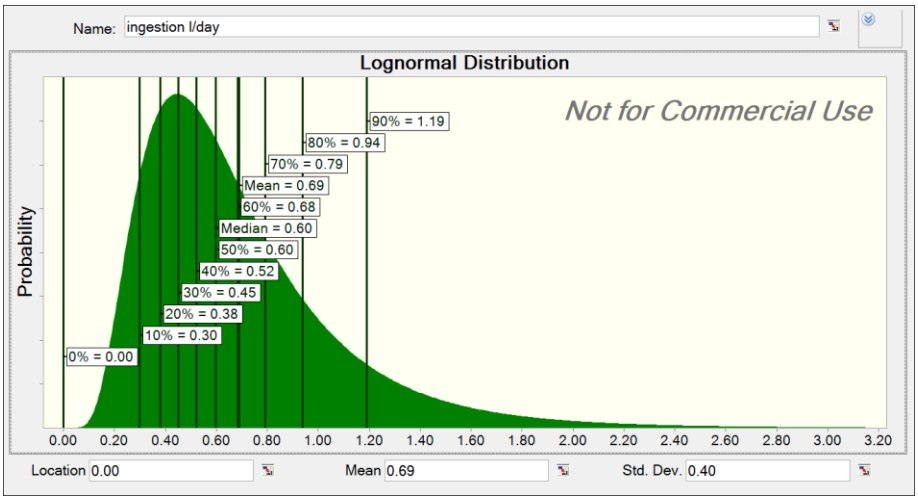
<u>Children Group (age 1-6)</u>

$$\text{RfD} = \frac{4{,}351{,}000\ \text{ng/L} \times 0.00014\ \text{L/kg/day}}{300\ \text{UF}} = 2.03\ \text{ng/kg/day}$$

$$\text{MCL} = \frac{2\ \text{ng/kg/day} \times 16.8\ \text{kg} \times 0.2}{1.19\ \text{L/day}} = 5.65\ \text{ng/L}\ \text{(rounded to 6 ng/L)}$$

a. These values were determined using EPA 2011 Exposure Factor Handbook data, taking smaller increments of age groups and gender, combined by weighting the means of group increments, and pooling variances to determine means and standard deviations.)

b. Following EPA's default criteria of 90th percentile distribution of water intake, we found a 1.19 L/day water intake rate for children 1-6 at the 90th percentile, based on derivation of a lognormal distribution of water intake for this combined age group, shown in the graph below.



Lognormal Distribution of Water Intakes for Children Group Ages 1-6

Based on the above the MCL for PFOA should be 6 ng/L.

Conclusion

NJDWQI's Health Effects Subcommittee's work in developing a MCL for PFOA demonstrates a considerably sounder scientific basis than EPA's recent drinking water advisory for PFOA, where a 70 ng/L MCL is developed (USEPA 2016). However, NJDWQI's reliance upon Increased Relative Liver Weight in animal studies as an endpoint to develop a RfD disregards more sensitive toxicity endpoints. We believe that animal studies showing significant delayed mammary gland development are sufficient and appropriate to use in the MCL determination, irrespective of whether there is absence of precedence, where benchmark dose modeling allows calculation of an approximate MCL of 1 ng/L. Substantial epidemiological evidence showing a range of toxic effects should also be taken into account versus reliance solely upon animal studies. One such study, the Immunotoxicity study by Grandjean and Budtz-Jørgensen 2013 showing a significant association between PFOA and suppression of antibody responses in children, provides benchmark dose response data to calculate a MCL of ≤1 ng/L.

In addition, the proposed MCL of 14 ng/l calculated using adult default values for body weight results in a PFOA dose to children (ages 1-6) that is 50% higher at mean water intake levels, and 2½ times higher at 90th percentile water intake levels, than the reference dose (RfD) allowed to assure that serum levels remain below a protective maximum target level. Thus, the proposed

MCL of 14 ng/L using default adult exposure values is not protective of all age groups. This is concerning since, based on animal developmental studies that likely relate to humans, toxic effects from PFOA exposures in early childhood may persist into adulthood and could result in more profound disease in later life.

Absent lowering the proposed MCL to 1 ng/L, the MCL should be no higher than 6 ng/L.

** The views expressed in this report do not necessarily reflect those of the Harvard T.H. Chan School of Public Health, Harvard University, of which one of the authors is affiliated as a Research Fellow.*

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Maximum Contaminant Level Recommendation for Perfluorooctanoic Acid in Drinking Water

Basis and Background

New Jersey Drinking Water Quality Institute

March 15, 2017

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State of New Jersey

DEPARTMENT OF ENVIRONMENTAL PROTECTION
DRINKING WATER QUALITY INSTITUTE
401 EAST STATE STREET
P.O. BOX 420
Trenton, NJ 08625-0420

CHRIS CHRISTIE
Governor

KIM GUADAGNO
Lt. Governor

BOB MARTIN
Commissioner

March 15, 2017

Commissioner Bob Martin
New Jersey Department of Environmental Protection
P.O. Box 402
Trenton, NJ 08625-0402

Dear Commissioner Martin:

The members of the New Jersey Drinking Water Quality Institute (Institute) are pleased to submit their recommendation for a Maximum Contaminant Level (MCL) for perfluorooctanoic acid (PFOA) in drinking water.

The occurrence of PFOA and other perfluorinated compounds (PFCs) in public water supplies (PWS) has been evaluated more extensively in New Jersey than in most or all other states. More than 1,000 samples from 80 NJ PWS were analyzed with relatively low Reporting Levels (RLs; generally, < 5 ng/L) in 2006-2016. PFOA was the most frequently detected PFC and was found in samples from approximately 60% of the 80 NJ PWS tested. In the 2013-2015 USEPA Unregulated Contaminant Monitoring Rule 3 (UCMR3) survey of all large (>10,000 users) and a subset of smaller PWS in the U.S., PFOA was detected more than five times more frequently in New Jersey PWS (10.3%) than in the rest of the U.S. (2.1%). The RL in UCMR3 was 20 ng/L, much higher than the RLs for most other NJ PWS monitoring for PFOA. PFOA has also been detected in NJ private wells near sources of industrial discharge.

As you are aware, three subcommittees within the Institute were established to address the essential considerations for development of MCLs as outlined in the New Jersey Safe Drinking Water Act (N.J.S.A. 58:12A-20). The Health Effects Subcommittee is responsible for recommending health-based levels (Health-based MCLs) for contaminants of concern, the Testing Subcommittee is responsible for evaluating and recommending appropriate analytical methods and developing Practical Quantitation Levels (PQLs; the levels to which a contaminant can be reliably measured by drinking water laboratories), and the Treatment Subcommittee is responsible for evaluating best available treatment technologies for removal of the contaminants of concern from drinking water.

At your request, the three subcommittees thoroughly reviewed the available scientific information relevant to the health effects, analytical methods, and treatment options associated with PFOA in drinking water, including the information received through a public request for relevant technical information in the summer of 2014. The

three subcommittees then developed detailed technical reports to support the recommendations that are described below. These recommendations were presented at a public meeting of the Institute on September 22, 2016, and the draft subcommittee reports were posted for a 60-day public comment period following the September meeting.

The Health Effects Subcommittee used a risk assessment approach intended to protect for chronic (lifetime) exposure to develop a Health-based MCL of 14 ng/L, and the Testing Subcommittee determined a PQL of 6 ng/L for PFOA. The Treatment Subcommittee concluded that PFOA can be removed to levels below the recommended Health-based MCL of 14 ng/L and the recommended PQL of 6 ng/L with treatment technologies, such as granulated activated carbon (GAC) and reverse osmosis. GAC has been successfully installed at New Jersey public water systems to treat PFCs including PFOA. An additional benefit of the treatment technologies used to remove PFOA is that they also remove many other contaminants that may also be present.

The conclusions reached by the three subcommittees, which are detailed in the documents attached, were approved by a unanimous vote at an Institute meeting on February 16, 2017. Therefore, the Institute recommends that the Department propose and adopt a Health-based MCL of 14 ng/L for PFOA in drinking water.

Please feel free to contact me if you have any questions or need additional information related to these recommendations.

Respectfully,

Keith R. Cooper, Ph.D.
Chair

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Appendices

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Appendix B: Report on the Development of a Practical Quantitation Level for Perfluorooctanoic Acid (PFOA) in Drinking Water

Appendix C: Addendum to *Appendix C: Recommendation on Perfluorinated Compound Treatment Options for Drinking Water*

Appendix D: Responses to Comments on DWQI Health Effects Subcommittee Report: "*Public Review Draft- Health-Based Maximum Contaminant Level Support Document: Health-Based Maximum Contaminant Level Support Document: Perfluorooctanoic Acid (PFOA)*"

Executive Summary

The New Jersey Drinking Water Quality Institute (the Institute) was established by the 1984 amendments to the New Jersey Safe Drinking Water Act (SDWA) at N.J.S.A. 58:12A- 20. It is charged with developing standards (Maximum Contaminant Levels; MCLs) for hazardous contaminants in drinking water and for recommending those standards to the New Jersey Department of Environmental Protection (NJDEP). In 2014, New Jersey Department of Environmental Protection Commissioner Bob Martin requested that the Institute recommend MCLs for perfluorooctanoic acid (PFOA) and two other long-chain perfluorinated compounds (PFCs), perfluorononanoic acid (PFNA) and perfluorooctanesulfonic acid (PFOS). The Institute recommended an MCL for PFNA on July 1, 2015, and it now recommends an MCL for PFOA herein.

Three subcommittees are established within the Institute to address the essential considerations for development of MCLs as outlined in the New Jersey SDWA. The Health Effects Subcommittee is responsible for recommending health-based levels (Health-based MCLs) for contaminants of concern, the Testing Subcommittee is responsible for evaluating and recommending appropriate analytical methods and developing Practical Quantitation Levels (PQLs; the levels to which a contaminant can be reliably measured by drinking water laboratories), and the Treatment Subcommittee is responsible for evaluating best available treatment technologies for removal of the contaminants of concern from drinking water.

The three Institute subcommittees have reviewed the available scientific information relevant to the health effects, analytical methods, and treatment options associated with PFOA. Detailed documents presenting the technical basis for each of the subcommittee's recommendation are attached in Appendices A, B, and C.

Also attached is an additional document presenting the Health Effects Subcommittee's response to technical public comments. As the Drinking Water Quality Institute (DWQI) serves as an advisory body which makes recommendations to the NJDEP and DWQI's recommendation is not a rulemaking that is subject to the requirements of the Administrative Procedure Act, a formal response to public comments received on draft subcommittee documents is not required. However, the subcommittee wanted an opportunity to address public comments in more detail than a presentation would allow, in order to provide clarification with respect to its draft document and to address any changes made to the draft document based on those comments when appropriate.

The Health Effects Subcommittee used a risk assessment approach intended to protect for chronic drinking water exposure to develop a Health-based MCL of 14 ng/L (0.014 µg/L), and the Testing Subcommittee developed an analytical PQL of 6 ng/L (0.006 µg/L). The Treatment Subcommittee recommended that granular activated carbon or an equally efficient treatment removal technology can be used when PFOA is detected above the recommended MCL, subject to on-site pilot testing performance results, and concluded that the availability of treatment options is not anticipated to be a limiting factor in the development of a recommended MCL for PFOA or the other two PFCs (PFNA and PFOS) that have been, or will be, evaluated by the Institute. An additional benefit of the treatment technologies used to remove PFOA is that they also remove other synthetic organic chemicals, natural organic compounds, and other compounds affecting taste and odor that may be present.

Since neither treatment removal nor analytical methods are limiting factors for achieving the Health-based MCL of 14 ng/L (0.014 µg/L), the Institute recommends an MCL for PFOA of 14 ng/L (0.014 µg/L) to the Department as both health protective and technically feasible.

Introduction

A. Background

In 2014, Commissioner Bob Martin of the New Jersey Department of Environmental Protection (NJDEP) requested that the New Jersey Drinking Water Quality Institute (the Institute) recommend a drinking water standard for perfluorooctanoic acid (PFOA), the subject of this recommendation, as well as two other long-chain perfluorinated compounds (PFCs), perfluorononanoic acid (PFNA) and perfluorooctanesulfonic acid (PFOS).

The New Jersey Safe Drinking Water Act at N.J.S.A. 58: 12A-20, established the New Jersey Drinking Water Quality Institute, consisting of six *ex officio* and nine appointed members, to make recommendations to the NJDEP regarding drinking water quality. The members represent the public, the academic community, the water purveyors, NJDEP, New Jersey Department of Health, and the New Jersey Water Supply Advisory Council.

The Institute is responsible for providing recommendations to the Commissioner of NJDEP on implementation of the State's drinking water quality program, including MCLs. Three subcommittees are established to address the important considerations in the development of an MCL. The Health Effects Subcommittee recommends Health-based Maximum Contaminant Levels; these are target drinking water levels based solely on health effects. The Testing Subcommittee reviews existing analytical methods to identify those methods with practical quantitation levels (PQLs). The Treatment Subcommittee evaluates best available treatment technologies for removal of contaminants from drinking water.

The Institute has accepted the recommendations from each of its three subcommittees that are presented in this Basis and Background document and its Appendices. These recommendations form the basis for the recommended MCL for PFOA.

B. Drinking Water Quality Institute Membership

Chair
Keith R. Cooper, Ph.D., Rutgers University

Health Effects Subcommittee
Chair: Jessie A. Gleason, M.S.P.H., NJ Department of Health
Keith R. Cooper, Ph.D., Rutgers University
Judith B. Klotz, Dr.P.H., Adjunct Associate Professor, Rutgers University and Drexel University
Gloria B. Post, Ph.D., DABT, NJ Department of Environmental Protection
George Van Orden, Ph.D., Adjunct Professor, Rutgers University and Drew University

Testing Subcommittee
Chair: Bahman Parsa, Ph.D., NJ Department of Health
Sandra Krietzman, M.S., NJ Department of Environmental Protection
Daniel Salvito, Ph.D, Research Institute for Fragrance Materials
Sheng-Lu Soong, Ph.D., Suez Water

Treatment Subcommittee
Chair: Vacant
Patricia Gardner, NJ Department of Environmental Protection
Anthony Matarazzo, NJ American Water
Norm Nelson, Van Note-Harvey Associates
Carol T. Storms, Aqua NJ

Health Effects Considerations and Recommendations

A Health-based Maximum Contaminant Level (Health-based MCL) for PFOA was developed using a risk assessment approach intended to protect for chronic (lifetime) drinking water exposure. A public health-protective approach in developing a Health-based MCL based on animal toxicology data is supported by associations of PFOA with a number of health effects in the general population and communities with drinking water exposure, as well as PFOA's biological persistence and bioaccumulation from drinking water in humans. PFOA was described as "likely to be carcinogenic to humans" by the USEPA Science Advisory Board, "possibly carcinogenic to humans" by the International Agency for Research on Cancer (IARC), and as having "suggestive evidence of carcinogenic potential" by the USEPA Office of Water. Both non-carcinogenic and carcinogenic effects were evaluated for Health-based MCL development. Delayed mammary gland development and increased liver weight were the most sensitive non-carcinogenic endpoints with data needed for dose-response modeling. For each of these endpoints, benchmark dose modeling of serum PFOA levels from mouse studies was performed and appropriate uncertainty factors were applied to develop a Target Human Serum Level (analogous to a Reference Dose but on a serum level basis). A clearance factor (1.4×10^{-4} L/kg/day) which relates serum PFOA concentrations to human PFOA doses was applied to the Target Human Serum Levels to develop Reference Doses. For delayed mammary gland development, the Target Human Serum Level is 0.8 ng/ml, which is below the median serum PFOA level in the U.S. general population. The Reference Dose for this endpoint is 0.11 ng/kg/day. Because the use of delayed mammary gland development as the basis for quantitative risk assessment is a currently developing topic, a Health-based MCL using this endpoint as its primary basis was not recommended. However, it was concluded that an uncertainty factor for sensitive endpoints is needed to protect for this and other effects that occur at similarly low doses. A Health-Based MCL protective for increased relative liver weight was derived based on a study in which male mice were exposed to PFOA for 14 days. For increased relative liver weight, the Target Human Serum Level is 14.5 ng/ml and the Reference Dose is 2 ng/kg/day. This Target Human Serum Level and Reference Dose incorporate uncertainty factors to protect sensitive human subpopulations, to account for toxicodynamic differences between human and experimental animals, and to protect for more sensitive endpoints that occur from developmental exposures (delayed mammary gland development, persistent hepatic toxicity, and others). Default values for drinking water exposure assumptions (2 L/day water consumption; 70 kg body weight) and Relative Source Contribution factor (20%) were used to develop a Health-based MCL of 14 ng/L based on the Reference Dose for increased relative liver weight. A cancer slope factor of 0.021 $(mg/kg/day)^{-1}$ was developed based on increased incidence of testicular tumors in a chronic rat study. This slope factor was used to develop a Health-based MCL protective for cancer effects at the 1×10^{-6} (one in one million) lifetime cancer risk level of 14 ng/L, identical to the Health-based MCL based on non-cancer endpoints. The recommended Health-based MCL is therefore 14 ng/L (0.014 µg/L).

Analytical Considerations and Recommendations

The role of the Testing Subcommittee in the recommendation of an MCL for PFOA was to identify acceptable methods for PFOA analysis and to develop a PQL for PFOA. A PQL is the minimum concentration to which the contaminant under review can be reliably quantitated within acceptable limits of uncertainty. PQL development involves researching analytical methods that are reliable and sufficiently sensitive to measure the contaminant at, or as close as possible, to the Health-based MCL developed by the Health Effects Subcommittee

In determining the availability of analytical methods with adequate sensitivity, the Testing Subcommittee queried the existing NJDEP database for PFCs in NJ public water systems for PFOA data collected from June 2006 through April 2016. Due to the limited number of laboratories performing analyses of the PFOA samples in the NJDEP PFC database, the Testing Subcommittee also reviewed analytical information from other laboratories performing analyses of PFCs in drinking water.

When developing the PQL, the Testing Subcommittee considered analytical methods and laboratory performance. A summary of laboratory performance data is found in the full Testing Subcommittee report (Appendix B). In addition, if the Health-based MCL is available, the goal of the Testing Subcommittee is to establish the PQL at a level less than the Health-based MCL. However, the Health-based MCL for PFOA was not available while the Testing Subcommittee was developing the PFOA PQL. In the absence of a Health-based MCL to use as a goal, the Testing Subcommittee considered both the reporting limit recommendation of 10 ng/L provided by the NJDEP Office of Quality Assurance prior to the 2006 NJDEP study of PFCs in NJ public water systems, and the MRL of 20 ng/L required for the USEPA UCMR3. Therefore, while the UCMR3 MRL demonstrated that national laboratories could reliably report PFOA at an MRL of 20 ng/L, the 2006 and 2009-10 NJDEP studies of PFCs in NJ public water systems, and other data in the NJDEP PFC database, establish that lower reporting levels are achievable for PFOA.

The Subcommittee evaluated four approaches for calculating the PQL, described in detail in the report found at Appendix B. To derive the PFOA PQL, the Testing Subcommittee decided to use an approach that considers both the precision and accuracy of the analytical method. Therefore, the Testing Subcommittee relied on the actual reporting limits from laboratories currently performing PFOA analyses for determining its recommendation of a PQL of 6 ng/L for PFOA.

Treatment Considerations and Recommendations

The Treatment Subcommittee is responsible for identifying available treatment technologies or methods for removal of hazardous contaminants from drinking water. In June 2015, the Treatment Subcommittee issued a final report on treatment options for all three PFCs under consideration by the Institute (PFNA, PFOA, and PFOS), as the treatment options are not expected to differ from compound to compound due to their similar properties (e.g. persistence, water solubility, similar structure, strong carbon-fluorine bonds, and high polarity). This approach contrasts with the other two subcommittees, which evaluate each compound separately. According to published literature, long-chain PFCs such as PFNA, PFOA and PFOS can be removed from water with varying success using a number of treatment options, which are described in detail in the Subcommittee report found in Appendix C. The most common treatment approach for PFC removal both in the literature and in practice is granulated activated carbon (GAC). The attached addendum to Appendix C updates and supplements the June 2015 Treatment Subcommittee report for the recommendation of an MCL for PFNA. The update is based on a more recent 2016 Water Research Foundation (WRF) document entitled, “Treatment Mitigation Strategies for Poly- and Perfluoroalkyl Substances” (also referred to as WRF Web Report #4322), and takes into consideration the recommendations for PFOA made by the Health Effects and Testing Subcommittees.

The Treatment Subcommittee concluded, based on case studies of full scale operations including at sites in New Jersey, that removal to levels below the recommended PQL for PFOA can be consistently achieved using GAC. This method of treatment has been successfully used in New Jersey for removal of PFCs including PFOA, as well as for removal of synthetic organic chemicals, natural organic compounds, and other compounds affecting taste and odor. Based on these successful applications, the use of GAC is therefore considered practical and feasible. The Treatment Subcommittee therefore recommends that GAC or an equally efficient technology, as identified in the Subcommittee report, can be used for treatment of PFOA when detected above the DWQI recommended MCL, subject to the on-site pilot testing performance results. The Subcommittee concluded that the ability of treatment options to remove these contaminants is not anticipated to be a limiting factor in the development of a recommended MCL for PFOA or the other two PFCs (PFNA and PFOS) that have been, or will be, evaluated by the Institute.

MCL Recommendation

A Health-based MCL for PFOA of 14 ng/L (0.014 µg/L) is recommended by the Health Effects Subcommittee. The Testing and Treatment Subcommittees concluded that analytical limitations and treatment removal are not

limiting factors for achieving this Health-based MCL.

The Institute has accepted the recommendations of each of the three subcommittees, and these recommendations form the basis for the recommended MCL for PFOA. Accordingly, the Institute recommends an MCL for PFOA of 14 ng/L (0.014 µg/L) to the Department as both health protective and technically feasible.

Public Review Draft

Addendum to Appendix C: Recommendation on Perfluorinated Compound Treatment Options for Drinking Water

New Jersey Drinking Water Quality Institute

Treatment Subcommittee

August 2016

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Introduction

In June 2015 the Drinking Water Quality Institute (DWQI) issued a recommendation for a Maximum Contaminant Level (MCL) for perfluorononanoic acid in response to a request from the Department of Environmental Protection to recommended MCLs for three long-chain perfluorinated compounds (PFC), also known as poly- and perfluoroalkyl substances (PFAS): perfluorononanoic acid (PFNA), perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS). Due to their similar properties (e.g. persistence, water solubility, similar structure, strong carbon-fluorine bonds, and high polarity) the treatment options are not expected to differ for each of these three compounds. Accordingly, the DWQI Treatment Subcommittee reported on treatment options for all three compounds in one document found in Appendix C of the DWQI PFNA Recommendation and entitled "Recommendation on Perfluorinated Compound Treatment Options for Drinking Water."

This document is intended to update and supplement the June 2015 Treatment Subcommittee report, based on the 2016 publication of the Water Research Foundation (WRF) document entitled, "Treatment Mitigation Strategies for Poly- and Perfluoroalkyl Substances," (also referred to as WRF Web Report #4322) and in light of draft recommendations for PFOA by the Health Effects and Testing Subcommittees.

The Treatment Subcommittee of the DWQI is responsible for identifying available treatment technologies or methods for removal of hazardous contaminants from drinking water and for identifying whether a recommended MCL can be feasibly and reliably met using available technologies.

Addendum

1. PFNA Treatment Clarification

It should be clarified that the 2014 WRF report referenced throughout the Treatment Subcommittee report included a table that described the "assumed" removal rates for PFNA with respect to anion exchange and granular activated carbon (GAC) rather than observed removal rates. The referenced case studies for New Jersey American (Logan System Birch Creek) and Amsterdam demonstrated actual removal of PFNA using full-scale GAC installations.

2. Treatment Design

The treatment techniques described in the Treatment Subcommittee document may not be effective for all perfluorinated compounds. As noted in the document, some treatment methods are more effective for long-chain PFCs than for short-chain PFCs. Further information on the removal of various PFASs is included in the 2016 WRF document (Dickenson & Higgins, 2016). The Treatment Subcommittee recommends that consideration should be given when designing the bench study evaluations if other PFASs are present in the source water, even if they are not currently being regulated.

3. Anion Exchange

In a 2015 WRF publication, Dudley et al. (2015) indicated that anion exchange "demonstrated great promise for PFAS removal, provided that resins are regenerated in a manner that restores, at least periodically, the PFAS removal capacity of the resin." The same report also suggested consideration of "[a] possible alternative for PFAS removal could be a hybrid adsorption/anion exchange treatment approach, in which more strongly adsorbing PFASs are initially removed by activated carbon and more weakly adsorbing PFASs subsequently by anion exchange (Dudley et al., 2015)."

On Page 9 of the Treatment Subcommittee report, WRF Project #4322 is cited and referred to as an "ongoing" project. It should be noted that this project has been concluded, and the results note that anion exchange, among other treatment methods, "show promising results for the removal of these chemicals (Dickenson & Higgins, 2016).

5. Advanced Oxidation:

The statement on page 9 of the June 2015 Treatment Subcommittee report regarding the ineffectiveness of conventional oxidation is further supported by recent WRF publications, which concludes that these processes "proved mostly ineffective" and "unable to oxidize PFASs because of the strength of the carbon-fluorine bond" (Dickenson & Higgins, 2016 and Dudley et al., 2015).

6. Membrane Filtration:

Membrane filtration, specifically nanofiltration/reverse osmosis, was also evaluated by WRF and found to be effective (Dickenson & Higgins, 2016).

7. Granular Activated Carbon:

In the 2016 WRF publication, GAC is considered to be an effective method of treatment, as two out of three sites that tested for PFAS concentration observed removal, with empty bed contact time ranging from 10-13 minutes (Dickenson & Higgins, 2016). However, consideration must be

given to the background matrix of the source water. WRF refers to some instances where PFASs measured at higher concentrations in the effluent than in the influent. According to the WRF publication, "this is believed to be due to competitive effects with other sorbing species (perhaps longer chain PFASs and/or natural organic matter) leading to desorption …" (Dickenson & Higgins, 2016). Additionally, during rapid small-scale column tests, the effluent concentration for PFOS never reached more than 2% of influent concentration (Dickenson & Higgins, 2016).

Conclusion

The Treatment Subcommittee concludes that it has been demonstrated that PFOA can be reliably and feasibly removed by carefully designed GAC treatment to below the recommended health-based MCL of 14 ng/L.

References

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NJ Drinking Water Quality Institute
Testing Subcommittee

Report on the Development of a Practical Quantitation Level for Perfluorooctanoic Acid (PFOA) in Drinking Water

8/29/2016

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Testing Subcommittee Report on PQL Development for Perfluorooctanoic Acid in Drinking Water

Background:

In February 2006 the New Jersey Department of Environmental Protection (NJDEP) first became aware of the presence of perfluorinated compounds (PFC) in New Jersey drinking waters. This finding was a result of the Penns Grove Water Supply Company of Salem County, NJ sharing their perfluorooctanoic acid (PFOA) water test results with the NJDEP. A concentration of 64 nanograms per liter (ng/L or parts per trillion) of PFOA was found in the Penns Grove Water Supply Company's finished drinking water. These concentrations of PFOA prompted the NJDEP to conduct a study for determining the occurrence and levels of PFOA and perfluorooctane sulfonic acid (PFOS) in NJ water systems' raw and finished drinking water. Of the 23 New Jersey drinking water systems monitored in the NJDEP 2006 PFC study, 65% had concentrations of PFOA and 30% had concentrations of PFOS in their finished drinking water or drinking water sources. A PFC follow-up study conducted in 2009 determined the occurrence of PFOA, PFOS and eight additional PFCs in drinking water sources of 30 public water systems throughout New Jersey. In contrast to the 2006 PFC study which included samples from both raw (untreated) and treated water sources, the 2009 PFC study included only *untreated* water samples. In the 2009 study, between one and eight PFCs were detected in 21 of 30 NJ public water systems sampled, with total PFC concentrations ranging between 5 ng/L and 174 ng/L.

In 2014, the Drinking Water Quality Institute (DWQI) was tasked with recommending a Maximum Contaminant Level (MCL) for PFOA to the Commissioner of the New Jersey Department of Environmental Protection (NJDEP). This advisory panel comprised of 15 members from academia, regulated water systems, governmental agencies, and public health experts is responsible for providing MCL recommendations as part of the regulatory process in setting an MCL specific to New Jersey. The DWQI recommendations are a result of the collaboration of three DWQI Subcommittees: the Health Effects Subcommittee, the Treatment Subcommittee and the Testing Subcommittee. The Health Effects Subcommittee is responsible for recommending "health-based MCL[1]" levels for contaminants. The Treatment Subcommittee is responsible for evaluating the best available treatment technologies for removal of the contaminant from drinking water supplies. The Testing Subcommittee is responsible for developing Practical Quantitation Levels (PQL) for the contaminants. (A PQL is the minimum concentration for which the contaminant under review can be reliably quantitated within acceptable limits of uncertainty.)

Developing a PQL involves researching analytical methods that are reliable and have the sensitivity to detect the contaminant at or as close as possible to the health-based MCL developed by the

[1] Health-based MCLs are goals, not enforceable standards, similar to USEPA Maximum Contaminant Level Goals (MCLG). For carcinogens, health-based MCLS are set at levels that are not expected to result in cancer in more than one in one million persons ingesting the contaminant for a lifetime, and for non-carcinogens, at levels not expected to result in "any adverse physiological effects from ingestion" for a lifetime. The enforceable MCLs consider other factors such as analytical quantitation limits and availability of treatment removal technology and may be set higher than the MCLGs.

Health Effects Subcommittee. When developing a PQL, the Testing Subcommittee considers available analytical methods and laboratory performance. In 2009, the EPA published Method 537, a solid-phase extraction and liquid chromatography/ tandem mass spectrometry (LC/MS/MS) method for perfluorinated alkyl acids (which includes PFOA). Although published and required for the analysis of UCMR3 samples by the USEPA (2013-2015), this method has not been promulgated in federal regulation, as PFOA does not yet have an MCL. In response to the lack of a promulgated method, some laboratories developed their own proprietary methods or modified EPA 537 to enhance performance of the method. The PQL for PFOA recommended by the Testing Subcommittee in this document was developed solely on the performance data of a group of drinking water laboratories that meet certain criteria established by the Testing Subcommittee.

If the health-based MCL for a contaminant is known, the Testing Subcommittee will attempt to establish a PQL at a level less than that health-based MCL. This is not always feasible and ultimately it is the performance data from robust analytical methods and accredited laboratories that determine the PQL. In the current process of developing a PFOA MCL recommendation, the health-based MCL and the PQL were being developed simultaneously.

In 2007, in response to a request by the Penns Grove Water Supply Company, a health-based guidance for PFOA was developed by the NJDEP Office of Science and Research to provide guidance in assessing the public health implications of the PFOA concentrations detected in their drinking water. This health-based value was determined to be 40 ng/L and was developed "to provide preliminary guidance to the system within a reasonable timeframe and does not represent a comprehensive review of the toxicological literature on PFOA which will be needed as additional information becomes available." Since 2007, laboratories have demonstrated that lower reporting limits are achievable. Therefore, when requesting that water systems conduct additional sampling for PFOA, the NJDEP recommends a reporting limit (or MRL) of 10 ng/L or lower.

In the absence of an EPA developed MCL, as other states have discovered PFCs in their drinking water sources many have developed their own health advisories or guidance levels. For instance, in March 2016, Vermont's Agency of Natural Resources and Department of Health set a health advisory of 20 ng/L for PFOA in groundwater. The New York Department of Environmental Conservation (NYDEC) has been using 100 ng/L as a health advisory level which was developed by EPA Region 2 for the Hoosick Falls area. EPA-issued health advisories provide non-cancer health effects information on contaminants that may be found in drinking water, and are non-enforceable. New Hampshire Department of Environmental Services (NHDES) used 100 ng/L as their action level. In many cases the states use these health derived guidance or advisory values to establish the reporting limits for the analysis of those PFCs in their state. Vermont uses laboratories that offer detection limits or reporting limits lower than their health advisory of 20 ng/L for their investigative studies. Because NYDEC had considered the possibility that the EPA would set a PFOA health advisory lower than the EPA Region 2 Hoosick Falls health advisory of 100 ng/L, they contracted the services of a laboratory capable of providing a PFOA reporting limit of 2 ng/L, thereby ensuring the usefulness of all or most of their previously acquired data.

In May 2016, the EPA released a lifetime health advisory for a combined PFOA and PFOS concentration of 70 ng/L.[2] Prior to this, the EPA had developed a provisional short-term health advisory of 400 ng/L for PFOA in January 2009, due to a contamination event in Alabama. Provisional health advisory values are developed to provide information in response to an urgent or rapidly developing situation. They reflect reasonable, health-based hazard concentrations above which action should be taken to reduce exposure to unregulated contaminants in drinking water and are developed with the intention that they will be updated as additional information becomes available for reevaluation. The short-term provisional drinking water health advisory is usually developed to be protective of a one-day or 10-day exposure timeframe. The EPA was not clear for which timeframe their value was developed

It had already been established through the USEPA Unregulated Monitoring Rule 3 (UCMR3), which will be discussed later in this document, that laboratories participating in this rule and performing the PFC analysis could detect, and reliably quantitate PFOA at and over 20 ng/L. Through conversations with these UCMR3 participating laboratories, 65% were already reporting PFOA lower than 20 ng/L or, if requested, were confident that they would be able to provide a client with a lower reporting limit. The above information further corroborates that the PFOA reporting limits for PFOA are generally client driven.

Data Sources for PQL Determination:

As a first step in the PQL development process, data from drinking water laboratories with adequate sensitivity for reliably analyzing PFOA were compiled from the following sources:

1) Laboratories that analyzed water samples for PFOA for NJDEP PFC studies (2006 and 2009) and as requested by water systems;
2) Laboratories that are certified for the analysis of PFOA in drinking water by the NJDEP Office of Quality Assurance (OQA); and
3) National laboratories that have obtained US Environmental Protection Agency (EPA) approval to analyze six PFCs under the Unregulated Contaminant Monitoring Rule 3 (UCMR3) program using EPA Method 537 and that have demonstrated that they are capable of reporting PFOA lower than the required UCMR3 minimum reporting level (MRL) of 20 ng/L.

The PQL for PFOA has been determined as a result of performance data compiled from these three data sources.

1) The NJDEP PFC Database:

The NJDEP PFC Database was originally set up to house the results of the NJDEP 2006 and 2009 PFC studies. The laboratories and methods which generated the study data were reviewed and sanctioned by both the NJDEP OQA and the NJDEP Bureau of Safe Drinking Water (BSDW). Subsequently, PFC results from public water systems conducting their own PFC monitoring were

[2] https://www.epa.gov/ground-water-and-drinking-water/drinking-water-health-advisories-pfoa-and-pfos

added to this database. Public water system data includes follow up testing results based on recommendations made by the NJDEP, as a result of findings of the 2006 and 2009 studies, and data from water systems that voluntarily initiated PFC testing.

The NJDEP PFC database contains 911 PFOA results, 4% of which were generated by STL-Denver, 20% by MWH Laboratories, 35% by Eurofins Eaton Analytical, 34% by Test America-Denver and 7% by Underwriters Laboratories. Severn Trent Laboratory (STL-Denver) became Test America-Denver in 2007. Similarly, MWH Laboratories became Eurofins Eaton Analytical (California) in 2012, and in 2014, part of the Underwriters Laboratory in South Bend, Indiana became Eurofins Eaton Analytical, Inc. (Indiana). Using the current names of the laboratories, the above information would breakdown to the following: 38% Test America-Denver (Colorado), 55 % Eurofins Eaton Analytical (California), and 7% Eurofins Eaton Analytical (Indiana).

In 2006, PFCs were considered emerging contaminants and few laboratories had developed analytical methods capable of analyzing PFOA. The federal safe drinking water regulations required the use of EPA approved drinking water methods for the analyses of regulated contaminants, and although preferred, it was not required that emerging contaminants be analyzed with EPA approved methods. In the absence of an EPA published analytical method for PFOA, the OQA reviewed and approved as a Department Sanctioned Analytical Method (DSAM) an acceptable method for the 2006 PFC study. In addition to reviewing and approving an analytical method, the BSDW requested that the OQA determine the lowest reporting limit (or minimum reporting limit) for PFOA that could be achieved with sufficient precision and accuracy. As a result of the research performed by OQA, the value of 10 ng/L was determined to be a reliable minimum reporting limit for PFOA based on the proprietary methods of Axys Laboratory, STL-Denver (Test America-Denver) and Exygen Laboratory.[3] By 2009, EPA Method 537 was available, although any of the NJDEP OQA sanctioned PFC laboratory methods were acceptable to NJDEP at that time.

Test America-Denver

The NJDEP selected STL-Denver (Test America-Denver) for the 2006 PFC study. STL-Denver's proprietary method, SOP DEN-LC-0012 Revision 4, had been reviewed and approved by OQA as a DSAM for PFOA analysis. They used a Reporting Limit (RL) of 10 ng/L for PFOA and the lowest PFOA calibration standard was 4 ng/L. Any result between 4 ng/L and 10 ng/L was considered a reportable and quantifiable value. (Although the laboratory reports stated 10 ng/L as the RL, 4 ng/L was used as the RL for the 2006 study results). In addition to PFOA, the STL-Denver method was capable of reporting PFOS to 10 ng/L. STL-Denver's certification with NJDEP OQA was specifically for PFOA, however the PFOS concentrations were also reported with the 2006 NJDEP PFC study results.

In February 2009, Revision 7 of the Test America-Denver SOP, DEN-LC-0012, included 14 additional PFC compounds to the analyte target list. Although Revision 7 of SOP DEN-LC-0012 expanded the capability of Test America-Denver to analyze and report 16 PFCs, in

[3] OQA referred to this reporting limit as the "practical quantitation limit."

many cases only PFOA and PFOS results were reported based upon instructions provided to the laboratory by the water system (client).

During the seven years in which Test America-Denver analyzed NJ water system drinking water samples, several different revisions of the method were used. Revision 4 was used for the 2006 PFC study while the latest version currently being used is Revision 12.[4] Table 1 shows that over the course of seven years Test America-Denver reported PFOA data using different RLs with the most frequently occurring RL in the PFC database being 15 ng/L. This RL in addition to the lowest calibration standard of 4 ng/L used in the 2006 PFC study (where the actual laboratory reporting limit was 10 ng/L) will be the values selected from the NJDEP database to represent Test America-Denver's performance data in the consideration of a PQL value (see Table 2). The lowest Test America-Denver Method Detection Limit (MDL)[5] reported, 1.1 ng/L.

Table 1.
Test America PFOA Data from the NJDEP Historical Database using their Proprietary Method, SOP DEN-LC 0012
June 2006 to April 2016

Reporting Limit (ng/L)	Method Detection Limit (ng/L)	# of Results
4	NP	32
9	NP, 2.1	30
10	NP, 1.1,1.9, 2.1	112
12	1.2	3
15	NP, 1.1, 2.1, 2.6, 3.1, 3.6	123
19	1.2, 4.4, 9.3,	7
20	NP, 1.2, 4.1, 4.4, 4.5, 4.6, 9.7, 9.8, 12	35

NP =Information not provided in the PFC database.

MWH Laboratory

The MWH Laboratories proprietary method, MWH SOP-HPLC 12 (also referred to as MWH PFC Extra), was the analytical method used in the 2009 NJDEP PFC Study. This method was approved by OQA and offered lower reporting limits for the PFCs of concern. The MWH PFC Extra method includes PFOA and nine other PFCs. The MWH-PFC Extra reporting limit for

[4] The revision number of the method was not noted in the PFC database. However, if needed, this could be determined based on the date of the analysis. The solid phase extraction method used to prepare the water samples for analysis exists as a stand-alone extraction method (DV-OP-0019).

[5] An MDL is a measurement used by a laboratory to determine specific minimum detection capabilities for a particular method. It is the minimum concentration of a substance that can be measured and reported with 99% confidence that the true value is greater than zero (See 40 CFR 136 Appendix B for procedure).

PFOA is 5 ng/L. Eurofins Eaton Analytical (formally MWH Labs) also offers the method MWH PFC which analyzes PFOA, PFOS and PFBA using the same reporting limits as in MWH PFC Extra.

Underwriters Laboratory

Several water systems used Underwriters Laboratory for their follow up testing. PFC proprietary method L400 was developed by Underwriters Laboratory and was capable of reporting both PFOA and PFOS to 10 ng/L. (The official name for L400 was UL-SBN-LCMS-013-03 and subsequently, 06-LO-S0442). Underwriters Laboratory was certified by NJDEP OQA for analysis of PFOA and PFOS by L400 until 2014 when Eurofins purchased the South Bend, Indiana Underwriters Laboratory. Eurofins Eaton Indiana no longer utilizes the Underwriter's L400 method.

As a result of the of the NJDEP 2006 and 2009 PFC studies, those water systems found to have PFOA and/or PFOS in their drinking water were requested by the NJDEP to conduct follow-up testing within each quarter of the calendar (or every three months). Letters from NJDEP to water systems following the 2006 PFC study included a recommendation to use a method approved by the NJDEP OQA (DSAM), a PFOA RL of 10 ng/L or lower and a low PFOA calibration standard of 4 ng/L. Letters sent to water systems following the 2009 PFC study did not specify reporting limits however, the NJDEP's PFOA health-based level of 40 ng/L developed by the NJDEP Office of Science in 2007 was provided as guidance.

Table 2 provides a summary of PFOA laboratory information obtained from the NJDEP PFC database for samples collected between June 2006 and April 2016. It includes analytical methods, RLs, MDLs, and the number of analyses performed with those RLs/MDLs by three laboratories.

Table 2.
Laboratories/Methods with Reporting Limits and MDLs from the NJDEP PFC Database
PFOA Data from the NJDEP Historical Database[6] June 2006 to April 2016

Laboratory	Method	Reporting Limit (ng/L)	MDL (ng/L)	# of Analyses
Test America Denver	SOP DEN-LC-0012	10	NP, 1.1, 1.9, 2.1	112
		15*	NP, 1.1, 2.1, 2.6, 3.1, 3.6	123
Underwriters Laboratories	L400	10	NP	66
MWH Laboratories/Eurofins Eaton Analytical CA	EPA 537 PFAA	2.5	NP	205
	MWH PFC EXTRA	5	NP	266
	MWH PFC	5	NP	2

NP =Information not provided in the PFC database.
* The most frequent reporting limit from Test America Denver (see Table 1).

[6] The laboratories presented in this table are those that analyzed samples for the NJDEP PFC occurrence studies and those that analyzed follow up samples for those water systems that had detections of PFCs in the NJDEP occurrence studies. This does not include all laboratories capable of performing PFC analysis, only those that analyzed New Jersey public water systems samples during June 2006-April 2016.

2) OQA Sanctioned Methods and Laboratories Certified to Report PFOA in Drinking Water

There are currently three drinking water analytical methods that have been approved by NJDEP OQA as DSAMs for the analysis of PFOA. These consist of EPA 537 and two proprietary methods: DV-LC-0012 Rev 12 developed by Test America-Denver and MWH SOP-HPLC12 Rev 4.0 developed by Eurofins Eaton Analytical (California). The three DSAMs are similar in that they utilize solid phase extraction, isotope dilution and electrospray ionization with LC/MS/MS. Eurofins Eaton Analytical (California), Eurofins Lancaster Laboratories Environmental, Eurofins Eaton Analytical (Indiana), SGS Accutest-Orlando and Test America- Sacramento are certified for analysis of PFOA in drinking water using EPA 537.

Table 3 below lists the six laboratories that are currently certified by the NJDEP OQA to analyze and report PFOA in drinking water with their corresponding NJDEP approved analytical methods (DSAM), RLs and MDLs. Eurofins Lancaster Laboratories Environmental (Pennsylvania), Eurofins Eaton Analytical (Indiana), Test America Sacramento (California) and SGS Accutest-Orlando (Florida) were contacted for their MDLs and RLs. MDLs were not always provided since it is not required with EPA 537.

While PFOA was still an emerging contaminant of concern and EPA 537 was in development, the NJDEP OQA reviewed various proprietary analytical methods and approved them as DSAMs. The vetting and certification by the OQA of a PFC method was necessary in order for NJDEP to initiate the sampling for the 2006 PFC study. Since 2006, various laboratories have obtained OQA approval of their proprietary methods as DSAMS for the analysis of PFOA and other PFCs in New Jersey drinking water samples. After EPA 537 was published, a number of laboratories obtained OQA certification for EPA 537. Should the EPA regulate PFOA, only those PFOA analytical methods approved by the EPA would be allowed for the analysis of regulatory drinking water samples. Laboratories preferring to use their proprietary PFC method for regulatory purposes would then be required obtain EPA approval for their proprietary method as an alternate test method (ATP).

3) UCMR3 EPA Approved Laboratories for PFC Analysis

The Unregulated Contaminant Monitoring Rule (UCMR) is a national monitoring program administered every five years by the EPA in which community water systems serving 10,000 and over throughout the country are required to test their drinking water for a specific set of 30 unregulated contaminants. The UCMR analytes are usually chosen from the corresponding EPA Candidate Contaminant List (CCL) as was the case with the selection of most of the UCMR3 analytes from the CCL3. Besides PFOA and PFOS, the UCMR3 (third list of UCMR contaminants) includes perfluorononanoic acid (PFNA), perfluorohexane sulfonate (PFHxS), perfluoroheptanoic acid (PFHpA), and perfluorobutane sulfonate (PFBS) in the List 1 Assessment Monitoring part of the required monitoring. These four additional PFCs were not selected from the CCL3.

Table 3.
Laboratories Certified by NJDEP Office of Quality Assurance for Analysis of PFOA in Drinking Water with their NJDEP Department Sanctioned Analytical Method (DSAM) Reporting limits and MDLs

OQA Certified Lab	Location (State)	DSAM	Reporting Limit (ng/L)	MDL (ng/L)
Eurofins Eaton Analytical, Inc.	CA	EPA 537	2.5	0.23
		User Defined MWH SOP-HPLC 12, Rev 4.0 (MWH PFC EXTRA)	5	0.327
Eurofins Eaton Analytical, Inc.	IN	EPA 537	20	NR
Eurofins Lancaster Laboratories Environmental	PA	EPA 537	2	1
SGS Accutest Inc. - Orlando	FL	EPA 537	20	8
Test America-Denver	CO	User Defined DV-LC-0012 Rev 12	20	9.79
Test America-Sacramento	CA	EPA 537	20	NR

NR=Not Required.

The Testing Subcommittee identified the UCMR3 participating laboratories as potential sources of data to consider in the PFOA PQL determination, as long as these laboratories were able to provide better performance data than was required for UCMR3. As part of the UCMR3 rule, laboratories performing analyses for any of the UCMR3 contaminants were required to obtain approval from the EPA. Among other requirements, this approval included proficiency testing and on-site audits. The laboratories that applied for UCMR3 analyses were not required to have NELAP or state certification for the analytical methods used for the UCMR3. The EPA established the specific analytical methods to be used for analyzing the UCMR3 contaminants. The PFOA analysis, which also included the analysis of the other five PFCs mentioned above, was performed exclusively with EPA Method 537 version 1.1 for the UCMR3.

EPA 537 version 1.1, "Determination of Selected Perfluorinated Alkyl Acids in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)" was developed by the EPA. The quantitation level term, Minimum Reporting Level (MRL) used in EPA 537, was defined as "the minimum concentration that can be reported as a quantitated value for a method analyte in a sample following analysis." The MRL could be no lower than the concentration

of the lowest calibration standard for that analyte and could only be used if acceptable quality control (QC) criteria for this standard were met. The MRL used in EPA 537 is a term that is more specific than a RL due to the additional requirement of meeting the verification criteria with a one-time demonstration of capability step in Section 9.2.5 of EPA 537. Laboratories using EPA 537 could not report results to a specific MRL unless it was verified with this procedure. Although the EPA required an MRL of 20 ng/L for UCMR3 reporting, a laboratory running EPA 537 had the option of using a different MRL provided that they met the QC requirements for reporting at that MRL. For example, Eurofins Eaton Analytical in Monrovia, California could report PFOA either at 2.5 ng/L or 20 ng/L depending on client requests.

The EPA's MRL of 20 ng/L for PFOA was statistically determined from three laboratories' Lowest Concentration MRLs (LCMRL) which were generated using the procedure described in the Environmental Science Technology article, *Statistical Procedures for Determination and Verification of MRLs for Drinking Water Methods* (Winslow et. al., 2006). The LCMRL is defined as the lowest spiking concentration at which recovery of between 50 and 150% is expected 99% of the time by a single analyst. The EPA determines an MRL using a Bayesian bootstrap of the LCMRL estimator using the LCMRL study data from each of several experienced drinking water laboratories. The Bayesian bootstrap replicates that were generated from each laboratory's data, serve to estimate the distribution of estimated LCMRL values that each laboratory might generate on repeated performance of the LCMRL study. The distribution of pooled Bayesian bootstrap replicates, generated from the LCMRL study data from a sample of experienced drinking water laboratories, approximates the distribution of estimated LCMRL values which might be generated from the *population* of experienced drinking water laboratories. The EPA statistical software, the LCMRL Calculator, performing this process was designed such that the MRL would be an estimate of the LCMRL that is achievable with 95% confidence by a capable analyst/laboratory at least 75% of the time.[7] For PFOA, three laboratory LCMRLs of 18 ng/L, 5.4 ng/L and 0.54 ng/L were integrated into the EPA statistical software resulting in the MRL of 20 ng/L.

The EPA's goal in developing this MRL was to establish a reporting concentration where laboratories across the nation would be able to reliably analyze PFCs for the UCMR3. When discussing the MRLs for the six UCMR3 PFCs, the EPA states in the May 2, 2012 Federal Register, "While particular laboratories may be able to meet MRLs lower than those proposed, the selected MRLs reflect those achievable by the national array of laboratories that support the program."

On behalf of the Testing Subcommittee, the NJDEP conducted a phone inquiry of those EPA laboratories approved for PFC analysis for the UCMR3. The intention of this inquiry was to determine if any of these laboratories with experience analyzing PFCs are also reporting PFOA lower than 20 ng/L for purposes other than the UCMR3. Of the 20 UCMR3 participating laboratories that were solicited for information, five stated that they are reporting PFOA lower than 20 ng/L and five stated that they do not report lower than 20 ng/L. Two labs did not respond. Of the remaining eight labs, five stated either that they were in the process of lowering the reporting

[7] Technical Basis for the Lowest Concentration Minimum Reporting Level (LCMRL) Calculator (EPA 815-R-11-001). http://water.epa.gov/scitech/drinkingwater/labcert/analyticalmethods_ogwdw.cfm

limit or were confident that they could achieve lower reporting limits if client requested and three actually conducted low level calibrations or MRL confirmations in response to our inquiry.

Those UCMR3 participating laboratories reporting PFOA less than 20 ng/L are listed in Table 4. Although these laboratories use EPA 537 for the UCMR3, they may use a modified EPA 537 method or a proprietary method for reporting PFOA to lower concentrations.

Table 4.
UCMR3 Laboratories using PFC Analytical Methods with Reporting Limit or Lowest Calibration Standard Lower than 20 ng/L

UCMR3 Participating Laboratory	State	Analytical Method	Reporting Limit (ng/L)	Lowest Calibration Standard	MDL (ng/L)
American Water Central Laboratory	IL	EPA 537	10	NA[8]	0.382
Columbia Analytical Services ALS	WA	Modified EPA 537	2	2	0.27
Eurofins Eaton Analytical	CA	EPA 537	2.5	2.5	0.23
Eurofins Eaton Analytical	CA	MWH-PFC Extra	5	5	0.550
Orange County Water District Advanced Water Quality Assurance Lab	CA	EPA 537	20	10	NR
Pace Analytical Services-Ormond Beach	FL	S-Fl-O-045 Rev.00	2	2	0.67
State Hygienic Laboratory-Coralville	IA	EPA 537	15	6	NR
Test America- Sacramento	CA	WS-LC-0025	2	1	0.748
Weck Laboratories	CA	Modified EPA 537	5	5	1.81

NA=Not Applicable; NR=Not Required.

This information supports the conclusion that the current reporting limits being used for PFOA are mostly client driven and that if needed, many of these laboratories would be able to accommodate a lower reporting limit than the MRL required by the UCMR3.

[8] American Water Central laboratory conducted an MRL Confirmation Study found in Section 9.2.5 of EPA 537 to prove that they can use an MRL of 10 ng/L. Since that reporting limit had not been requested by any clients, they had not pursued changing their calibration curve and other QAQC requirements for reporting to 10 ng/L.

PQL Determination

In developing the PQL for PFOA, the DWQI Testing Subcommittee considered the RLs, lowest calibration standards and MDLs from laboratories that meet at least one of the criteria below:

1) The laboratories that analyzed water samples for PFOA during the NJDEP 2006 and 2009 studies.;
2) The laboratories must use PFOA methods that have been vetted by the NJDEP OQA, NELAP or EPA; and
3) The laboratories must be EPA UCMR3 approved and demonstrated capability of reporting PFOA lower than the UCMR3 MRL of 20 mg/L using EPA 537 or modifications of EPA 537.

Table 5 consolidates the RL, the lowest calibration standard and MDL data from Tables 2, 3 and 4. Table 2 consists of laboratories that have generated PFOA data that had been entered into the NJDEP's PFC Database. Table 3 consists of laboratories certified by NJDEP OQA for PFOA in drinking water and Table 4 consists of EPA UCMR3 approved laboratories for EPA 537 that are capable of reporting to a lower MRL or RL with either EPA 537, a modification of EPA 537 or a proprietary method. Eurofins Eaton Analytical (Indiana) and Test America-Denver which report to 20 ng/L are included in Table 5. They are included in the consideration of the PQL because water systems that are monitoring for PFCs are directed to the list of OQA certified laboratories which include these laboratories. The NJDEP has been recommending a reporting limit of 10 ng/L for PFOA, however, this was not a requirement.

Determination of the PQL using MDLs

The determination of the PQL using MDLs requires a sample size of at least five MDLs from which to obtain an inter-laboratory MDL value. The individual MDL value from each laboratory for a given method is used to obtain a median MDL value as a representative inter-laboratory MDL. This inter-laboratory MDL is then multiplied by a factor of five. In 1993, a research project was conducted by NJDEP to determine if the MDL multiplied by a certain factor could yield a supportable PQL value. The outcome of this research concluded that a factor of 4, 5 or 6 could be used to derive a PQL (Eaton, et. al., 1993). In 1994, the Testing Subcommittee chose to use a multiplier of five to determine the PQLs generated as part of the NJ DWQI MCL contaminant recommendations. This multiplier approach for determination of a PQL is also consistent with that outlined in the Ground Water Quality Standards (N.J.A.C. 7:9-6).

For PFOA, the Testing Subcommittee was able to derive a PQL from a sample size of 13 MDLs, from ten laboratories and eight different methods. As seen in Table 6 the median MDL value of these 13 MDLs is 1 ng/L. This median value 1 when multiplied by 5 is 5 ng/L.

Table 5.
Consolidation of Laboratory Performance Data Meeting Established Criteria for Determining the PQL[9]

Laboratory	Method	Reporting Limit (ng/L)	Lowest Calibration Standard (ng/L)	MDL (ng/L)
American Water Central Laboratory	EPA 537	10	NA	0.382
Columbia Analytical Services Washington	Modified EPA 537	2	2	0.27
Eurofins Eaton Analytical California	Proprietary MWH PFC EXTRA	5	5	0.550
Eurofins Eaton Analytical California	EPA 537	2.5	2.5	0.23
Eurofins Eaton Analytical Indiana	EPA 537	20	20	NR
Eurofins Lancaster Laboratories Environmental	EPA 537	2	2	1
Orange County Water District Advanced Water Quality Assurance Laboratory	Modified EPA 537	10	10	NP
Pace Analytical Services Inc. Florida	Modified EPA 537	2	2	0.67
SGS Accutest – Orlando	EPA 537	20	20	8.0
State Hygienic Laboratory-Coralville	EPA 537	15	6	NR
Test America-Denver	DV-LC-0012 Rev 4	10	4	2
Test America-Denver	DV-LC-0012 Rev 8	15	4	1.1
Test America-Denver	DV-LC-0012 Rev 12	20	4	9.79
Test America-Sacramento	EPA 537	20	20	NR
Test America-Sacramento	Proprietary WS-LC-0025 Rev 1.2	2	1	0.748
Underwriters Laboratory	L400	10	5	2.9
Weck Labs	Modified EPA 537	5	5	1.81

NA=Not Applicable; NP=Not Provided; NR=Not Required.

[9] Table 5 is a consolidation of Table 2: PFOA Reporting Limits and MDLs from Laboratory Data in the NJDEP PFC Database, Table 3: Laboratories Certified by NJDEP Office of Quality Assurance for Analysis of PFOA Reporting Limit and MDL Information Acquired by Phone or Email (2015) and Table 4: UCMR3 Laboratories with Reporting Limits Lower than 20 ng/L.

Table 6.
Laboratories Used for PQL Calculation in order of Increasing MDL Values

Laboratory	Analytical Method	MDL (ng/L)
Eurofins Eaton Analytical CA	EPA 537	0.23
Columbia Analytical Services	Modified EPA 537	0.27
American Water Central Laboratory	EPA 537	0.382
Eurofins Eaton Analytical CA	Proprietary MWH PFC EXTRA	0.550
Pace Analytical Services, Inc.	S-FL-O-045 Rev.00	0.67
Test America-Sacramento	Proprietary WS-LC-0025 Rev 1.2	0.748
Eurofins Lancaster Laboratories Environmental	EPA 537	1
Test America-Denver	DV-LC-0012 Rev 8	1.1
Weck Laboratories	Modified EPA 537	1.81
Test America-Denver	DV-LC-0012 Rev 4	2
Underwriters Laboratory	L400	2.9
SGS Accutest – Orlando	EPA 537	8
Test America-Denver	DV-LC-0012 Rev 12	9.79
Median of the MDLs		**1**
PQL = Median of MDLs x 5		**5**

Determination of PQL Using Reporting Limits or Lowest Calibration Standards

Analytical terminology based on multiples of the standard deviation such as the MDL, does not account for non-ideal instrumental and analytical occurrences of interference, analyte degradation, matrix enhancement and background contamination which can, particularly at low concentrations, contribute to false positive and false negative results. The MRL in EPA 537 differs from an MDL in that it accounts for both accuracy and precision as a quantitation level. Laboratories using EPA 537 report results to a MRL which is a concentration equal to or greater than the lowest calibration standard, but must also meet the QC criteria at Section 9.2.5 of EPA 537. This criterion is a verification of laboratory proficiency at the laboratory's designated MRL. EPA 537 does not require laboratories to perform the previously discussed LCMRL procedure, but does require this less rigorous MRL confirmation. Both the LCMRL procedure and the confirmation MRL procedure account for the combined effect accuracy and precision have on these quantitation levels.

An MRL can be established either by the laboratory for their own specific purpose or by a regulatory agency as with the required MRL of 20 ng/L for the EPA UCMR3 program. Since EPA 537 describes the MRL as the lowest analyte concentration that meets the Data Quality Objectives developed for the intended use of this method, the MRL would be an important factor in determining the PQL for PFOA. It would follow that, in addition to using inter-laboratory MDLs, the PQL should be assessed by considering the MRLs used by these laboratories. Of the ten laboratories that use either EPA 537 or a modified EPA 537, eight have performed the method's MRL confirmation procedure.

In most cases proprietary methods such as those sanctioned by the NJDEP OQA were developed prior to the publication of EPA 537 and do not include the MRL confirmation procedure. It was helpful to the Testing Subcommittees that several laboratories not certified by the NJDEP OQA provided details on their proprietary methods even though they were not obligated to do so. Each of the proprietary methods required confirmation of the reporting limit within the analytical batch analysis.

If different than the MRL or reporting limit, the laboratories' lowest calibration standard was considered in the PQL assessment. As previously stated since the RLs are mostly client driven it is not obvious that greater sensitivity can be achieved. For this reason, in cases where the lowest calibration standard was lower than the reporting limit, the lowest calibration standard was used in lieu of the reporting limit when deriving the PQL. Three different reporting limits were considered for Test America-Denver since the data was generated using different versions of the original method. In Table 7 the lower of the RL or the lowest calibration standard was used to determine the median. This median was determined to be 5. Likewise, the calculated average (mean) was determined to be 7.2 ng/L.

The data in Table 7 indicates that reporting limits of 2 ng/L are achievable. In considering the PQL, the Testing Subcommittee was aware of background contamination issues with PFOA.

Bootstrap Estimate of a Confidence Interval of a Mean

Basic statistics were used in determining the median from the 13 MDL values in Table 6. The minimum criteria of five laboratories was met for the PQL calculation using the median of the MDLs and the value was determined to be 5 ng/L following the convention of multiplying the inter-laboratory MDL value of 1 ng/L by a factor of five (5).

Another approach that has been used most recently by the EPA for LCMRL range calculation is a statistical technique called "Bootstrap Estimate of a Confidence Interval of the Mean." This technique was applied to generate a normal distribution and associated 95 % upper and lower confidence intervals from the inter-laboratory MDL values from Table 6 and the RLs or the lowest calibration standard from Table 7.

Table 7.
Reporting Limit and Lowest Calibration Standard for Each PFOA Laboratory/Method Combination

Laboratory	State	Method	Reporting Limit (ng/L)	Lowest Calibration Standard (ng/L)
Eurofins Eaton Analytical	IN	EPA 537	20	20
SGS Accutest- Orlando	FL	EPA 537	20	20
Test America-Sacramento	CA	EPA 537	20	20
American Water Central Laboratory	IL	EPA 537	10	NA
Orange County Water District Advanced Water Quality Assurance Lab	CA	EPA 537	20	10
State Hygienic Laboratory Coralville	IO	EPA 537	15	6
Eurofins Eaton Analytical	CA	MWH-PFC	5	5
Weck Laboratories	CA	Modified EPA 537	5	5
Underwriters Laboratory	IN	L400	10	5
Test America-Denver	CO	DV-LC-0012 REV 12	20	4
Test America-Denver	CO	DV-LC-0012 REV 8	15	4
Test America-Denver	CO	DV-LC-0012 REV 4	10	4
Eurofins Eaton Analytical	CA	EPA 537	2.5	2.5
Columbia Analytical Services	WA	EPA 537	2	2
Eurofins Lancaster Laboratories Environmental	PA	EPA 537	2	2
Pace Analytical Services	FL	S-FL-O-045 Rev.00	2	2
Test America-Sacramento	CA	WS-LC-0025 Rev 1.2	2	1
Mean of underlined values[10]	**7.2**			
Median of underlined values	**5**			

NA=Not Applicable

[10] The underlined values are the lower of the reporting limit or MRL and the lowest calibration standard that was used in the 17 lab-method combinations to determine the mean and the median.

Bootstrap Analysis using Inter-laboratory MDLs

A bootstrap analysis of the MDL data presented in Table 6 resulted in a distribution where the upper confidence limit for MDL values reported by laboratories was 4.1 ng/L. The results of this data analysis are shown below in Table 8.

Table 8.
Bootstrap Estimate of Inter-laboratory MDLs

Lower Confidence Limit (ng/L)	Mean (ng/L)	Upper Confidence Limit (ng/L)	Confidence Level Range	Number of Randomly Selected Values[11]
0.9	2.3	4.1	95%	2000

Two laboratories reported MDL values above the upper confidence limit of 4.1 ng/L and were not included in a second iteration of the bootstrap analysis. The second iteration bootstrap analysis resulted in a distribution where the upper confidence limit was 1.6 ng/L. This data are presented in Table 9.

Table 9.
Second Iteration: Bootstrap Estimate of Inter-laboratory MDLs
(Excluding two laboratories with MDLs above the UCL of 4.1 ng/L)

Lower Confidence Limit (ng/L)	Mean (ng/L)	Upper Confidence Limit (ng/L)	Confidence Level Range	Number of Randomly Selected Values[11]
0.6	1.1	1.6	95%	2000

One laboratory reported an MDL above the upper confidence limit of 1.6 ng/L value and was not included in a third iteration of the bootstrap analysis. The third iteration bootstrap analysis resulted in a distribution where the upper confidence limit was 1.2 ng/L. These data are presented in Table 10.

Table 10.
Third Iteration: Bootstrap Estimate of Inter-laboratory MDLs
(Excluding one laboratory with an MDL above the UCL of 1.6 ng/L)

Lower Confidence Limit (ng/L)	Mean (ng/L)	Upper Confidence Limit (ng/L)	Confidence Level Range	Number of Randomly Selected Values[12]
0.5	0.9	1.3	95%	2000

[11] The Bootstrap Technique uses a default value of 2000 iterations to calculate the statistics presented.
[12] The Bootstrap Technique uses a default value of 2000 iterations to calculate the statistics presented.

Using the 95% upper confidence level from the bootstrap method, a PQL value (5 times the Upper Confidence Limit of the MDL) can be calculated following the regulatory convention that has been used by the NJDEP in the past. This value would be 1.3 ng/L x 5 which would be 6.5 ng/L. This MDL value and the PQL calculated from this value is achievable by 95% of the laboratory community that voluntarily provided the performance data presented in this recommendation.

Bootstrap Analysis using MRLs or Reporting Limits

To incorporate more recent techniques of calculating quantification levels, the bootstrap technique can also be applied to evaluate the consistency of the 17 laboratory reporting limits (RLs) or lowest calibration standards found in Table 7. This generated distribution of 2000 randomly selected values produced an upper confidence limit of 10.3 ng/L as a reporting level that 95% of the laboratory community should be able to achieve. The data generated by this bootstrap analysis is in Table 11.

Table 11.
Bootstrap Estimate of Reporting Levels or Lowest Calibration Standards

Lower Confidence Limit (ng/L)	Mean (ng/L)	Upper Confidence Limit (ng/L)	Confidence Level Range	Number of Randomly Selected Values
4.4	7.2	10.3	95%	2000

Three laboratories from Table 7 have RL values above the upper confidence level of 10.3 ng/L. These three laboratories reported RL values and lowest calibration standards information equivalent to the requirements of the UCMR3; the remaining 14 laboratories provided data to demonstrate performance better than that required of the UCMR3. Therefore, because these three laboratories have RL values outside of the 95% confidence interval and did not report their lowest calibration standard information as less than their RL, the RL values were excluded and the statistical analysis was rerun, producing the following information in Table 12.

Table 12.
Second Iteration: Bootstrap Estimate of Reporting Levels or Lowest Calibration Standards
(Excluding three laboratories with Reporting Levels above the Upper Confidence Level of 10.3 ng/L)

Lower Confidence Limit (ng/L)	Mean (ng/L)	Upper Confidence Limit (ng/L)	Confidence Level Range	Number of Randomly Selected Values
3.4	4.6	6.0	95%	2000

This bootstrap analysis generated an upper confidence limit of 6.0 ng/L. This distribution shows that 95% of the laboratory community can achieve a RL level of 6 ng/L. This value of 6 ng/L agrees closely with:

1) the PQL value of 5 ng/L derived from the median of the MDLs from 13 laboratories (Table 6),

2) the PQL value of 7.2 ng/L as the average (or mean) of the17 reporting limits or lowest calibration standards used by actual laboratories (Table 7),

3) the PQL value of 5 ng/L derived from the median of 17 reporting limits or lowest calibration standards used by actual laboratories (Table 7),

4) the PQL value of 6.5 ng/L derived from the bootstrap analysis of the MDLs multiplied by 5 (Table 10), and

5) the PQL value of 6.0 ng/L derived from the bootstrap analysis of the RLs (Table 12).

The median of the values above is 5.9 ng/L; when rounded to one significant figure, the value is 6 ng/L.

Summary and Recommendations

The decision by the Drinking Water Quality Institute to recommend an MCL for perfluorooctanoic acid (PFOA) required the Testing Subcommittee to develop a drinking water Practical Quantitation Limit (PQL) for PFOA. This PQL would then be used in conjunction with the information generated by the Health Effects Subcommittee and Treatment Subcommittee in recommending the PFOA MCL.

The three Drinking Water Quality Institute Subcommittees were tasked with developing values and researching treatment options for PFOA at the same time, therefore a drinking water Health-based MCL was unavailable to the Testing Subcommittee as guidance for determining analytical sensitivity requirements. As a result, several approaches were used to derive a PQL and the resulting PQLs from those approaches were considered in the final determination of the PQL. MDLs from 13 laboratories were used in the determination of the PFOA PQL and determine the PFOA PQL which included those laboratories that generated PFC data found in the NJ PFC database, the New Jersey Office of Quality Assurance certified laboratories and a subset of UCMR3 participating laboratories that analyze and report PFOA lower than the UCMR3 MRL of 20 ng/L. The median value of the MDL values (1 ng/L) multiplied by the factor of 5 resulted in a value of 5 ng/L and a calculated PQL of 5 ng/L. In addition to using the MDLs for determining the PQL, the median value of the lower of the MRLs or minimum reporting limits and lowest calibration standards for the 17 laboratory/method combination of performance data resulted in a PQL value of 5 ng/L. The "Bootstrap Estimate of a Confidence Interval of a Mean" was used to confirm that the calculated values were consistent with the statistically derived values for a PFOA PQL.

The Testing Subcommittee is basing the PQL recommendation to the DWQI on the MRL, using either the reporting limit or lowest calibration standard; whichever is lower. The Testing Subcommittee is not recommending a PQL based on the MDL because the MDL is a statistical value while the others are actual concentrations verified within the analysis. The RLs of the laboratories performing PFOA analysis, however, may be higher than what the laboratory is truly capable of achieving since performance data on emerging contaminants such as PFOA is largely client-driven.

For PFOA, the Testing Subcommittee recommends that the PQL be that derived using the bootstrap upper confidence limit of the RL to account for the lack of laboratory performance data.

Table 13.
Summary of approaches for calculating the PFOA PQL

PQL Approach	Value (ng/L)
Mean of RL (Table 7)	7.2
Median of RL (Table 7)	5.0
Bootstrap Upper Confidence Limit of RL or lowest calibration standard (Table 12)	6.0

The median of the values in Table 13 above that summarizes the approaches used for the PQL derivation and the PQL values derived from each is 6.1 ng/L; when rounded to one significant figure, the value is 6 ng/L. Therefore, the Testing Subcommittee recommends a PQL of 6 ng/L for PFOA to the Drinking Water Quality Institute.

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Socioeconomic Value of the Delaware River Basin in Delaware, New Jersey, New York, and Pennsylvania

The Delaware River Basin, an economic engine for over 400 years

October 11, 2011

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Executive Summary

What do the Guggenheim Museum, New York Yankees, Boeing, Sunoco, Campbell's Soup, DuPont, Wawa, Starbucks, Iron Hill Brewery, Philadelphia Phillies, Camelback Ski Area, Pt. Pleasant Canoe Livery, Salem Nuclear Power Plant, and United States Navy all have in common? They all depend on the waters of the Delaware River Basin to sustain their businesses.

The Delaware River Basin is an economic engine that supplies drinking water to the 1st (New York City) and 7th (Philadelphia) largest metropolitan economies in the United States and supports the largest freshwater port in the world. The Delaware Basin's water supplies, natural resources, and ecosystems in Delaware, New Jersey, New York, Pennsylvania and a small sliver of Maryland:

- Contribute $25 billion in annual economic activity from recreation, water quality, water supply, hunting/fishing, ecotourism, forest, agriculture, open space, potential Marcellus Shale natural gas, and port benefits.

- Provide ecosystem goods and services (natural capital) of $21 billion per year in 2010 dollars with net present value (NPV) of $683 billion discounted over 100 years.

- Are directly/indirectly responsible for 600,000 jobs with $10 billion in annual wages.

The Basin

The Delaware River Basin occupies almost 13,000 sq mi (not including the river and bay) in Delaware, Maryland, New Jersey, New York, and Pennsylvania. In 2010, over 8.2 million residents lived in the basin including 654,000 people in Delaware, 2,300 in Maryland, 1,964,000 in New Jersey, 131,000 in New York, and 5,469,000 in Pennsylvania. Nearly 3,500,000 people work in the basin with 316,000 jobs in Delaware, 823,000 jobs in New Jersey, 70,000 jobs in New York, and 2,271,000 jobs in Pennsylvania. An additional 8 million people in New York City and northern New Jersey receive drinking water from the Delaware River via interbasin transfers. The Delaware Basin occupies just 0.4% of the continental U.S. yet supplies drinking water to 5% of the U.S. population.

The Delaware Basin population exceeds 8.2 million which if counted together would be the 12th most populous state after New Jersey but ahead of Virginia. The Delaware Basin occupies:

- Delaware (50% of the State's area and 74% of the First State's population)
- New Jersey (40% of the State's area and 22% of the Garden State's population)
- New York (5% of the State's area and 0.7% of the Empire State's population)
- Pennsylvania (14% of the State's area and 43% of the Keystone State's population.

Between 2000 and 2010, the population in the Delaware Basin increased by 6.1% or 472,066 people. Over the last decade, the population increased by 30% in Pike County, Pa.; by over 20% in Kent and Sussex counties, Del. and Monroe County, Pa.; and by over 10% in Gloucester and Ocean counties, NJ, Orange County, NY, and Chester, Lehigh, and Northampton counties, Pa. For the first time in two generations, Philadelphia gained population. Several counties in the basin lost population since 2000: Cape May, NJ; Broome, Delaware, and Greene counties, NY; and Lackawanna, Luzerne, and Schuylkill counties, Pa.

Watersheds of the Delaware River Basin

East-West Branch Watersheds

Lackawaxen Watersheds

Neversink-Mongaup Watersheds

Upper Central Watersheds

Lower Central Watersheds

Lehigh Valley

Schuylkill Valley

Upper Estuary Watersheds

Lower Estuary Watersheds

Delaware Bay Watersheds

N

0 10 20

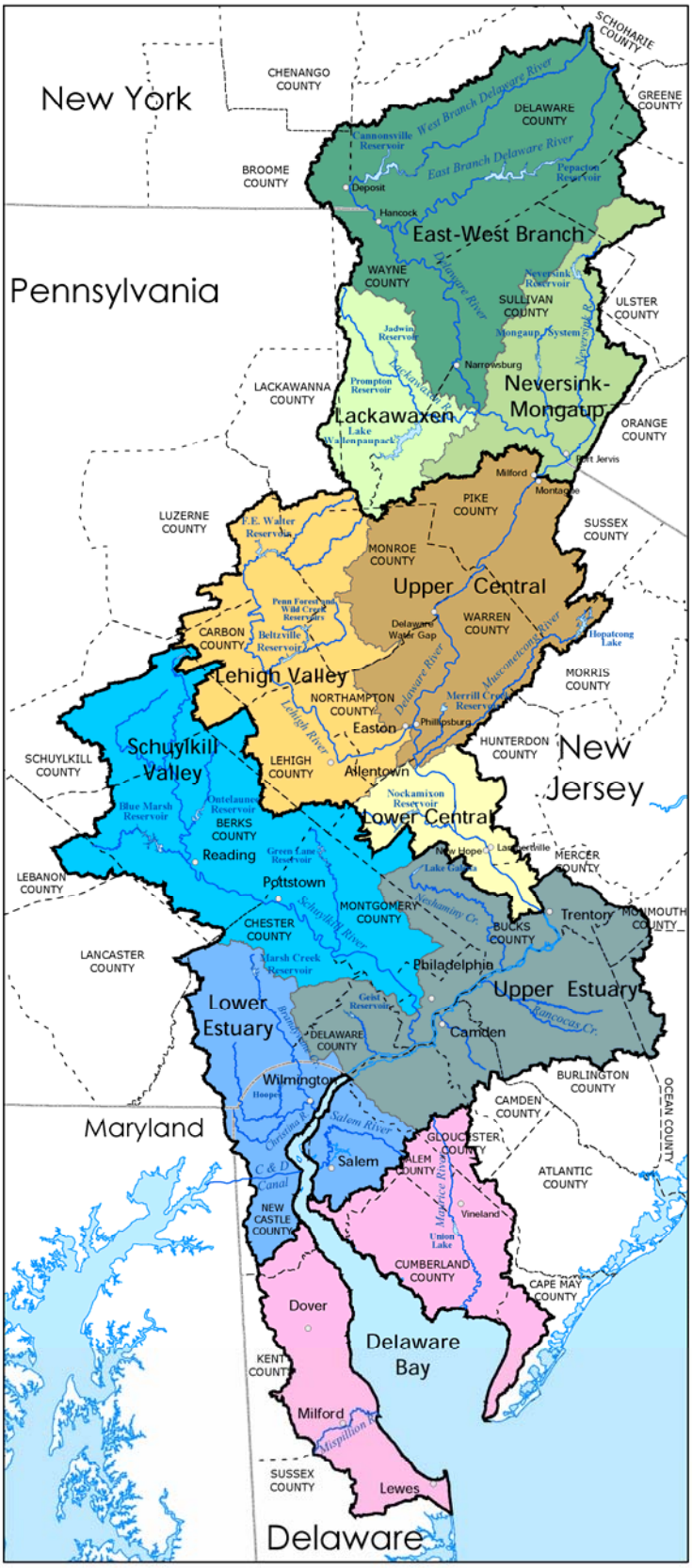
Miles

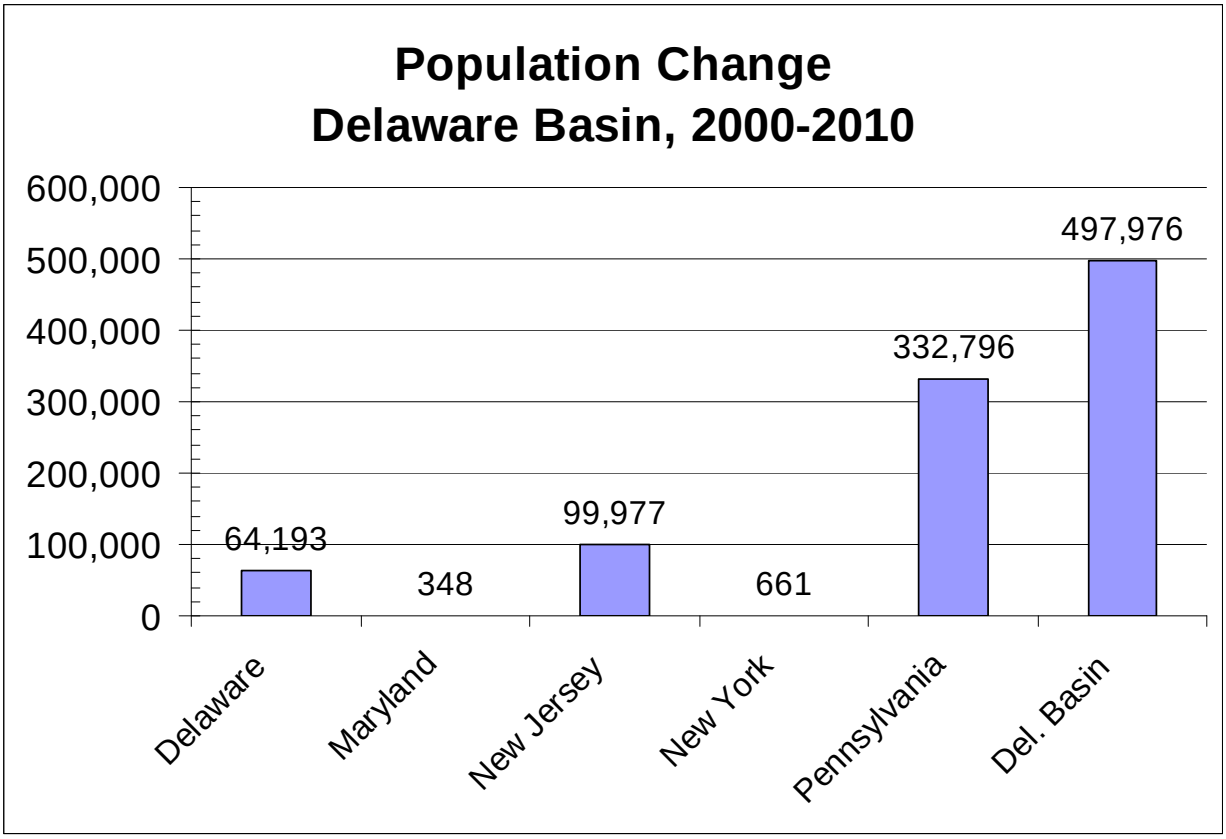
Delaware River Basin Commission

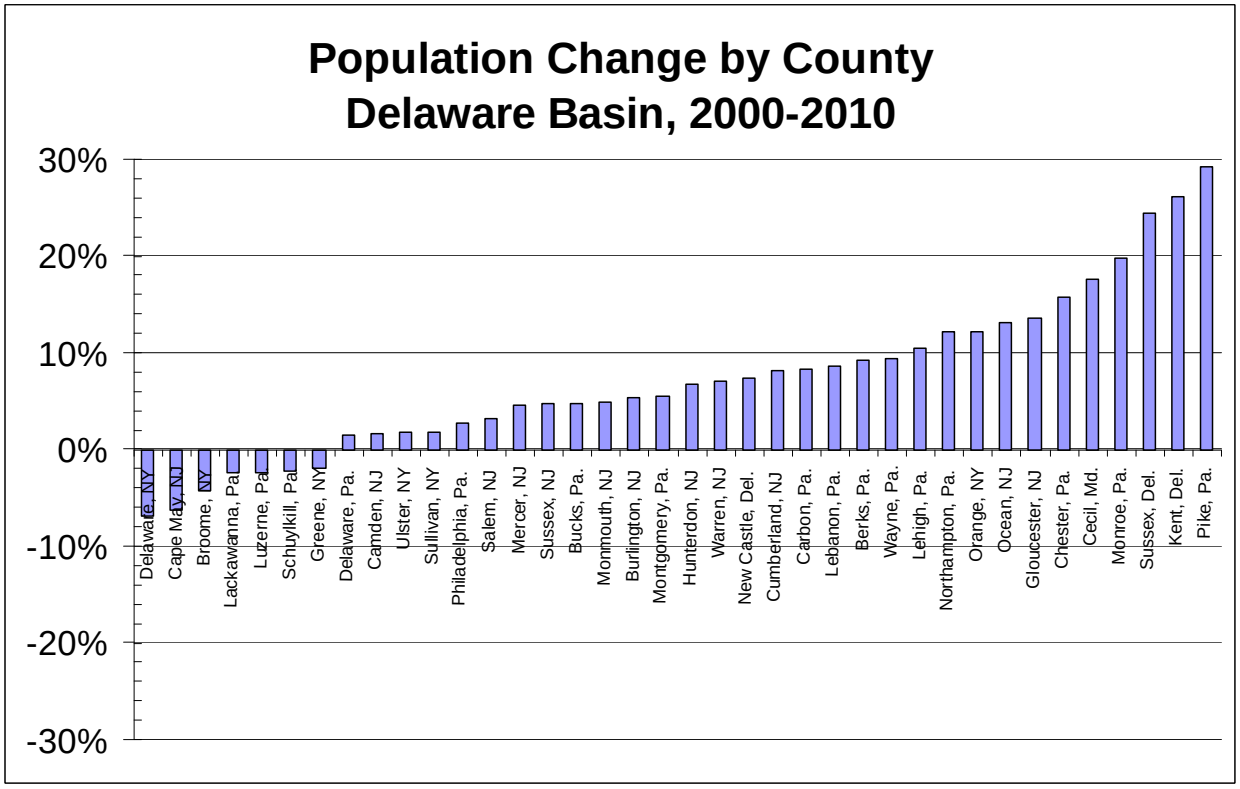
DELAWARE · NEW JERSEY

PENNSYLVANIA · NEW YORK

UNITED STATES OF AMERICA




Population Change
Delaware Basin, 2000-2010
600,000
500,000
400,000
300,000
200,000
100,000
0
64,193
348
99,977
661
332,796
497,976
Delaware
Maryland
New Jersey
New York
Pennsylvania
Del. Basin


Population Change by County
Delaware Basin, 2000-2010
30%
20%
10%
0%
-10%
-20%
-30%
Delaware, NY
Cape May, NJ
Broome, NY
Lackawanna, Pa.
Luzerne, Pa.
Schuylkill, Pa.
Greene, NY
Delaware, Pa.
Camden, NJ
Ulster, NY
Sullivan, NY
Philadelphia, Pa.
Salem, NJ
Mercer, NJ
Sussex, NJ
Bucks, Pa.
Monmouth, NJ
Burlington, NJ
Montgomery, Pa.
Hunterdon, NJ
Warren, NJ
New Castle, Del.
Cumberland, NJ
Carbon, Pa.
Lebanon, Pa.
Berks, Pa.
Wayne, Pa.
Lehigh, Pa.
Northampton, Pa.
Orange, NY
Ocean, NJ
Gloucester, NJ
Chester, Pa.
Cecil, Md.
Monroe, Pa.
Sussex, Del.
Kent, Del.
Pike, Pa.

Annual Economic Activity

The Delaware Basin contributes over $25 billion in annual market/non-market value to the regional economy from the following activities:

- Recreation $1.22 billion
- Fish and Wildlife $1.55 billion
- Public Parks $1.83 billion
- Water Quality $2.46 billion
- Navigation/Ports $2.62 billion
- Marcellus Shale Natural Gas (potential) $3.30 billion
- Agriculture $3.37 billion
- Water Supply $3.82 billion
- Forests $5.13 billion

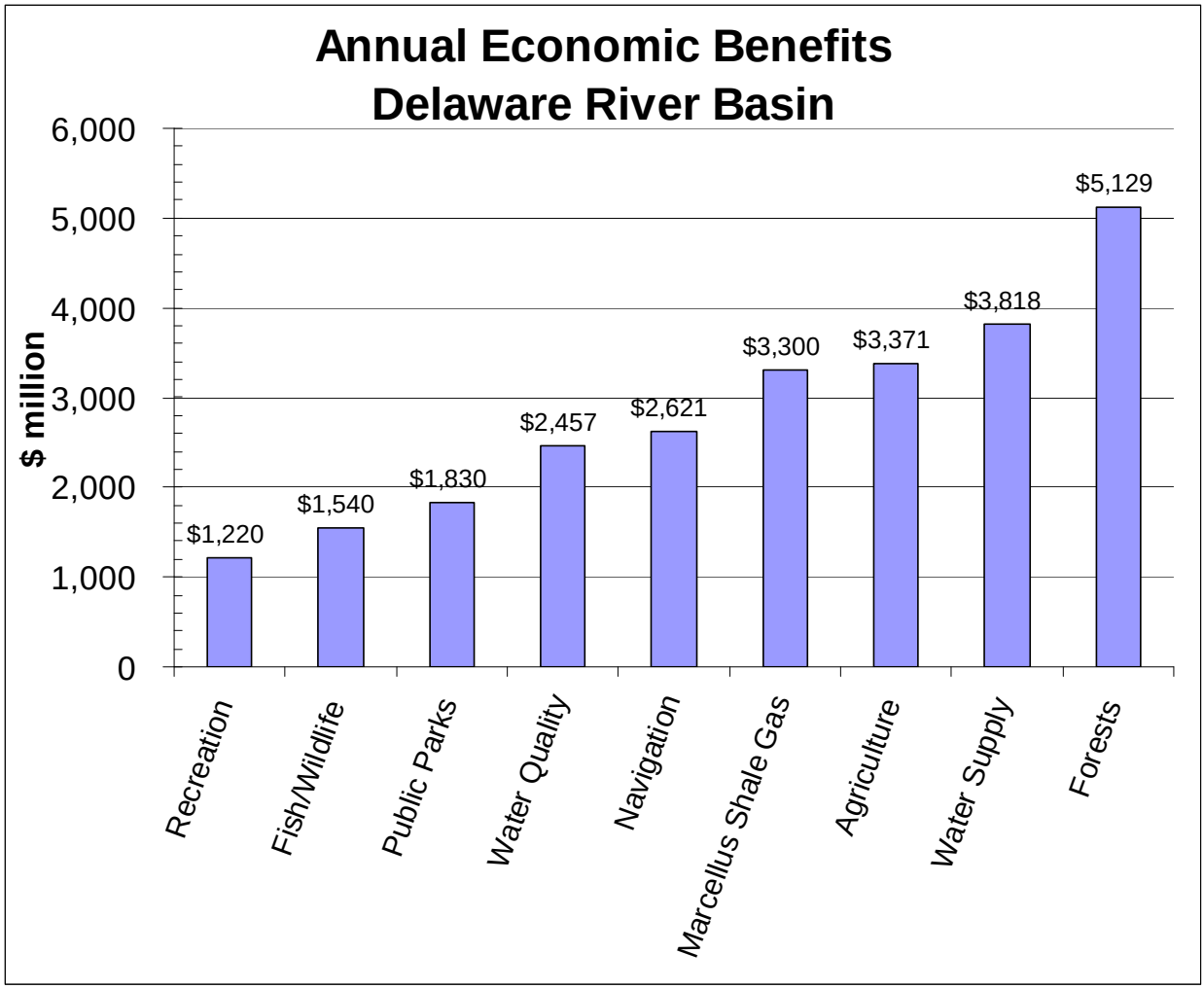


Table E1. Annual economic value supported by the Delaware River Basin.

Market Value	2010 ($ million)	Sources
Recreation (Boating, Fishing, Swimming)		
Skiing (1.9 million ski-days @ $45/day)	325	Penna Ski Areas Association (2010
Paddling-based Recreation (620,860 paddlers)	362	Outdoor Industry Association (2006)
Del. Water Gap River Recreation (267,000 visits)	41	U.S. Forest Service, Nat'l Park Service (1990)
Canoe/Kayak/Rafting (225,000 visits)	9	Canoe and Kayak Liveries (2010)
Powerboating (232,000 boat registrations)	395	National Marine Manufacturers Assoc. (2010)
Water Quality		
Water Treatment by Forests ($96/mgd)	63	Trust for Public Land, AWWA (2004)
Wastewater Treatment ($4.00/1000 gal)	1,722	DRBC and USEPA
Increased Property Value (+8%, 2000 ft of river)	13	EPA (1973), Brookings Institute (2010)
Water Supply		
Drinking Water Supply ($4.78/1000 gal)	3,145	UDWRA and DRBC (2010)
Reservoir Storage ($0.394/1000 gal)	145	UDWRA and DRBC (2010)
Irrigation Water Supply ($300/ac-ft)	32	Resources for Future (1996), USDA (2007)
Thermoelectric Power Water Supply ($44/ac-ft)	297	EIA (2002), NETL (2009)
Industrial Water Supply ($200/ac-ft)	179	Resources for Future (1996), DRBC (2010)
Hydropower Water Supply ($32/ac-ft)	20	Resources for Future (1996), DRBC (2010)
Fish/Wildlife		
Commercial Fish Landings ($0.60/lb)	34	NMFS, Nat'l. Ocean Econ. Program (2007)
Fishing (11-18 trips/angler, $53/trip)	576	U. S. Fish and Wildlife Service (2001)
Hunting (16 trips/hunter, $50/trip)	340	U. S. Fish and Wildlife Service (2001)
Wildlife/Bird-watching (8-13 trips/yr, $27/trip)	561	U. S. Fish and Wildlife Service (2001)
Shad Fishing (63,000 trips, $102/trip)	6	Pennsylvania Fish & Boat Comm. (2011)
Wild Trout Fishing	29	Sportfishing Assn./Trout Unlimited (1998)
Agriculture		
Crop, poultry, livestock value ($1,180/ac)	3,371	USDA Census of Agriculture 2007 (2009)
Public Parks		
Del. Water Gap Natl. Rec. Area (4.9 million visits)	100	U.S. National Park Service (2002)
Marcellus Shale		
Natural Gas (potential)	3,300	USGS (2011), EIA (2011)
Maritime Transportation		
Navigation ($15/ac-ft)	220	Resources for the Future (1996)
Port Activity	2,400	Economy League of Greater Phila. (2008)
Delaware Basin Market Value	**≈ $17.7 billion**	
Non-Market Value		
Recreation (Boating, Fishing, Swimming)		
Clean Water Act Restoration		
Viewing/Aesthetics ($0.58/person)	5	University of Delaware (2003)
Boating ($0.76/person)	6	University of Delaware (2003)
Fishing ($2.95/person)	24	University of Delaware (2003)
Swimming ($6.88/person)	57	University of Delaware (2003)
Water Quality		
WTP for Clean Water ($38/nonuser-$121/user)	659	University of Maryland (1989)
Forests		
Carbon Storage ($827/ac)	3,592	U.S. Forest Service, Del. Center Hort. (2008)
Carbon Sequestration ($29/ac)	126	U.S. Forest Service, Del. Center Hort. (2008)
Air Pollution Removal ($266/ac)	1,155	U.S. Forest Service, Del. Center Hort. (2008)
Building Energy Savings ($56/ac)	243	U.S. Forest Service, Del. Center Hort. (2008)
Avoided Carbon Emissions ($3/ac)	13	U.S. Forest Service, Del. Center Hort. (2008)
Public Parks		
Health Benefits ($9,734/ac)	1,283	Trust for Public Land (2009)
Community Cohesion ($2,383/ac)	314	Trust for Public Land (2009)
Stormwater Benefit ($921/ac)	121	Trust for Public Land (2009)
Air Pollution ($88/ac)	12	Trust for Public Land (2009)
Delaware Basin Non-Market Value	**≈ $7.6 billion**	

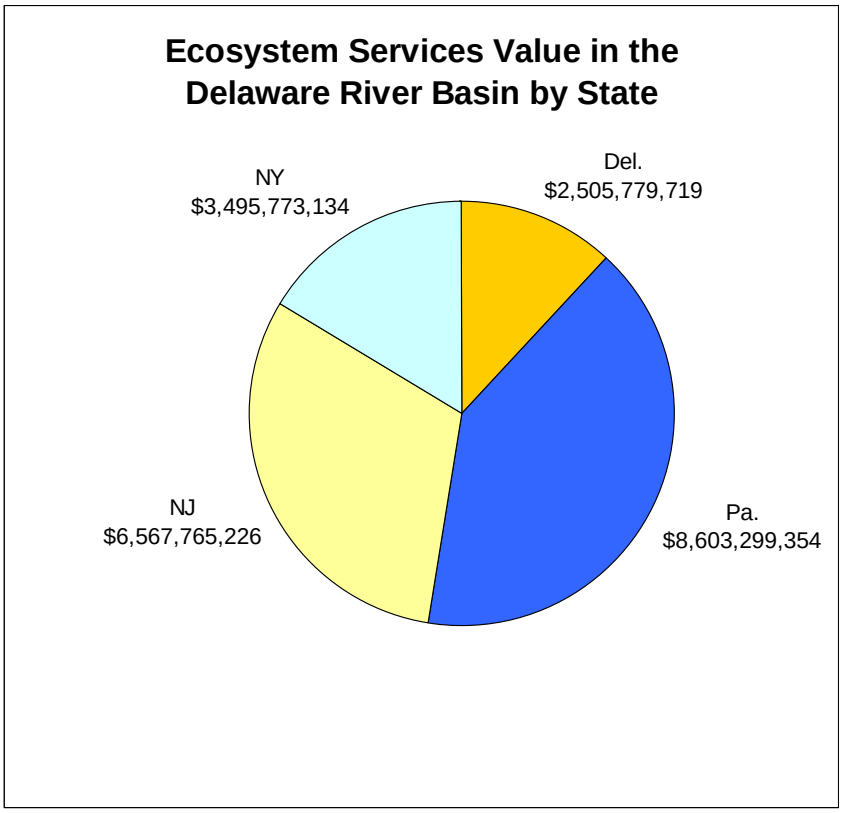
Ecosystem Services

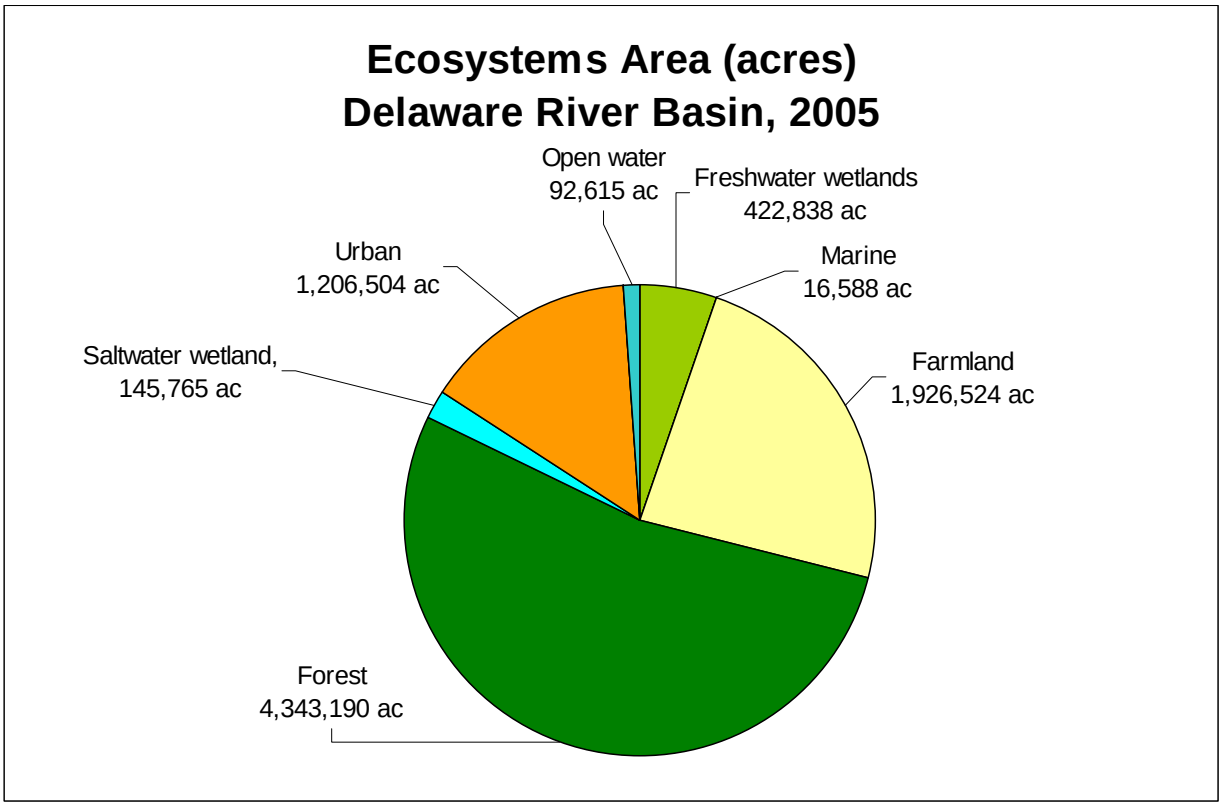
The value of natural goods and services from ecosystems in the Delaware Basin is $21 billion ($2010) with net present value (NPV) of $683 billion using a discount of 3% over 100 years. The contributions of ecosystem services by state include:

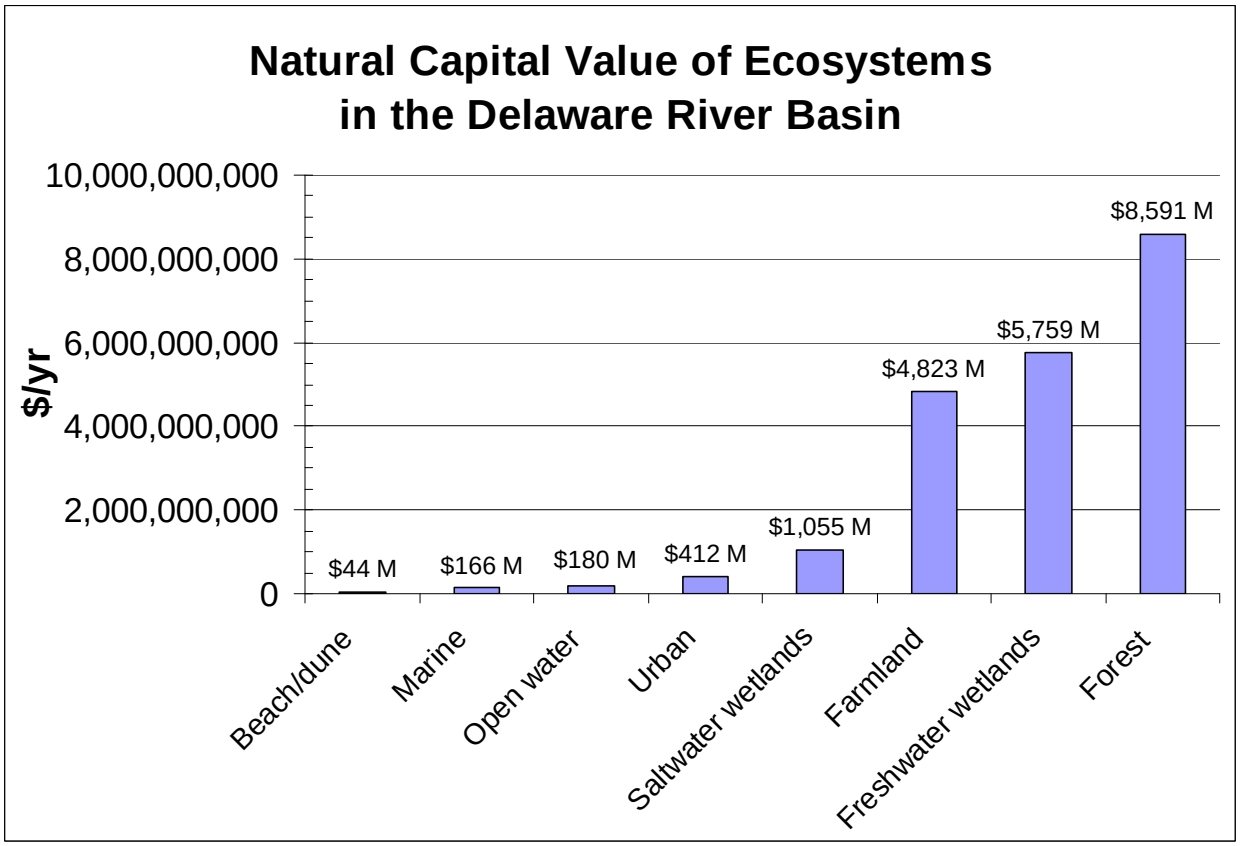
- Delaware ($2.5 billion, NPV $81.4 billion)
- New Jersey ($6.6 billion, NPV $213.4 billion)
- New York ($3.5 billion, NPV $113.6 billion)
- Pennsylvania ($8.6 billion, NPV $279.6 billion)

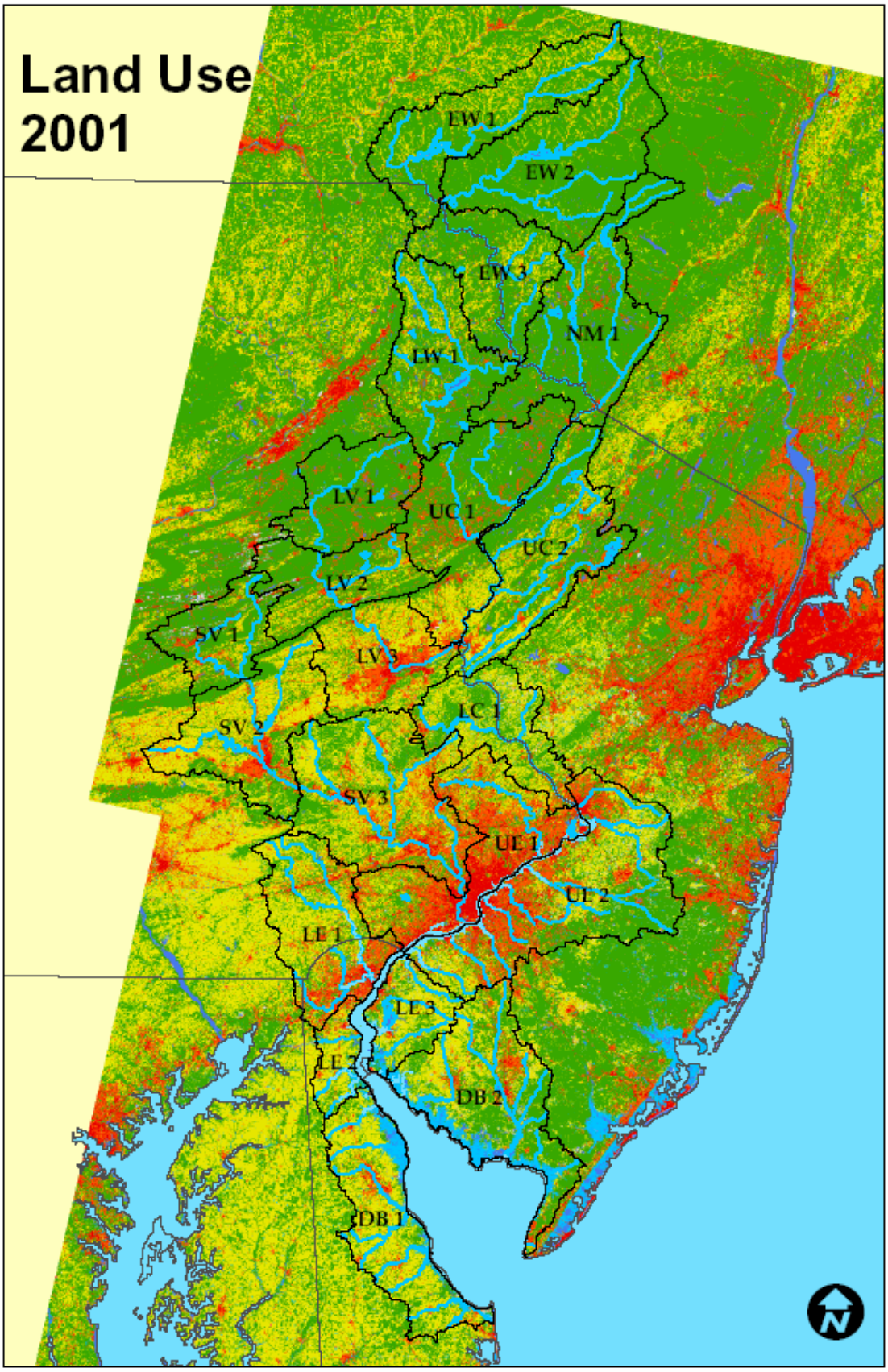
Table E2. Ecosystem goods and services provided by the Delaware River Basin

Ecosystem	Area (ac)	$/ac/yr 2010	$/yr 2010	NPV $
Freshwater wetlands	422,838	13,621	5,759,329,048	187,178,194,067
Marine	16,588	10,006	165,982,947	5,394,445,767
Farmland	1,926,524	2,503	4,823,030,404	156,748,488,136
Forest land	4,343,190	1,978	8,591,367,360	279,219,439,184
Saltwater wetland	145,765	7,235	1,054,617,851	34,275,080,170
Urban	1,206,504	342	412,157,579	13,395,121,322
Beach/dune	900	48,644	43,758,633	1,422,155,566
Open water	92,615	1,946	180,210,703	5,856,847,857
Total	**8,154,924**		**$21,030,454,525**	**$683,489,772,069**




Ecosystems Area (acres)
Delaware River Basin, 2005
Open water
92,615 ac
Freshwater wetlands
422,838 ac
Urban
1,206,504 ac
Marine
16,588 ac
Saltwater wetland,
145,765 ac
Farmland
1,926,524 ac
Forest
4,343,190 ac


Natural Capital Value of Ecosystems
in the Delaware River Basin
$/yr
10,000,000,000
8,000,000,000
6,000,000,000
4,000,000,000
2,000,000,000
0
$44 M
$166 M
$180 M
$412 M
$1,055 M
$4,823 M
$5,759 M
$8,591 M
Beach/dune
Marine
Open water
Urban
Saltwater wetlands
Farmland
Freshwater wetlands
Forest


Land Use
2001
EW 1
EW 2
EW 3
NM 1
LW 1
LV 1
UC 1
UC 2
LV 2
SV 1
LV 3
LC 1
SV 2
SV 3
UE 1
UE 2
LE 1
LE 3
LE 2
DB 2
DB 1
N

Jobs and Wages

The Delaware River Basin is a jobs engine that supports 600,000 direct/indirect jobs with $10 billion in annual wages in the coastal, farm, ecotourism, water/wastewater, ports, and recreation industries.

Table E3. Jobs and wages directly and indirectly supported by the Delaware River Basin

Sector	Jobs	Wages ($ million)	Source
Direct Basin Related	240,621	4,900	U.S. Bureau of Labor Statistics, 2009
Indirect Basin Related	288,745	4,000	U.S. Census Bureau, 2009
Coastal	44,658	947	National Coastal Economics Program, 2009
Farm	45,865	1,376	USDA Census of Agriculture, 2007
Fishing/Hunting/Birding	44,941	1,476	U.S. Fish and Wildlife Service, 2008
Water Supply Utilities	8,750	485	UDWRA and DRBC, 2010
Wastewater Utilities	1,298	61	UDWRA and DRBC, 2010
Watershed Organizations	201	10	UDWRA and DRBC, 2010
Ski Area Jobs	1,753	88	Penna. Ski Areas Association
Paddling-based Recreation	4,226		Outdoor Industry Association (2006
River Recreation	448	9	U. S. Forest Service/Nat'l. Park Service, 1990
Canoe/Kayak/Rafting	225		Canoe Liveries and UDWRA, 2010
Wild Trout Fishing	350	4	Maharaj, McGurrin, and Carpenter, 1998
Del. Water Gap Nat'l. Rec. Area	7,563	101	Stynes and Sun, 2002
Port Jobs	12,121	772	Economy League of Greater Phila., 2008
Delaware Basin Total	**> 600,000**	**>$10 billion**	

Within the Delaware Basin are 3,480,483 jobs earning $172.6 billion in wages including:

- Delaware (316,014 jobs earning $16.5 billion in wages)
- New Jersey (823,294 jobs, $38.1 billion in wages)
- New York (69,858 jobs earning $2.5 billion in wages)
- Pennsylvania (2,271,317 jobs earning $115.5 billion in wages)

Jobs directly associated with the Delaware River Basin (such as water/sewer construction, water utilities, fishing, recreation, tourism, and ports) employ 240,621 with $4.9 billion in wages including:

- Delaware (15,737 jobs earning $340 million in wages)
- New Jersey (62,349 jobs earning $1.3 billion in wages)
- New York (32,171 jobs earning $550 million in wages)
- Pennsylvania (130,364 jobs earning $2.8 billion in wages)

Jobs indirectly related to the waters of the Delaware Basin (based on multipliers of 2.2 for jobs and 1.8 for salaries) employ 288,745 people with $4.0 billion in wages including:

- Delaware (18,884 jobs earning $270 million in wages)
- New Jersey (74,819 jobs earning $1.0 billion in wages)
- New York (38,605 jobs earning $400 million in wages)
- Pennsylvania (156,437 jobs earning $2.2 billion in wages)

According to the National Coastal Economy Report (2009), coastal employment sectors within the Delaware River Basin are responsible for 44,658 jobs earning $947 million in wages with contributions of $1.8 billion toward the GDP including:

- Delaware (12,139 jobs, $214 million in wages, $392 million toward the GDP)
- New Jersey (4,423 jobs, $140 million in wages, $235 million toward the GDP).
- Pennsylvania (28,096 jobs, $593 million in wages, $1.2 billion toward the GDP.

Over 21,800 farms provide 45,865 jobs with $1.9 billion in wages in the Delaware Basin including:

- Delaware (3,140 farm jobs earning $129 million in wages)
- New Jersey (14,305 farm jobs earning $587 million in wages)
- New York (2,410 farm jobs earning $99 million in wages)
- Pennsylvania (26,010 farm jobs earning $1.1 billion in wages)

Fishing, hunting, and bird watching/wildlife associated recreation employ 44,941 jobs with $1.5 billion in wages in the Delaware Basin including:

- Delaware (4,080 jobs earning $134 million in wages)
- New Jersey (17,477 jobs earning $574 million in wages)
- New York (4,872 jobs earning $160 million in wages)
- Pennsylvania (18,512 jobs earning $608 million in wages)
-

Public and private water utilities that withdraw drinking water from the Delaware River Basin employ 8,750 people with wages of $485 million including:

- Delaware (141 jobs earning $7.8 million in wages)
- New Jersey (823 jobs earning $46 million in wages)
- New York (5,600 jobs earning $310 million in wages)
- Pennsylvania (2,186 jobs earning $121 million in wages)

Wastewater utilities that treat and discharge wastewater to the Delaware River Basin employ 1,298 people with wages of $61 million including:

- Delaware (108 jobs earning $5 million in wages)
- New Jersey (257 jobs earning $12 million in wages)
- New York (20 jobs earning $1 million in wages)
- Pennsylvania (913 jobs earning $43 million in wages)

Over 100 nonprofit watershed and environmental organizations employ at least 200 staff who earn at least $9.5 million in wages to restore the watersheds in the Delaware River Basin.

In the Pocono Mountains of Pennsylvania, 9 ski resorts support 1,753 direct jobs in the Delaware Basin from aggregate annual revenues of $87,655,063 from 1,908,228 skier visits.

Paddling-based recreation in the Delaware Basin is responsible for 620,860 participants and 4,226 jobs according to data prorated from the Outdoor Industry Association (2006).

The U. S. Forest Service and U.S. National Park Service estimated river recreation along the Upper Delaware River and Delaware Water Gap was responsible for 448 jobs with wages of $8.8 million in $1986.

The 37 canoe/kayak liveries along the Delaware, Lehigh, and Schuylkill, and Brandywine Rivers have earnings of $9 million per year and employ 225 people to lease watercraft to 225,000 visitors.

Along the Beaverkill, East Branch, West Branch, and upper main stem of the Delaware River in New York, wild trout fishing provides for 350 jobs with $3.6 million in wages.

The Delaware Water Gap National Recreation Area recorded 4,867,272 recreation visits in 2001 that generated $106 million in sales and 7,563 direct/indirect jobs with $100 million in wages.

Delaware River ports from Wilmington to Philadelphia to Trenton are collectively the 5th largest port in the U.S. based on imports and the 20 largest U.S. port based on exports. These ports:

- Employ 4,056 workers who earn $326 million in wages.
- Provide port jobs that support an additional two jobs each in port activity and employee spending for a total of 12,121 port related jobs with $772 million in wages.
- Most of the 4,056 direct port jobs are in cargo handling and warehousing with petroleum port jobs adding up to less than 10% of employment
- Provides good jobs, the average salary of a port employee (with benefits) is over $80,000.

1. Introduction

Objectives

This report summarizes the socioeconomic value of water, natural resources and ecosystems in the Delaware River Basin in Delaware, New Jersey, New York, and Pennsylvania estimated as:

- Economic activity including market use and nonuse value of water supply, fishing, hunting, recreation, boating, ecotourism, agriculture, and navigation/port benefits in the basin.

- Natural capital or ecosystem services value of natural goods and services provided by habitat such as wetlands, forests, farms and open water.

- Jobs and wages directly and indirectly associated with the Delaware River Basin.

Two decades ago, researchers conducted a series of studies that indicated the Delaware River and Bay was worth hundreds of millions if not billions of dollars. Latham and Stapleford (1990) from the University of Delaware estimated total contributions of Delaware Estuary (the tidal river and bay) activities within the State of Delaware accounted for 10,500 jobs with $222 million in annual wages, each direct estuary job created 2.2 indirect jobs, and the multiplier of direct/indirect wages was 1.8. The Greeley-Polhemus Group (1993) estimated the Delaware Estuary supported 123,000 jobs, $4.3 billion in wages, $24 billion in sales, $25 million in sport fishing non-market value, $1 million in commercial fish landings, and wetlands replacement values up to $638 million.

This report is designed to update economic analyses for the Delaware River and Bay conducted 20 years ago and incorporate more recent valuation data from the emerging fields of ecological economics and ecosystem services.

The Value of a Watershed

Studies for the Chesapeake Bay, Great Lakes, and Florida Everglades conclude that watersheds have significant economic value and restoration can result in green jobs and favorable cost-benefit investment ratios. The University of Maryland reported in 1988 that the Chesapeake Bay was worth $678 billion and the Chesapeake Blue Ribbon Panel (2003) reported with inflation the present value of the bay would exceed $1 trillion.

The Brookings Institution (Austin et al. 2007) found restoration of the Great Lakes would cost $26 billion in present value and aggregate economic benefits would exceed $50 billion (2:1 B/C ratio). Great Lakes benefits include $6.5-11.8 billion in tourism, fishing, and recreation dollars, $12-19 billion increase in property values from contaminated sediment cleanup, $50-125 million in reduced municipal water treatment costs, and $30 billion in short time multiplier benefits. The Great Lakes Coalition (2010) concluded investment in watershed restoration creates good paying jobs and leads to economic benefits while restoring the environment (Table 1).

The Everglades Foundation estimated that the Comprehensive Everglades Restoration Plan (CERP) would result in $6 billion in benefits and 443,000 jobs over 50 years (McCormick 2010). Net present

value of the Everglades's restoration benefits would be $46 billion resulting from investments of $11.5 billion or a benefit to cost ratio of 4:1.

Table 1. Jobs and salaries created by watershed restoration work
(Great Lakes Coalition (2010) from U. S. Bureau of Labor Statistics)

Job	Mean Salary	Job	Mean Salary
Wetland scientist	$45,730	Fisheries Biologist	$60,670
Research scientist	$45,730	Archeologist	$57,230
Construction manager	$93,290	Operating Engineer	$44,180
Biologist	$69,430	Environmental Engr.	$80,750
Toxicologist	$70,000	Hydrogeologist	$92,710
Chemist	$72,740	Environmental Planner	$64,680
Geologist	$58,000	Plumber/Pipefitter	$9,870
Helicopter Pilot	$90,000	Carpenter	$43,640
Info. Technology	$70,930	Electrician	$50,850
Admin. Staff	$32,990	Truck Driver	$39,260
Mechanics	$37,000	Concrete Workers	$39,410
Excavator	$38,540	Dredge Operator	$38,330
Landscape Architect	$65,910	Conservation Scientist	$61,180
Civil Engineer	$81,180	Biological technician	$41,140
General Laborer	$33,190	Pile Drive Operator	$51,410

An Economic Engine

What do the Guggenheim Museum, Boeing, Sunoco, Campbell's Soup, DuPont, Wawa, Starbucks, Iron Hill Brewery, Philadelphia Philadelphia Phillies, New York Yankees, Camelback Ski Area, Pt. Pleasant Canoe Livery, Salem Nuclear Power Plant, and the United States Navy have in common? They all depend on the waters of the Delaware River Basin to sustain their businesses.

Most economists agree that water is an undervalued resource. The astronomer Copernicus and Adam Smith of the invincible hand of the economy fame both considered the "diamond-water paradox". If water is more valuable to society than a precious gem, then why is water sold for a fraction of a penny per gallon for drinking water or not even valued at all as an ecological resource in the river or bay? Just as under-compensated police officers or teachers are more valuable to society than multimillion dollar movie stars, perhaps the value of water is just as marginalized. We tend to underprice water based on its marginal value for single uses (i.e. drinking water) and not consider the full value of water for all its myriad uses. This report attempts to quantify the highest multiobjective value of water *in toto* for its wide range of habitat, recreation, ecological, and industrial benefits in the Delaware River Basin.

If water is society's most valuable chemical, then the Delaware River with a mean annual flow of 2.7 trillion gallons per year at Trenton is the Delaware Valley's (and by aqueduct Manhattan Island's) most invaluable economic asset. For 400 years, the Delaware River has been an economic engine ever since Henry Hudson discovered the bay off Cape May in August 1609 for commerce and the Dutch East India Company during his unsuccessful quest for an inner trade route to Asia.

When William Penn founded the City of Brotherly Love in 1681 seeking refuge from religious persecution in Europe, he also found a safe harbor between the Delaware and the Schuylkill in a colony rich with lumber, fertile land, beaver pelts, and in later centuries coal and oil reserves. By the 18th century frugal yet prosperous Philadelphia Quaker merchants established triangle trade route connections to Europe and the Caribbean from their home port along the Delaware. During the American Revolution, Philadelphia was the largest city in the colonies and the 3rd largest port in the British Empire after London and Liverpool. In 1790 Ben Franklin, America's first environmentalist, was so concerned about pollution in the river that he willed funds to build the first municipal water system in the United States at Philadelphia to tap the Delaware and Schuylkill for drinking water.

The economic engine kicked into high gear during the 19th century with hydropower and steam power during the Industrial Revolution. In 1802, the DuPont family searched up and down the Atlantic Seaboard and established gunpowder mills along the falls of the Brandywine River above Wilmington as one of the first industries in the Delaware Valley. Delaware River ports grew when anthracite coal was discovered in the Lehigh Valley in 1792 and steam railroads were built in the 1830s. By the Gay Nineties, every Philadelphia wharf had railroad access and the advent of steam ships made for faster transatlantic shipping. In 1895, the Corps of Engineers dredged the Delaware River to 26 feet from the natural depth of 17 feet (Economy League 2008).

By the end of the 19th century, the Delaware River supported the largest commercial American shad and sturgeon fishery along the Atlantic coast. The sturgeon was such a lucrative fish that boom town Caviar (Bayside) near Greenwich, New Jersey was founded to process the roe for worldwide export. By the 1880s, 1,400 sailing vessels harvested 22 million pounds of oysters from the Delaware Bay. In 1886, nationally famous hotels in Gloucester, N. J. served 10,000 planked shad dinners at events that resembled modern day blue crab feasts. In 1896 over 14 million pounds of shad were caught with a value of $400,000 ($10 million in 2008 dollars). In 1896, a fisheries report to the governor of Pennsylvania listed the catch of a 76-pound striped bass above Gloucester, NJ.

At the turn of the 20th century, Delaware River ports supported a premier ship building industry. By the First World War the Delaware was known as the "Clyde of America" with ship building and repair production that rivaled its Scottish cousin. By 1912, Philadelphia and environs built and manufactured 5% of all goods in the United States. Export markets included coal, iron, cotton, leather, grain, lumber and tobacco, and gunpowder from Wilmington. By 1914, the Panama Canal opened access from the East Coast to Hawaii sugar cane fields and Philadelphia refined and shipped 500,000 tons of raw sugar or 1/6 of all sugar refined in the United States.

After the Delaware River ship channel was deepened to 41 feet in 1941, the port economy boomed during World War II as the Philadelphia Navy Yard employed 40,000 workers who built 53 ships and repaired over 500 vessels. After the war, the "Arsenal of America" manufacturing and export base declined due to decreased demand for Pennsylvania coal and decline of Lehigh Valley steel industries. In 1995, the Department of the Navy closed the Philadelphia Navy Yard and decommissioned the ghost fleet due to decreased ship building needs in the "New Navy."

During the 19th Century, the Delaware Water Gap along the Blue Mountain near Stroudsburg, Pa. was a resort that grew with the railroads from Philadelphia and New York City. In 1965, Congress authorized the National Park Service to form the Delaware Water Gap National Recreation Area that now receives 5 million visits per year, the 8th most visited unit in the National Park System.

In 1931 and amended in 1954, the U. S. Supreme Court issued a decree authorizing New York City to divert 800 mgd of water from three Catskill Mountain reservoirs in the Delaware Basin to the Hudson River Basin. The Delaware River delivers over half the drinking water to New York City.

By 1986, the Salem and Hope Creek nuclear power plants were built on Artificial Island in Salem County, New Jersey that pump 3 billion gallons per day of cooling water to provide 3,500 megawatts of electricity to the tri-state region. In 2010, a billion gallons per day of drinking water and industrial process water were withdrawn from streams and aquifers in the Delaware Basin to sustain the region's jobs and domestic, commercial, and industrial economy. The river, bay, beaches, wetlands, and forests support a billion dollar tourism, recreation, and hunting/fishing/birding economy.

After the turn of the 21st Century, new horizontal drilling and hydraulic fracturing technology kicked off the Marcellus Shale natural gas drilling boom in a 50,000 square mile basin stretching from Kentucky to Pennsylvania and New York. The Marcellus Shale occupies about 36% or 4700 square miles under the upper Delaware Basin. A 2011 USGS report indicates 7 trillion cubic feet of natural gas may be recoverable under the Delaware Basin, a potential multi-billion dollar natural resource.

The Delaware River Basin supplies drinking water to the 1st (New York City) and 5th (Philadelphia) largest metropolitan economies in the United States. The following report tabulates the substantial economic value and worth of this irreplaceable asset for over 8 million residents in Delaware, New Jersey, New York, and Pennsylvania who live in the basin and an additional 8 million people in New York City and northern New Jersey who receive drinking water from the Delaware River.

Governance

For the last fifty years, Federal, state, and local governments, nonprofits, and the private sector have focused efforts on restoring the Delaware River Basin. In 1961, JFK signed the Delaware River Basin Compact that appointed the Governors of Delaware, New Jersey, New York, and Pennsylvania as Commissioners as the first ever Federal-state watershed accord. In 1968 a full four years before the Clean Water Act was passed by Congress, the DRBC issued waste load allocations to reduce pollutant discharges from over 80 wastewater treatment plants. In 1988, the Delaware Estuary was nominated by the Governors of Delaware, New Jersey, and Pennsylvania for the National Estuary Program per Section 320 of the Federal Clean Water Act. In 1996, the Delaware Estuary was designated by Congress as one of only 28 National Estuary Programs in the United States and is now the only tri-state estuary program in the nation. In 1996, the nonprofit Partnership for the Delaware Estuary was established to implement a Comprehensive Conservation and Management Plan (CCMP). In 2011, the DRBC celebrates the 50th anniversary of its founding by JFK, Congress, and the Governors of Delaware, New Jersey, New York, and Pennsylvania.

The Watershed

The Delaware River Basin (Figure 1 and Table 2) occupies 12,769 sq mi (not including the river and bay) in Delaware (8%), New Jersey (23%), New York (20%), and Pennsylvania (49%). In 2010, 8,255,013 residents lived in the basin including 643,418 people in Delaware (9%), 2,324 in Maryland, 1,951,047 in New Jersey (24%), 124,969 in New York (2%), and 5,533,254 in Pennsylvania (66%). In 2009, nearly 3,500,000 people worked in the Delaware Basin with 316,014 jobs in Delaware (9%), 1,172 jobs in Maryland, 823,294 jobs in New Jersey (24%), 69,858 jobs in New York (2%), and 2,271,317 jobs in Pennsylvania (65%).

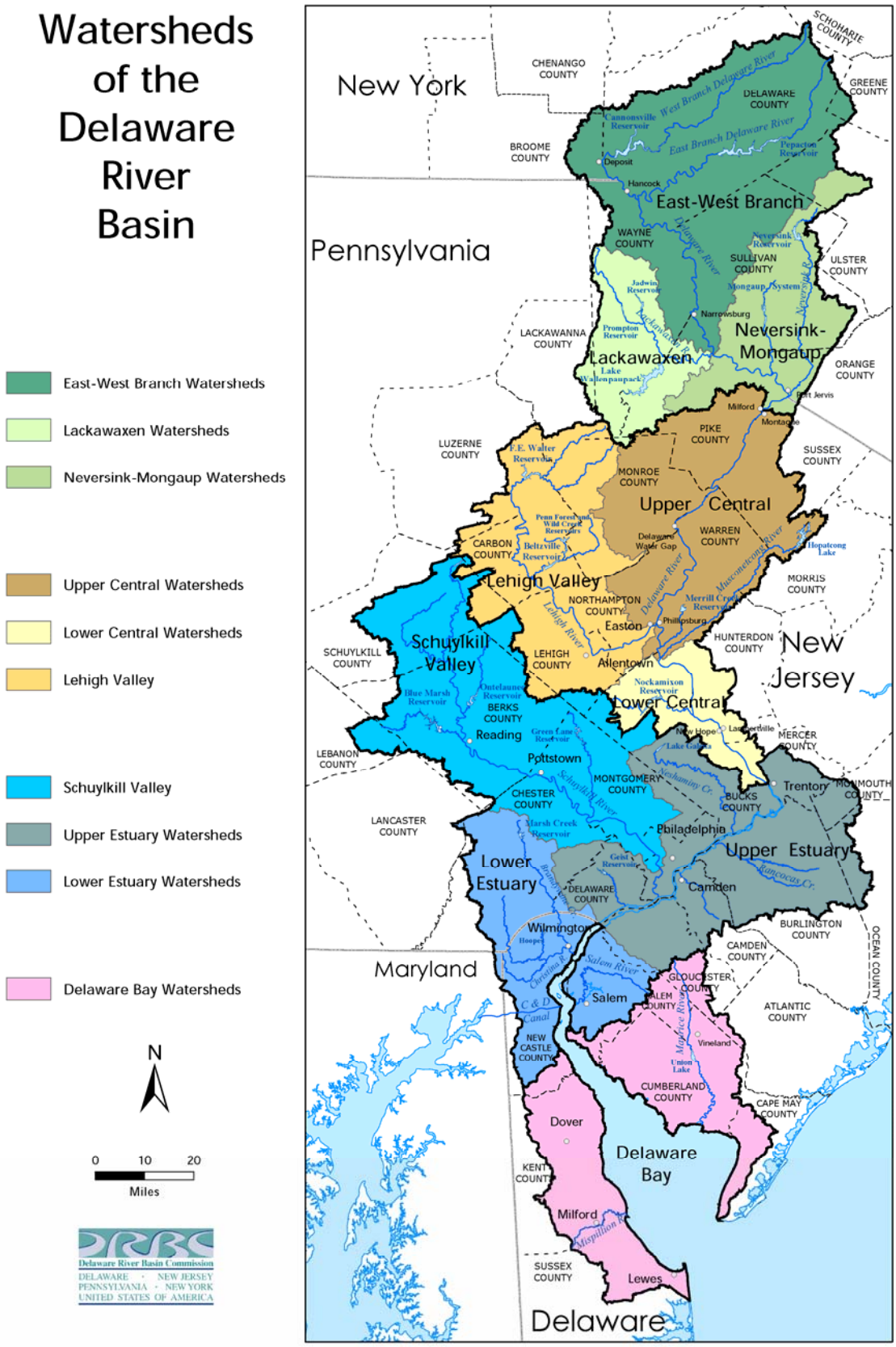


Figure 1. The Delaware River Basin. (DRBC)

Table 2. Land area, population, and employment in the Delaware River Basin

State	Area (sq mi)	Population[1] 2010	Employment[2] 2009
Delaware	965	643,418	316,014
Maryland	8	2,324	1,172
New Jersey	2,961	1,951,047	823,294
New York	2,555	124,969	69,858
Pennsylvania	6,280	5,533,254	2,271,317
Total	12,769	8,255,013	3,481,655

1. U.S. Census Bureau 2009. 2. U.S. Bureau of Labor Statistics

Table 3 summarizes the area, population, and employment by state and county in the Delaware Basin. In Delaware, the basin covers 50% of the land area yet includes 74% of the First State's population. The New Jersey portion of the basin covers 40% of the State's land area and includes 22% of the Garden State's population. New York State covers 5% of the State's land area and the basin includes 0.7% of the Empire State's population. The Pennsylvania part of the basin covers just 14% of the State's area yet includes 43% of the Keystone State's population.

The population of the Delaware Basin now exceeds 8.2 million which if considered as a single jurisdiction, it would be the 12th most populous state in the U.S. after North Carolina and New Jersey but ahead of Virginia and Massachusetts. Between 2000 and 2010, the population in the Delaware Basin increased by 6.1% or 472,066 people (Table 4 and Figure 2). Over the last decade, population increased by 30% in Pike County, Pa.; by over 20% in Kent and Sussex counties, Del. and Monroe County, Pa.; and by over 10% in Gloucester and Ocean counties, NJ, Orange County, NY, and Chester, Lehigh, and Northampton counties, Pa (Figure 3). For the first time in twp generations, Philadelphia gained population. Several counties in the basin lost population since 2000: Cape May, NJ; Broome, Delaware, and Greene counties, NY; and Lackawanna, Luzerne, and Schuylkill counties, Pa.

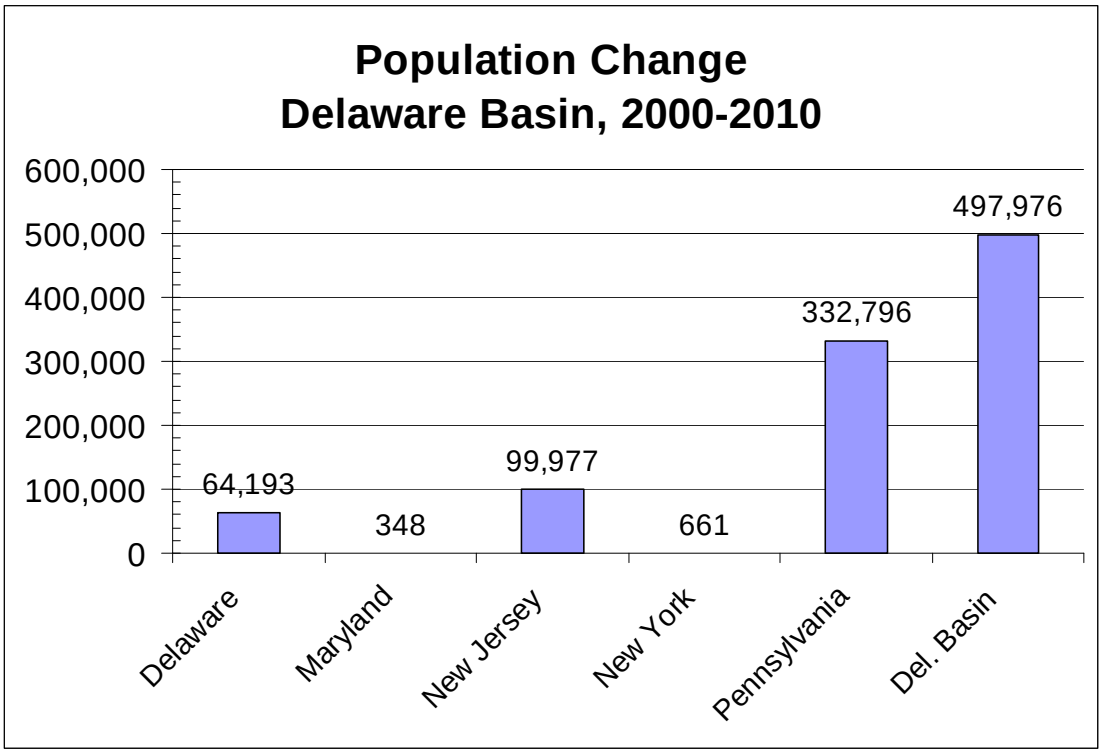
Table 3. Land area, population, and employment by county in the Delaware River Basin

State/county	Area 2005[1] (sq mi)	Population[2] 2010	Employment[3] 2009
Kent	389	108,025	50,412
New Castle	381	493,428	252,534
Sussex	195	41,965	13,068
Delaware	**965**	**643,418**	**316,014**
Cecil	**8**	2,324	**1,172**
Maryland	**8**	**2,324**	**1,172**
Burlington	495	367,157	187,758
Camden	123	432,315	169,909
Cape May	104	52,209	14,545
Cumberland	490	158,289	61,868
Gloucester	279	271,332	89,183
Hunterdon	215	65,132	23,650
Mercer	180	287,685	178,320
Monmouth	20	24,620	9,864
Ocean	30	23,616	7,495
Salem	347	66,342	21,900
Sussex	320	92,689	23,302
Warren	358	109,662	35,500
New Jersey	**2,961**	**1,951,047**	**823,294**
Broome	85	15,038	11,292
Delaware	1,295	26,111	14,240
Greene	25	1,207	572
Orange	65	19,887	10,456
Sullivan	940	47,563	25,511
Ulster	145	15,162	7,787
New York	**2,555**	**124,969**	**69,858**
Berks	777	407,843	150,665
Bucks	607	626,280	244,453
Carbon	381	63,640	16,730
Chester	616	491,070	212,996
Delaware	184	559,776	201,208
Lackawanna	25	11,335	4,830
Lebanon	20	7,221	2,750
Lehigh	347	344,571	166,932
Luzerne	50	17,491	8,074
Monroe	609	166,209	56,025
Montgomery	483	789,862	453,771
Northampton	374	299,646	96,536
Philadelphia	135	1,558,613	619,396
Pike	547	59,859	9,874
Schuylkill	420	79,358	27,077
Wayne	705	50,480	14,114
Pennsylvania	**6,280**	**5,533,254**	**2,271,317**
Delaware Basin	**12,761**	**8,255,013**	**3,481,655**

1. NOAA CSC 2005. 2. U. S. Census Bureau 2010. 3. U. S. Bureau of Labor Statistics 2009.

Table 4. Population change in the Delaware River Basin, 2000-2010 (U. S. Census)

State/ County	Population 2000	Population 2010	Change	%
Kent	85,680	108,025	22,345	26.1%
New Castle	459,829	493,428	33,599	7.3%
Sussex	33,716	41,965	8,249	24.5%
Delaware	**579,225**	**643,418**	**64,193**	**11.1%**
Cecil	1,976	2,324	348	17.6%
Maryland	**1,976**	**2,324**	**348**	**17.6%**
Burlington	348,729	367,157	18,428	5.3%
Camden	425,646	432,315	6,669	1.6%
Cape May	55,679	52,209	-3,470	-6.2%
Cumberland	146,442	158,289	11,847	8.1%
Gloucester	239,012	271,332	32,320	13.5%
Hunterdon	60,995	65,132	4,137	6.8%
Mercer	274,945	287,685	12,740	4.6%
Monmouth	23,465	24,620	1,155	4.9%
Ocean	20,887	23,616	2,729	13.1%
Salem	64,285	66,342	2,057	3.2%
Sussex	88,547	92,689	4,142	4.7%
Warren	102,438	109,662	7,224	7.1%
New Jersey	**1,851,070**	**1,951,047**	**99,977**	**5.9%**
Broome	15,713	15,038	-675	-4.3%
Delaware	28,030	26,111	-1,919	-6.8%
Greene	1,231	1,207	-24	-1.9%
Orange	17,722	19,887	2,165	12.2%
Sullivan	46,712	47,563	851	1.8%
Ulster	14,900	15,162	262	1.8%
New York	**124,308**	**124,969**	**661**	**0.5%**
Berks	373,638	407,843	34,205	9.2%
Bucks	597,632	626,280	28,648	4.8%
Carbon	58,795	63,640	4,845	8.2%
Chester	424,241	491,070	66,829	15.8%
Delaware	551,976	559,776	7,800	1.4%
Lackawanna	11,617	11,335	-282	-2.4%
Lebanon	6,648	7,221	573	8.6%
Lehigh	312,090	344,571	32,481	10.4%
Luzerne	17,916	17,491	-425	-2.4%
Monroe	138,690	166,209	27,519	19.8%
Montgomery	748,987	789,862	40,875	5.5%
Northampton	267,077	299,646	32,569	12.2%
Philadelphia	1,517,542	1,558,613	41,071	2.7%
Pike	46,303	59,859	13,556	29.3%
Schuylkill	81,159	79,358	-1,801	-2.2%
Wayne	46,147	50,480	4,333	9.4%
Pennsylvania	**5,200,458**	**5,533,254**	**332,796**	**6.2%**
Delaware Basin	**7,757,037**	**8,255,013**	**497,976**	**6.4%**



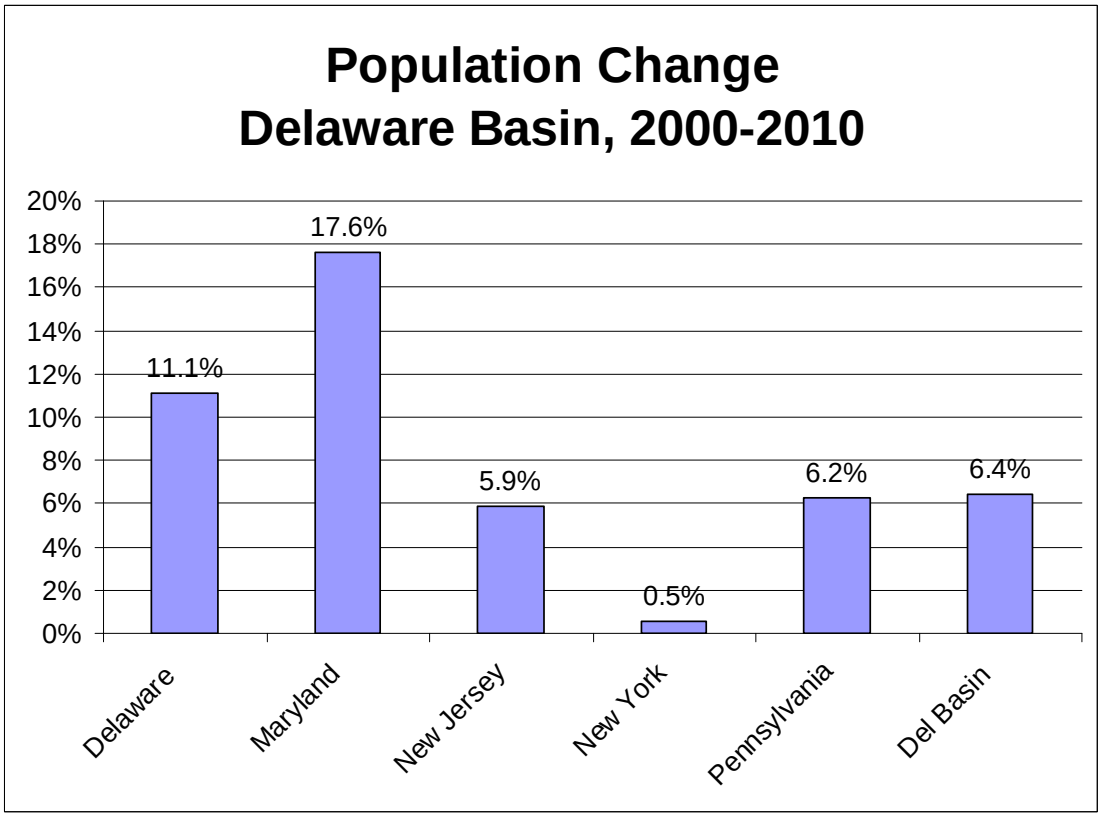
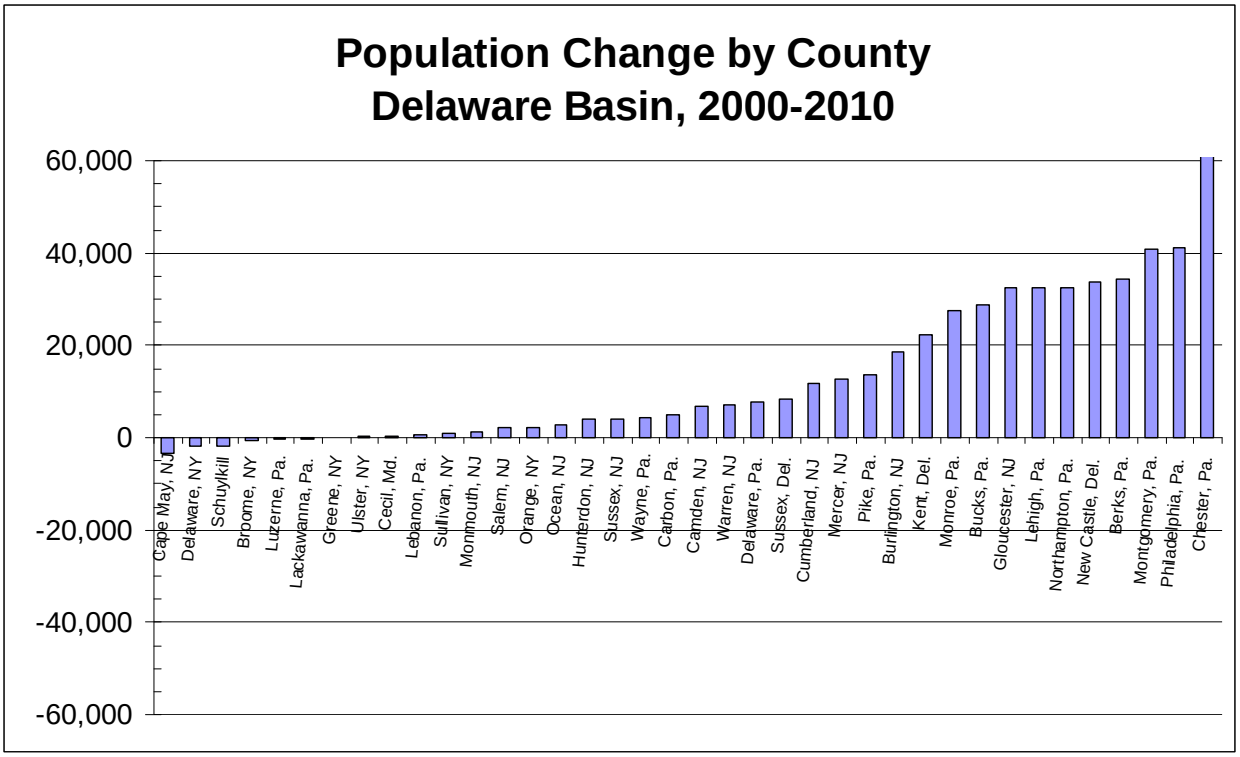


Figure 2. Population change in the Delaware River Basin, 2000-2010 (U.S. Census)



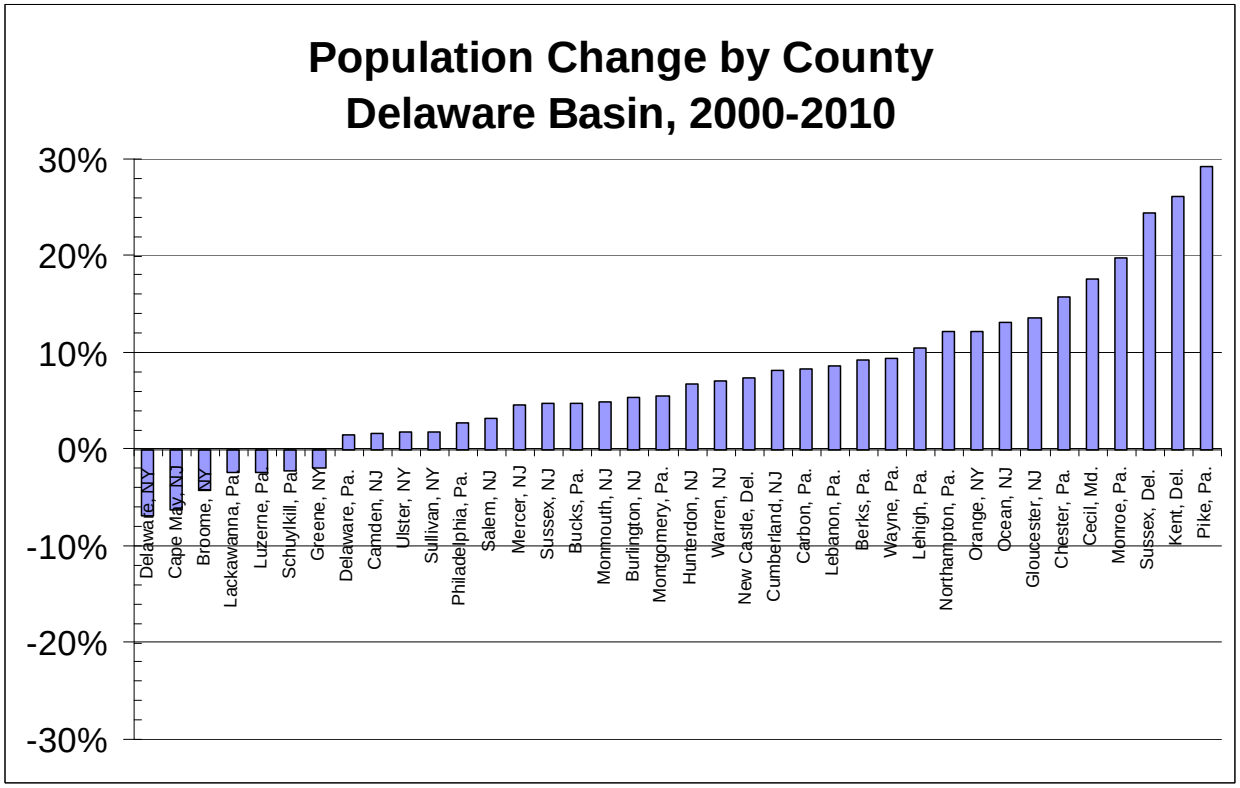


Figure 3. Population change in Delaware River Basin counties, 2000-2010 (U.S. Census)

The Delaware Basin includes 21 watersheds that flow to the river and bay (Table 5 and Figure 4).

Table 5. Watersheds in the Delaware River Basin

Watershed	Area (sq mi)	Population 2000	Pop. Density (pop./sq mi)
LE1 Brandywine/Christina	187	382,703	2,047
LE2 C&D Canal	152	54,960	362
DB1 Delaware Bay	626	141,562	226
Delaware	965	**579,225**	**600**
UC2 NJ Highlands	745	218,638	293
LC1 Del. R. above Trenton	159	55,880	351
UE2 New Jersey Coastal Plain	1,021	1,287,810	1,261
LE3 Salem River	254	54,290	214
DB2 Delaware Bay	782	234,480	300
New Jersey	**2,961**	**1,851,098**	**625**
EW1 East Branch Del. R.	666	23,040	35
EW2 West Branch Del. R.	841	19,263	23
EW3 Del. R. above Pt. Jervis	314	11,840	38
NM1 Neversink R.	734	70,164	96
New York	**2,555**	**124,307**	**49**
EW3 Del. R. above Pt. Jervis	210	7,894	38
NM1 Neversink R.	82	7,796	95
LW1 Lackawaxen R.	598	49,734	83
UC1 Pocono Mt.	779	208,478	268
LV1 Lehigh River above Lehighton	451	37,622	83
LV2 Lehigh River above Jim Thorpe	430	88,349	205
LV3 Lehigh River above Bethlehem	480	478,278	996
LC1 Del. R. above Trenton	295	103,771	352
SV1 Schuylkill above Reading	338	88,681	262
SV2 Schuylkill above Valley Forge	649	321,066	495
SV3 Schuylkill above Philadelphia	874	952,560	1,090
UE1 Penna Fall Line	693	2,579,100	3,722
LE1 Brandywine/Christina	401	277,129	691
Pennsylvania	**6,280**	**5,200,458**	**828**
Delaware Basin	**12,761**	**7,755,088**	**608**

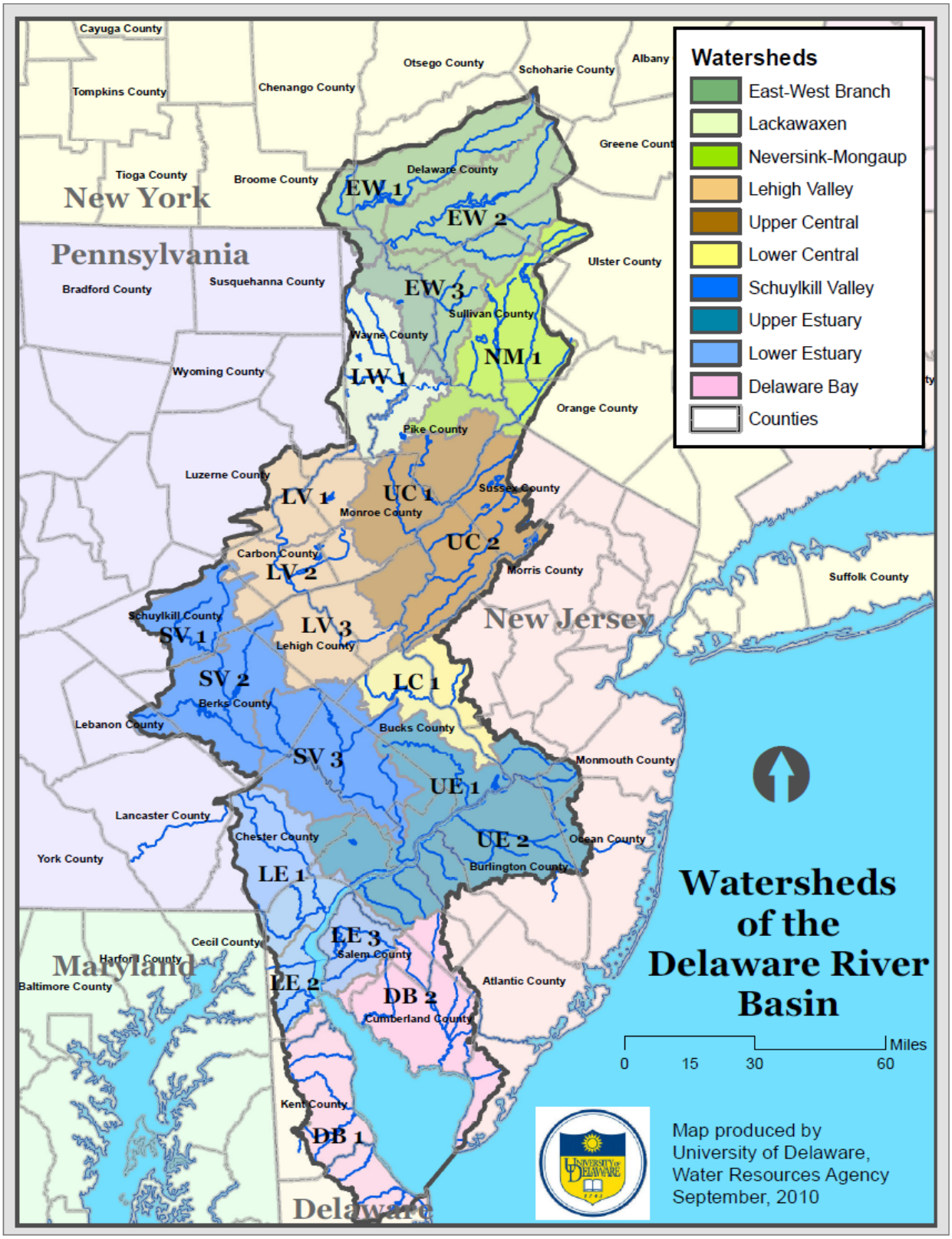


Figure 4. Watersheds in the Delaware River Basin (UDWRA 2010)

2. Methods

Valuation Techniques

The economic value of the Delaware River Basin in Delaware, New Jersey, New York, and Pennsylvania is derived from published studies and valuation methods such as:

Avoided Cost: Society sustains costs if certain ecosystems are not present or lost. For instance, the loss of wetlands may increase economic flood damages.

Replacement Cost: Natural services are lost and replaced by more expensive manmade systems, i.e. forests provide water filtration benefits that are replaced by costly water filtration plants.

Net Factor Income by Enhancement of Income: Improved water quality water enhances fisheries and crabbing industries and, in turn, boosts jobs and wages.

Travel Cost: Visitors are willing to pay to travel and visit ecosystems and natural resources for hunting, fishing, and birding.

Hedonic Pricing Process: Residents may be willing to pay more for property values that are higher along scenic bay and river coastlines.

Contingent Valuation: Valuation by survey of individual different preferences to preserve ecosystems. People may be willing to pay more in fees to preserve bay water quality.

Scope of Work

The socioeconomic value of the Delaware Basin was established by the following scope of work.

1. **Define and map area of interest:** The area of interest is defined as the Delaware River Basin from the headwaters in the Catskill Mountains of New York to the mouth of the bay at Cape Henlopen, Delaware. ArcGIS map layers of population census blocks, watershed boundaries, and land use/land cover were developed to perform the analysis.

2. **Literature review:** Gather a database of published literature and socioeconomic data relevant to the Delaware River Basin from the U. S. Census Bureau, U. S. Bureau of Labor Statistics, U.S. Department of Agriculture, U. S. Forest Service, and U. S. Fish and Wildlife Service.

3. **Economic activity:** Estimate the direct/indirect value of agriculture, water quality, water supply, fishing, hunting, recreation, boating, ecotourism, and navigation in the watershed from population, employment, industrial activity, and land use data. Total economic activity is defined as the sum of direct/indirect use, option, and non use values (Ingraham and Foster 2008). Direct use values are from natural goods such as drinking water, boating, recreation, and commercial fishing. Indirect values are benefits from ecosystems such as water filtration by forests and flood control/habitat protection from wetlands. Option demand is public willingness to pay for benefits from water quality or scenic value of the bay. Nonuse (existence) values accrue to a public who may never visit the resource but are willing to pay to preserve the existence of the resource.

4. Ecosystem Services: Tabulate the market value of natural resources (ecosystem services value) in the watershed for habitat such as wetlands, forests, farmland, and open water. Prepare GIS based data sets and mapping. Ecosystem services (ecological services) are provided by nature and represent benefits such as water filtration, flood reduction, and drinking water supply.

Using GIS, define ecosystem areas using 2006 NOAA Coastal Services Center land cover data in the following classifications: (a) Freshwater wetlands, (b) Marine, (c) Farmland, (d) Forest, (e) Barren, (f) Saltwater wetland, (g) Urban, (h) Beach/dune, (i) Open freshwater, and (j) Riparian buffer.

Search research studies and gather value ($/acre) data for ecosystem services: (a) carbon sequestration, (b) flood control, (c) drinking water supply, (d) water quality filtration, (e) waste treatment and assimilation, (f) nutrient regulation, (g) fish and wildlife habitat, (h) recreation and aesthetics. Ecosystem services were estimated using value (benefits) transfer where published data and literature are reviewed and applied in the context of the resource in question. Value transfer is used to estimate ecosystem goods and services for the Delaware River Basin.

Compute ecosystem services value by multiplying land use area (acres) by ecosystem value ($/ac). The value transfer techniques employed here involves selecting data from published literature from another watershed or study area and applying the $ per ac values to land use areas computed by GIS. While primary research data from the watershed in question (the Delaware Basin) is preferable and is used in this report, value transfer is the next best practical way to value ecosystems especially when in the absence of such data the worth of ecosystems have previously been deemed zero. Future economic valuation survey research is recommended to develop primary ecosystem service values for the Delaware Basin in particular.

4. Jobs and salaries: Obtain employment and wage data from the U. S. Department of Labor, U. S. Census Bureau, and National Ocean Economics Program. Calculate direct/indirect jobs in the Delaware Basin by North American Industry Classification System (NAICS) codes such as shipbuilding, marine transportation/ports, fisheries, recreation, minerals, trade, agriculture, and others. Total jobs and salaries were summarized for each county within the watershed based on population census block data. NAICS data were supplemented with farm jobs data from the USDA Agricultural Statistics Bureau, U. S. Fish and Wildlife Service ecotourism jobs data, and jobs provided by water purveyors and wastewater treatment utilities.

5. Report: Prepare a report and GIS mapping summarizing the direct and indirect economic values of goods and services provided by the Delaware River Basin updated to 2010 dollars.

3. Annual Economic Activity

Estimated annual economic value of the Delaware River Basin from recreation, fish and wildlife, public parks, water quality, navigation/ports, potential Marcellus Shale natural gas, agriculture, water supply, and forest activities is over $25 billion (Table 6 and Figure 5).

- Recreation $1.22 billion
- Fish and Wildlife $1.55 billion
- Public Parks $1.83 billion
- Water Quality $2.46 billion
- Navigation/Ports $2.62 billion
- Marcellus Shale Natural Gas (potential) $3.30 billion
- Agriculture $3.37 billion
- Water Supply $3.82 billion
- Forests $5.13 billion

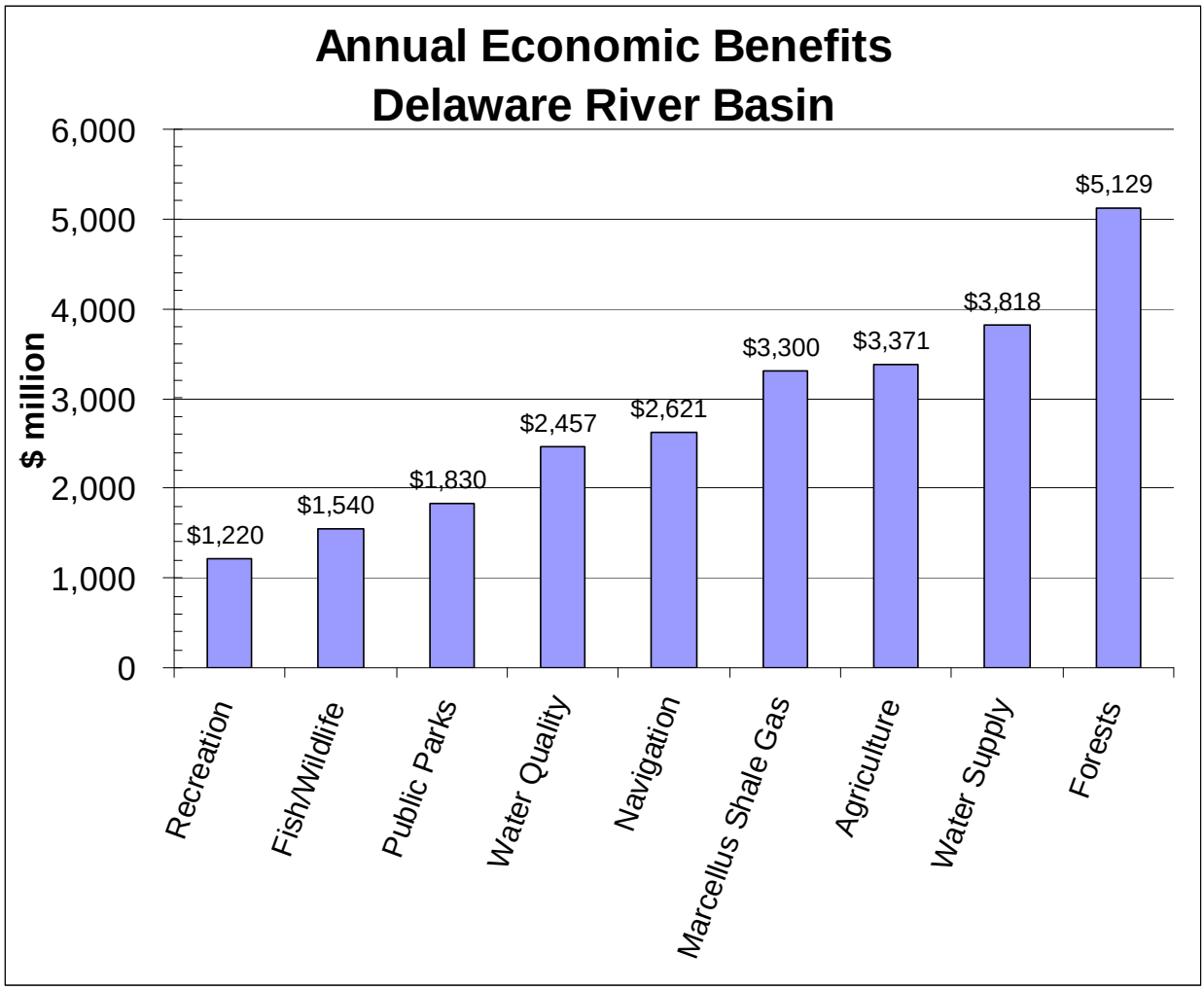


Figure 5. Annual economic activity related to the Delaware River Basin

Table 6. Annual economic activity in the Delaware River Basin, 2010

Activity	2010 ($ million)	Value Transfer Sources
Recreation (Boating, Fishing, Swimming)		
Clean Water Act Restoration		
Viewing/Aesthetics ($0.58/person)	5	University of Delaware (2003)
Boating ($0.76/person)	6	University of Delaware (2003)
Fishing ($2.95/person)	24	University of Delaware (2003)
Swimming ($6.88/person)	57	University of Delaware (2003)
Water Quality Based Recreation		
Swimming ($13.40/trip)	9	University of Rhode Island (2002)
Boating ($30/trip)	47	University of Rhode Island (2002)
Fishing ($62.79/trip)	52	University of Rhode Island (2002)
Wildlife/bird watching ($77.73/trip)	104	University of Rhode Island (2002)
Skiing (1.9 million ski-days @$45/day)	325	Pennsylvania Ski Areas Association (2010)
Paddling-based Recreation (620,860 paddlers)	362	Outdoor Industry Association(2006)
Del. Water Gap River Recreation (267,000 visitors)	41	U.S. Forest Service, U.S. Nat'l Park Service (1990)
Canoe/Kayak/Rafting (225,000 visits)	9	Canoe and Kayak Liveries (2010)
Powerboating (232,000 boat registrations)	395	National Marine Manufacturers Association (2010)
Water Quality		
Willing to Pay for Clean Water ($38-$121/user)	659	University of Maryland (1989)
Water Treatment by Forests ($96/mgd)	63	Trust for Public Land, AWWA (2004)
Wastewater Treatment ($4.00/1000 gal)	1,722	DRBC and USEPA
Increased Property Value (+8%)	13	EPA (1973), Brookings Institute (2010)
Water Supply		
Drinking Water Supply ($4.78/1000 gal)	3,145	UDWRA and DRBC (2010)
Reservoir Storage ($0.394/1000 gal)	145	UDWRA and DRBC (2010)
Irrigation Water Supply ($300/ac-ft)	32	Resources for the Future (1996), USDA (2007)
Thermoelectric Power Water Supply ($44/ac-ft)	297	EIA (2002), NETL (2009)
Industrial Water Supply ($200/ac-ft)	179	Resources for the Future (1996), DRBC (2010)
Hydropower Water Supply ($32/ac-ft)	20	Resources for the Future (1996), DRBC (2010)
Fish/Wildlife		
Commercial Fish Landings ($0.60/lb)	34	NMFS, Nat'l. Ocean Economics Program (2007)
Fishing (11-18 trips/angler, $17-$53/trip))	576	U. S. Fish and Wildlife Service (2001)
Hunting (16 trips/hunter, $16-50/trip)	340	U. S. Fish and Wildlife Service (2001)
Wildlife/Bird-watching (8-13 trip/yr, $15-$27/trip)	561	U. S. Fish and Wildlife Service (2001)
Shad Fishing (63,000 trips, $102/trip)	6	Pennsylvania Fish and Boat Commission (2011)
Wild Trout Fishing	29	Amer. Sportfishing Assn./Trout Unlimited (1998)
Agriculture		
Crop, poultry, livestock value ($1,180/ac)	3,371	USDA Census of Agriculture 2007 (2009)
Forests		
Carbon Storage ($827/ac)	3,592	U.S. Forest Service, Del. Ctr. Horticulture (2008)
Carbon Sequestration ($29/ac)	126	U.S. Forest Service
Air Pollution Removal ($266/ac)	1,155	U.S. Forest Service
Building Energy Savings ($56/ac)	243	U.S. Forest Service
Avoided Carbon Emissions ($3/ac)	13	U.S. Forest Service
Public Parks		
Health Benefits ($9,734/ac)	1,283	Trust for Public Land
Community Cohesion ($2,383/ac)	314	Trust for Public Land
Stormwater Benefit ($921/ac)	121	Trust for Public Land
Air Pollution ($88/ac)	12	Trust for Public Land
Del. Water Gap Natl. Rec. Area (4.9 million visits)	100	U.S. National Park Service (2002)
Marcellus Shale		
Natural Gas (7.3 trillion cf @ $11.21/1000 cf)	3,300	USGS (2011), EIA (2011)
Maritime Transportation		
Navigation ($15/ac-ft)	220	Resources for the Future (1996)
Port Activity	2,400	Economy League of Greater Philadelphia (2008)
Delaware River Basin	**≈$25 billion**	

Recreation

Clean Water Act Restoration

Parsons, Helm, and Bondelid (2003) from the University of Delaware measured the economic benefits of water quality improvements to recreational users in the northeastern states and found annual per person benefits for improvements due to the Clean Water Act ranged from $0.47 for viewing, $0.62 for boating, $2.40 for fishing, to $5.59 for swimming. Table 7 summarizes total water quality benefits to recreational users in the Delaware River Basin by transferring the benefits in $2003 to $2010 assuming an annual rate of 3% and then multiplying the $2010 benefits by the basin population. Total 2010 recreation benefits due to Clean Water Act water quality improvements in the Delaware Basin are $92 million per year or $11.17 per person. Swimming (62%) and fishing (26%) are the highest valued recreational benefits followed by boating (7%) and viewing (5%).

Table 7. Water quality benefits from Clean Water Act improvements in the Delaware River Basin

Recreational Benefit	$2003[1] (per person)	$2010[2] (per person)	Del. Basin Pop. 2010	Benefit/yr	% of Benefit
Viewing	$0.47	$0.58	8,255,013	$4,787,908	5%
Boating	$0.62	$0.76	8,255,013	$6,273,810	7%
Fishing	$2.40	$2.95	8,255,013	$24,352,288	26%
Swimming	$5.59	$6.88	8,255,013	$56,794,489	62%
Total	**$9.08**	**$11.17**	**8,255,013**	**$92,208,495**	**100%**

1. Parsons et al. 2003. 2. $2010 transferred from $2003 at annual rate of 3%.

Water Quality Based Recreation

Using travel cost demand methods, Johnston et al. (2002) from the University of Rhode Island computed the consumer surplus (economic use value per person) for swimming, boating, recreational fishing, and bird watching/wildlife viewing in the Peconic Estuary watershed on Long Island, New York. Swimming, boating, fishing, and wildlife viewing were valued at $8.59, $19.23, $40.25, and $49.83 per trip in $1995, respectively. Table 8 summarizes water quality benefits to recreational users of $211 million per year in the Delaware Basin (estuary only) by transferring unit values from the Peconic Estuary, converting $1995 to $2010 by an annual rate of 3%, and multiplying $2010 benefits by trips per year.

Table 8. Total annual value of recreational benefits in the Delaware River Basin

Recreational Benefit	$1995 Consumer surplus/trip[1]	$2010 Consumer surplus/trip[2]	Trips/year to Del. Estuary	Annual Value	% of Benefit
Swimming	$8.59	$13.40	670,000[3]	$8,978,000	4%
Boating	$19.23	$30.00	1,568,473[4]	$47,054,190	22%
Fishing	$40.25	$62.79	824,249[4]	$51,754,595	24%
Wildlife/bird watching	$49.83	$77.73	3,336,440[5]	$103,700,000	49%
Total				**$211,486,785**	**100%**

1. Johnston et al. 2002. 2. $2010 transferred from $1995 at 3%. 3. 10% of Delaware Estuary population swims. 4. NOEP 2009 for boating (16.8% of pop. and 1.4 trips/p./yr) and fishing (10.3% of pop. and 1.2 trips/p./yr). 5. USFWS 2006 wildlife/bird watching (Del. 427,500, NJ 2,070,900, & Pa. 838,000 trips/yr).

Skiing

In the Pocono Mountains of Pennsylvania, nine ski areas draw approximately 1 mgd from Delaware Basin water supplies for snowmaking on 1,005 skiable acres. The Pennsylvania Ski Areas Association (2009) estimated the economic value at 23 ski resorts statewide was $832,000,000. Prorating from PSAA statewide estimates, the economic value for 9 resorts in the Delaware Basin is $325,000,000. The nine ski resorts in the Delaware Basin have aggregate annual revenues of $87,655,063 from 1,908,228 skier visits based on a mid-week lift ticket rate of $45/day (Table 9).

Table 9. Revenues from ski resorts in the Delaware River Basin

Ski Resort	Ski Area (ac)	Annual Ski Visits	Lift Ticket ($/day)	Revenue ($)
Elk Mountain	235	446,203	$48	$21,417,722
Ski Big Bear	26	49,367	42	2,073,418
Ski Shawnee	125	237,342	43	10,205,696
Alpine Mountain	60	113,924	37	4,215,190
Camelback	160	303,797	48	14,582,278
Jack Frost	100	189,873	44	8,354,430
Big Boulder	55	104,430	44	4,594,937
Blue Mountain	158	300,000	49	14,700,000
Bear Creek	86	163,291	46	7,511,392
Total	**1,005**	**1,908,228**	**$45**	**$87,655,063**

Paddling-based Recreation

Canoeing, kayaking, and rafting are key drivers to the local economy along the Brandywine, Lehigh, Schuylkill, and middle/upper Delaware rivers in the Delaware Basin (Van Rossum, Carluccio, and Blankinship 2010). In the Mid-Atlantic census division (NY, NJ, PA), the Outdoor Industry Association (2006) estimates paddling-based recreation is practiced by 11% of the population and is responsible for 3,356,000 participants, $356 million in gear retail sales, $1.6 billion in trip related sales, and 22,844 jobs. Given the Delaware Basin is the home of 7,611,595 people in NJ, NY, and Pa. or 22% of New Jersey's population (1,951,047), 0.7% of New York State's population, (124,969), and 43% of Pennsylvania's population (5,533,254) or 18.5% of the three state's total population of 40,800,000 people, then prorated paddling-based recreation in the basin is responsible for 620,860 participants, $96 million in gear retail sales, $296 million in trip sales, and 4,226 jobs (Table 10).

Table 10. Economic value of paddling-based recreation in the Delaware River Basin

Paddling Based Recreation	States of NJ, NY, PA[1]	Del. Basin NJ, NY, PA[2]
Population	40,800,000	7,563,762
Participants	3,356,000	620,860
Gear retail sales	$356 million	$66 million
Trip related sales	$1.600 billion	$296 million
Total Sales	$1.956 billion	$362 million
Jobs	22,844	4,226

1. Outdoor Industry Association 2006. 2. Prorated by 18.5% given 40,800,000 people live in NJ, NY, and PA and 7,611,595 people live in these states in the Delaware Basin.

River Recreation

Cordel et al. (1990) from the U. S. Forest Service and U.S. National Park Service estimated river recreation along the Upper Delaware River and Delaware Water Gap was responsible for $13.3 million and $6.9 million in total economic output, respectively, in $1986 (Table 11). Adjusting for 3% annually, river recreation economic output along the Upper Delaware River and Delaware Water Gap is roughly $27.1 million and $14.1 million, respectively, or $41.2 million total in $2010.

Table 11. Economic impacts of river recreation along Upper Delaware and Delaware Water Gap

River	Participants	Jobs	Wages ($1986)	Economic Output ($1986)	Wages ($2010)	Economic Output ($2010)
Upper Delaware	232,000	292	5,582,800	13,351,000	11,408,000	$27,100,000
Del. Water Gap	135,400	156	3,246,300	6,929,000	6,633,743	$14,100,000
Total	**367,400**	**448**	**8,829,100**	**20,280,000**	**18,041,743**	**41,200,000**

1. Cordel et al. 1990. 2. Adjusted to $2010 at 3% annually.

Canoe/Kayak/Rafting

Thirty seven (37) canoe and kayak liveries along the Delaware, Lehigh, and Schuylkill, and Brandywine Rivers lease watercraft to approximately 225,000 visitors with earnings of $9 million per year assuming a daily rental fee of $40 per person (Table 12).

Table 12. Annual revenue from canoe and kayak liveries in the Delaware River Basin

Canoe/Kayak Livery	Address	Daily Rate ($)	Annual Visitors	Revenue ($)
Delaware River				
Adventure Sports Canoe/Raft	Marshalls Creek, PA	$40	9,000	$360,000
Bucks County River Country	Point Pleasant, PA	$40	13,500	$540,000
Catskill Mountain Canoe Rentals	Hankins, NY	$40	7,000	$280,000
Cedar Rapids Kayak/Canoe	Barryville, NY	$40	5,000	$200,000
Chamberlain Canoes Inc	Minisink Hills, PA	$40	5,000	$200,000
Delaware River Rafting/Canoeing	Delaware, NJ	$40	9,000	$360,000
Delaware River Tubing	Frenchtown, NJ	$40	7,000	$280,000
Driftstone on the Delaware	Mount Bethel, PA	$40	5,000	$200,000
GreenWave Paddling	Yardville, New Jersey	$40	3,000	$120,000
Indian Head Canoes & Rafts	Barryville, NY	$40	5,000	$200,000
Jerrys Three River Canoes	Pond Eddy, NY	$40	4,000	$160,000
Kayak East	East Stroudsburg, PA	$40	4,000	$160,000
Kittatinny Canoes, Inc.	Dingmanns Ferry, PA	$40	4,000	$160,000
Landers River Trips	Narrowsburg, NY	$40	15,000	$600,000
Lazy River Outpost	Phillipsburg, NJ	$40	4,000	$160,000
Pack Shack Adventures Inc	Delaware Water Gap, PA	$40	5,000	$200,000
Paint Island Canoe & Kayak	Bordentown, NJ	$40	4,000	$160,000
Portland Outfitters	Portland, PA	$40	5,000	$200,000
River Country	Point Pleasant, PA	$40	9,000	$360,000
Shawnee Canoe Trips	Shawnee on Delaware, PA	$40	12,000	$480,000
Silver Canoe Rentals	Port Jervis, NY	$40	4,000	$160,000
Upper Delaware Campgrounds	Callicoon, NY	$40	5,000	$200,000
Whitewater Willies Canoe Rentals	Pond Eddy, NY	$40	4,000	$160,000
Wild & Scenic River Tours/Rentals	Barryville, NY	$40	5,000	$200,000
Lehigh River				$0
Jim Thorpe River Adventures	Jim Thorpe, PA	$40	9,000	$360,000
Lehigh Rafting Rentals Inc	White Haven, PA	$40	9,000	$360,000
Lehigh River Bait and Bow	Allentown, PA	$40	3,000	$120,000
Northeast PA Kayak School	Lehighton, PA	$40	3,000	$120,000
Pocono Whitewater	Jim Thorpe, PA	$40	8,000	$320,000
Whitewater Challengers, Inc.	White Haven, PA	$40	9,000	$360,000
Whitewater Rafting Adventures Inc.	Nesquehoning, PA	$40	6,000	$240,000
Schuylkill				$0
Schuylkill River Outfitters	Birdsboro, PA	$40	4,500	$180,000
Brandywine River				$0
Brandywine Outfitters	Coatesville, PA	$40	3,000	$120,000
Northbrook Canoe	West Chester, PA	$40	9,000	$360,000
Wilderrness Canoe Trips	Wilmington, DE	$40	9,000	$360,000
Total			**225,000**	**9,000,000**

Powerboating

The National Marine Manufacturers Association (2010) announced that New York, Delaware, Pennsylvania, and New Jersey ranked 3rd, 7th, 17th, and 23rd in the U.S. respectively in total expenditures for new powerboats, outboard engines, boat trailers, and accessories. Table 13 summarizes powerboat expenditures by state and then prorated by percent population of each state within the Delaware Basin. Powerboat expenditures due to boating within the waters of the Delaware Basin are estimated at about $395 million/year

Table 13. Recreational powerboat expenditures in the Delaware River Basin (NMMA 2010)

State	Rank Expenditures	Total Powerboat Expenditures ($)	% Pop. of State in Basin	Del. Basin Powerboat Expenditures ($)
Delaware	7	343,743,963	74%	254,370,533
New Jersey	23	183,044,985	22%	40,269,897
New York	3	401,353,400	0.70%	2,809,474
Pennsylvania	17	226,281,490	43%	97,301,041
Total		**1,154,423,838**		**394,750,944**

New York, Pennsylvania, New Jersey, and Delaware are ranked 7th, 13th, 28th, and 40th in number of recreational boat registrations in 2009. The four states combined had just over $1 million boat registrations in 2009 with 232,000 registrations for boating in the Delaware River Basin (Table 14).

Table 14. Recreational boat registrations in the Delaware River Basin (NMMA 2010)

State	Rank Registrations	Total Boat Registrations	% Pop. of State in Basin	Del. Basin Boat Registrations
Delaware	40	61,523	0.74	45,527
New Jersey	28	173,994	0.22	38,279
New York	7	479,161	0.007	3,354
Pennsylvania	13	337,747	0.43	145,231
Total		**1,052,425**		**232,391**

Water Quality

Willingness to Pay for Clean Water

Bockstael, McConnell, and Strand (1989) from the University of Maryland estimated public annual willingness to pay for a moderate improvements in water quality of the Chesapeake Bay to be $10 to $100 million in 1984 dollars ($21.6 to $216 million in $2010 at 3% annually). The study found 43% of the respondents were users or visitors (boaters, fishermen) to the Chesapeake Bay and were willing to pay $121 per year to make the bay water quality "acceptable". About 57% of respondents were nonusers, those who do not visit or use the bay's resources but were willing to pay $38 per year to restore the bay. Transferring these values to the estuary watershed portion of the Delaware Basin

(pop. 6,700,000) and using proportions of 10% users or visitors to the estuary and 90% nonusers, aggregate willingness to pay to make the Delaware Estuary water quality acceptable to the public is $658 million in $2010 or $99 per person.

Total willingness to pay for acceptable Delaware Estuary water quality
= (0.10)(6,700,000)($121/yr) + (0.90)(6,700,000)($38/yr)
= $310 million ($1984) = $659 million ($2010 at 3% annually).

Water Treatment

The Trust for Public Land and American Water Works Association (2004) found for every 10% increase in forested watershed land, drinking water treatment and chemical costs are reduced by approximately 20% (Table 15). The public drinking water supply is 1,803 mgd and forests cover 6,786 sq mi or 53% of the Delaware River Basin. Loss of these forests would increase drinking water treatment costs by $96 per mil gal ($139 per mil gal @ 0% forested minus $43 per mil gal @ 53% forested) or $173,088 per day for 1,803 mgd = $63,177,120 per year.

Table 15. Drinking water treatment and chemical costs based on percent of forested watershed (Trust for Public Land and AWWA 2004)

% of Watershed Forested	Water Treatment/ Chemical Costs (per mil gal)	% Change in Costs
0%	$139	21%
10%	$115	19%
20%	$93	20%
30%	$73	21%
40%	$58	21%
50%	$46	21%
60%	$37	19%

Wastewater Treatment

The waters of the Delaware Basin provide significant wastewater treatment, discharge, and assimilation services. In accordance with Federal Clean Water Act, DRBC, and state water quality regulations, NPDES municipal wastewater dischargers hold permits to discharge up to 1,180 million gallons per day to the Delaware River Basin or 106 mgd in Delaware, 218 mgd in New Jersey, 7 mgd in New York, and 849 mgd in Pennsylvania (Table 16). The average wastewater rate in the basin is $4.00 per 1000 gal. The fee for an average residence of 4 people @ 50 gpcd is $290 per year. The value of treated wastewater in the Delaware Basin is $4.7 million per day or $1.7 billion per year.

Table 16. Value of NPDES wastewater treatment discharges in the Delaware River Basin

NPDES ID	Facility	Location	State	Flow[1] (mgd)	Value[2] ($/day)	Wastewater Value ($/year)
DE0020338	Kent Co. Levy Court WWTR	Frederica	DE	15.0	60000	21900000
DE0021512	Lewes City POTW	Lewes	DE	0.8	3,200	1,168,000
DE0020320	Wilmington Wastewater Plant	Wilmington	DE	90.0	360,000	131,400,000
Delaware			**DE**	**105.8**	**423,200**	**154,468,000**

NJ0027481	Beverly City Sewer Auth. STP	Beverly	NJ	1.0	4,000	1,460,000
NJ0024678	Bordentown Sewerage Auth.	Bordentown	NJ	3.0	12,000	4,380,000
NJ0024651	Cumberland Co. Auth. WWTP	Bridgeton	NJ	7.0	28,000	10,220,000
NJ0024660	Burlington City STP	Burlington	NJ	2.7	10,800	3,942,000
NJ0021709	Burlington Twp. DPW	Burlington	NJ	1.6	6,400	2,336,000
NJ0026182	Camden County MUA	Camden	NJ	80.0	320,000	116,800,000
NJ0021601	Carneys Point Twp. WWTP	Carneys Point	NJ	1.3	5,200	1,898,000
NJ0024007	Cinnaminson Sewerage Auth.	Cinnaminson	NJ	2.0	8,000	2,920,000
NJ0023701	Florence Twp. DPW Sewer Auth.	Florence	NJ	2.5	10,000	3,650,000
NJ0026301	Hamilton Twp. DPW	Hamilton Twp.	NJ	16.0	64,000	23,360,000
NJ0020915	Lambertville City Sewer Auth.	Lambertville	NJ	1.5	6,000	2,190,000
NJ0024759	Ewing Lawrence Sewer WWTP	Lawrenceville	NJ	16.0	64,000	23,360,000
NJ0069167	Maple Shade Twp. Util, Authority	Maple Shade	NJ	3.4	13,600	4,964,000
NJ0026832	Medford Twp. Sewer Auth. STP	Medford	NJ	1.8	7,200	2,628,000
NJ0029467	Millville City Sewer Auth.	Millville	NJ	5.0	20,000	7,300,000
NJ0024996	Moorestown Twp. WWTP	Moorestown	NJ	3.5	14,000	5,110,000
NJ0024015	Mount Holly Twp. MUA	Mount Holly	NJ	7.7	30,800	11,242,000
NJ0020184	Newton Town DPW	Newton	NJ	1.4	5,600	2,044,000
NJ0024821	Pemberton Twp. MUA STP	Pemberton	NJ	2.5	10,000	3,650,000
NJ0024023	Penns Grove Sewerage Auth.	Penns Grove	NJ	0.8	3,200	1,168,000
NJ0021598	Pennsville Twp. Sewer Auth.	Pennsville	NJ	1.9	7,600	2,774,000
NJ0024716	Phillipsburg Town STP	Phillipsburg	NJ	3.5	14,000	5,110,000
NJ0022519	Riverside Twp. DPW	Riverside	NJ	1.0	4,000	1,460,000
NJ0024856	Salem WWTP Facility	Salem	NJ	1.4	5,600	2,044,000
NJ0024686	Gloucester Co. Util. Auth. STP	Thorofare	NJ	24.1	96,400	35,186,000
NJ0020923	Trenton City DPW Sewer Auth.	Trenton	NJ	20.0	80,000	29,200,000
NJ0023361	Willingboro Twp. MUA	Willingboro	NJ	5.2	20,800	7,592,000
New Jersey				**217.8**	**871,200**	**317,988,000**
NY0020265	Delhi WWTP	Delhi	NY	0.8	3,200	1,168,000
NY0030074	Liberty WWTF	Liberty	NY	1.6	6,400	2,336,000
NY0022454	Monticello STP	Monticello	NY	3.1	12,400	4,526,000
NY0029271	Sidney WWTP	Sidney	NY	1.7	6,800	2,482,000
New York				**7.2**	**28,800**	**10,512,000**
PA0026867	Abington Twp. STP	Abington	PA	3.9	15,600	5,694,000
PA0026000	Allentown City WWTP	Allentown	PA	40.0	160,000	58,400,000
PA0026042	Bethlehem City STP	Bethlehem	PA	90.0	360,000	131,400,000
PA0021181	Bristol Borough Water and Sewer	Bristol	PA	1.2	4,800	1,752,000
PA0027103	Delaware Co. Reg. Water Auth.	Chester	PA	44.0	176,000	64,240,000
PA0026859	Coatesville WWTP	Coatesville	PA	3.8	15,200	5,548,000
PA0026794	Conshohocken Borough Auth.	Conshohocken	PA	2.3	9,200	3,358,000
PA0026531	Downingtown Regional WPCC	Downingtown	PA	7.1	28,400	10,366,000
PA0026549	Borough of Doylestown WWTP	Doylestown	PA	28.5	114,000	41,610,000
PA0027235	Easton Area Joint Auth. WWTP	Easton, PA	PA	10.0	40,000	14,600,000
PA0029441	Upper Dublin Twp. MS4 UA	Ft. Washington	PA	1.1	4,400	1,606,000
PA0051985	Horsham Twp. STP	Horsham	PA	1.0	4,000	1,460,000
PA0024058	Kennett Square Borough WWTP	Kennett Square	PA	1.1	4,400	1,606,000
PA0026298	Whitemarsh STP	Lafayette Hill	PA	2.0	8,000	2,920,000
PA0026182	Lansdale Borough STP	Lansdale	PA	2.6	10,400	3,796,000
PA0039004	U. Gwynedd/Towamencin STP	Lansdale	PA	6.5	26,000	9,490,000
PA0026468	Morrisville Municipal Authority	Morrisville	PA	10.0	40,000	14,600,000
PA0027421	Norristown Borough WWTP	Norristown	PA	9.8	39,200	14,308,000
PA0020532	Upper Montgomery Joint Sewer	Pennsburg	PA	2.0	8,000	2,920,000

PA0026689	Northeast WPCP	Philadelphia	PA	210.0	840,000	306,600,000
PA0026662	Philadelphia Southeast POTW	Philadelphia	PA	112.0	448,000	163,520,000
PA0026671	SW Water Pollution Control	Philadelphia	PA	200.0	800,000	292,000,000
PA0020460	Quakertown WWTP	Quakertown	PA	4.3	17,200	6,278,000
PA0026549	Reading WWTP	Reading	PA	28.5	114,000	41,610,000
PA0020168	East Stroudsburg Filtration Plant	Stroudsburg	PA	2.3	9,200	3,358,000
PA0029289	Stroudsburg STP	Stroudsburg	PA	2.5	10,000	3,650,000
PA0027031	Goose Creek STP	West Chester	PA	1.7	6,800	2,482,000
PA0026018	West Chester Taylor Run STP	West Chester	PA	1.8	7,200	2,628,000
PA0028584	West Goshen STP	West Chester	PA	6.0	24,000	8,760,000
PA0023256	Upper Gwynedd Twp. WWTP	West Point	PA	5.7	22,800	8,322,000
PA0025976	Upper Moreland Hatboro Sewer	Willow Grove	PA	7.2	28,800	10,512,000
Pennsylvania			**PA**	**848.9**	**3,395,600**	**1,239,394,000**
Delaware Basin			**Basin**	**1,179.7**	**4,718,800**	**1,722,362,000**

1. DRBC and USEPA. 2. Value at @ $4.00/1000 gal

Increased Property Values

Several studies along rivers, estuaries, and coasts throughout the United States indicate that improved water quality can increase shoreline property values by 6% to 25% (Table 17). The EPA (1973) estimated that improved water quality can raise property values by up to 18% next to the water, 8% at 1000 feet from the water, 4% at 2000 feet from the water, and 1.5% at 3000 feet from the water. Leggett, et al. (2000) estimated that improved bacteria levels to meet state water quality standards along the western shore of the Chesapeake Bay in Maryland raised shoreline property values by 6%. The Brookings Institution (2007) projected that investments of $26 billion to restore the Great Lakes would increase shoreline property values by up to 10%. For this analysis, shoreline property values within 2000 feet of the waterways are estimated to increase by an average of 8% due to improved water quality in the Delaware Estuary.

Shoreline property values within 2000 feet of the water due to water quality improvements in the Delaware Estuary watershed will increase by $256 million (Table 18). The average riverfront property value in Philadelphia is $92,000 per acre. Multiply this value by the area of property within a 2,000 feet corridor along the Delaware Estuary shore between the C&D Canal and head of tide at Trenton. Multiply by increased property value of 8% due to improved water quality in the Delaware Estuary. Since the increase in property value is a one time benefit, the annual value over a 20 year period where water quality has improved in the Delaware Estuary is estimated as $13 million.

Table 17. Increased property values resulting from improved water quality

Study	Watershed	Increased Value
EPA (1973)	San Diego Bay, CA Kanawha, OH Willamette R., OR	
Next to water		18%
1000 ft from water		8%
2000 ft from water		4%
3,000 ft from water		1.5%
Leggett, et al. (2000)	Chesapeake Bay	6%
Brookings Institution (2007)	Great Lakes	10%

Table 18. Increased shore property value due to improved water quality in the Delaware Basin

State	Length of shoreline (ft)	Area 2000 ft of water (sf)	Area 2000 ft of water (ac)	Property Value @ $92,000/ac ($)	Increased Property Value @ 8% ($)
Delaware	114,048	228,096,000	5,236	481,745,455	38,539,636
New Jersey	357,456	714,912,000	16,412	1,509,915,152	120,793,212
Pennsylvania	285,648	571,296,000	13,115	1,206,593,939	96,527,515
Delaware Estuary	757,152	1,514,304,000	34,764	3,198,254,545	255,860,364

Water Supply

Drinking Water Supply

The Delaware Basin covers just 0.4% of the continental United States (12,769 sq mi/3,000,000 sq mi) yet supplies drinking water to 5% of the U.S. population (16,000,000/309,000,000 people). Delaware Basin aquifers and streams supply drinking water to over 8 million people within the basin to cities like Wilmington, Philadelphia, Allentown, Camden, and Trenton, NJ. Through interbasin transfers, the Delaware Basin also supplies drinking water to an additional 8 million people who live outside the basin by allocated diversions through the New York City Catskill Reservoir system (800 mgd) and the Delaware & Raritan Canal in New Jersey (100 mgd). Table 19 summarizes the economic benefits of groundwater reserve stock to generate ecosystem services (USEPA 1995).

Table 19. Groundwater services and effects (USEPA 2005)

Services	Effects
Drinking Water	Increase of decrease in availability of drinking water Change in human health or health risks
Water for Crop Irrigation	Change in value of crops or production costs Change in human health or health risks
Water for Livestock/Poultry	Change in Value of livestock products or production Change in human health or health risks

The Delaware Basin provides significant public drinking water supplies (1,804 mgd) with 44% in NY (800 mgd), 38% from Pa. (679 mgd), 16% from NJ (284 mgd), and 2% from Del. (40 mgd), Figure 6. The largest public water supply allocations in the Delaware Basin include United Water Delaware and Wilmington in Del.; Delaware & Raritan Canal diversion, New Jersey American, Trenton, and Camden in NJ; New York City, and Philadelphia and Aqua Pennsylvania in Pa. (Table 20). Figure 7 depicts public water supply service areas in the Delaware River Basin.

The annual value of raw (untreated) public water supply allocations in the Delaware Basin (1,803 mgd) is $658 million. When treated and delivered to customers the annual value of drinking water supplies is $3.14 billion (Table 21). Water purveyors in Delaware estimate the value of raw water supply is $1.00/1000 gallons according to cost of services studies for rate setting by the Public Service Commission. In FY13, the New Jersey Water Supply Authority plans to sell raw water supplies from the Manasquan Reservoir system for $1.02/1000 gallons (NJWSA 2011). The average value of treated drinking water based on rates set by public/private water purveyors in Del., NJ, Pa., and Md. is $4.78/1000 gallon (Corrozi and Seymour 2008).

Table 20. Public water supply allocations in the Delaware River Basin (DRBC 2010)

Water Purveyor	Supply (mgd)	Water Purveyor	Supply (mgd)	Water Purveyor	Supply (mgd)
Delaware	**40.10**				
United Water Del.	18.46	Harrington	0.36	Frederica Perkiomen	0.05
Wilmington	10.40	Camden-Wyoming	0.31		
Dover	4.74	Milton	0.17		
Newark	2.22	Milford	0.17		
Lewes BPW	0.98	Georgetown	0.13		
Tidewater Utilities	0.64	Frederica	0.08		
Dover AFB	0.44	Felton	0.08		
New Castle MSC	0.41	Delaware State Fair	0.05		
Smyrna	0.37	Magnolia	0.05		
New Jersey	**284.19**				
Del. & Raritan Canal	100.00	Hackettstown MUS	2.57	Medford Twp.	1.29
NJ American Western	39.37	Millville Water Dept	2.55	NJ American Oxford	1.20
Trenton	26.10	Moorestown	2.51	Florence Twp.	1.17
Camden	10.89	Bordentown	2.21	Salem City	1.12
Vineland	8.33	Burlington Twp.	2.00	Mantua Twp.	1.04
Merchant.-Pennsauken	6.05	Mt. Laurel	1.96	Pennsville Twp.	1.04
Washington Twp.	4.79	Glassboro	1.95	Pemberton Twp.	1.01
Willingboro MUA	4.65	Collingswood	1.93	Gloucester City	0.95
NJ American Mt. Holly	4.48	Maple Shade	1.64	Lower Twp MUA	0.95
Bridgeton	3.63	West Deptford	1.57	Sparta Twp.	0.94
Wildwood	3.59	Woodbury	1.55	Audubon Twp.	0.91
Aqua NJ Phillipsburg	3.46	Burlington City	1.47	Haddon Twp.	0.90
Aqua NJ Hamilton Sq.	3.39	Pennsgrove	1.42	Bellmawr Twp.	0.86
Aqua NJ Blackwood	2.96	Deptford Twp.	1.38	Haddonfield	0.82
Evesham MUA	2.82	Nesqehoning Boro	1.30	Greenwich Twp	0.82
				Misc. Water Purveyors	16.65
New York State	**800.03**				
New York City	800.00				
Pennsylvania	**679.30**				
Philadelphia	287.77	Easton Suburb.Water	4.47	Falls Twp.	2.66
Aqua PA Main System	102.18	Schuylkill Co. Auth.	4.36	Northampton Bucks	2.55
Forest Park	20.16	Muhlenberg Twp.	4.31	Warminster Twp.	2.54
Bethlehem	15.69	Lehigh County	4.22	Horsham Water/Sewer	2.30
Allentown	15.46	PA American Nazareth	4.13	Newtown Artesian	2.24
North Wales Water	15.09	Hazelton	4.12	Milford	1.88
Bucks Co. Water	14.99	PA Amer. Coatesville	4.07	Tamaqua MWA	1.87
Reading Area Auth.	14.31	Allentown City	4.02	Lehighton MWA	1.77
Bucks County SW	13.79	Northampton Boro.	3.74	Ambler Boro	1.75
PA Amer. Norristown	10.10	East Stroudsburg	3.69	Brodhead Cr. Auth.	1.73
Lower Bucks County	8.66	PA American Yardley	3.20	South Whitehall Twp.	1.71
North Penn Water	8.59	Phoenixville	3.01	Emmaus Munic. Water	1.49
Easton	7.13	Morrisville	2.89	Warrington Twp.	1.45
Schuylkill Co. Auth.	5.15	PA American Home	2.88	Wyomissing Boro	1.44
Pottstown Water Auth.	4.64	PA American Penn	2.76	Schuylkill Haven Boro.	1.42
				Misc. Water Purveyors	50.93

Table 21. Value of public drinking water supply allocations in the Delaware River Basin

State	Withdrawal (mgd)	Value/day untreated ($1.00/1000 gal)	Value/year untreated ($1.00/1000 gal)	Value/year treated ($4.78/1000 gal)
Delaware	40	40,000	14,600,000	69,788,000
New Jersey	284	284,000	103,660,000	495,494,800
New York	800	800,000	292,000,000	1,395,760,000
Pennsylvania	679	679,000	247,835,000	1,184,651,300
Delaware Basin	**1,803**	**1,803,000**	**658,095,000**	**3,145,694,100**

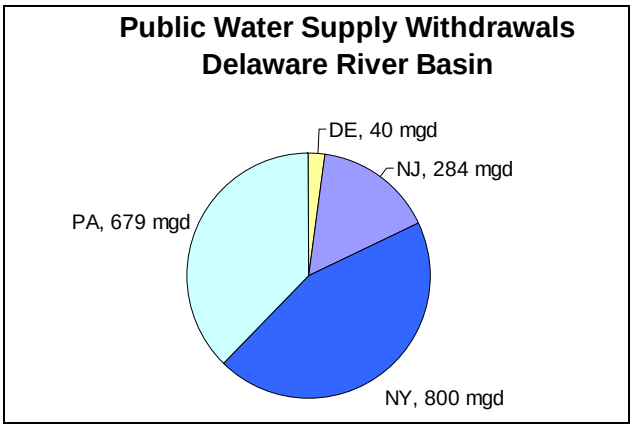


Figure 6. Public water supply withdrawals in the Delaware River Basin (DRBC)

Reservoir Storage

Almost 369 billion gallons of water is stored in reservoirs for interstate flow management and drinking water supply in the Delaware Basin (Table 22). The New Jersey Water Supply Authority operates a reservoir system and Delaware & Raritan Canal diversion from the Delaware River to New Jersey. The NJWSA delivers untreated water to public water purveyors from the Raritan River reservoir system at an estimated market price of $0.394/1,000 gallons (NJWSA 2011). Given the raw water value of drinking water before treatment) is $0.394/1000 gallons, the annual value of reservoir storage for flow management purposes in the Delaware Basin is $145 million.

Table 22. Economic value of reservoir storage in the Delaware River Basin

Reservoir	Storage (BG)	Value ($0.394/1000 gal)
Pepacton	140	55,160,000
Cannonsville	96	37,824,000
Neversink	35	13,790,000
Mongaup	15	5,910,000
Merrill Creek	16	6,304,000
Hoopes	2	788,000
Marsh Creek	4	1,576,000
Blue Marsh	6	2,561,000
Beltzville	13	5,122,000
F. E. Walter	11	4,334,000
L.Waullenpaupack	30	11,820,000
Total	**368**	**145,189,000**

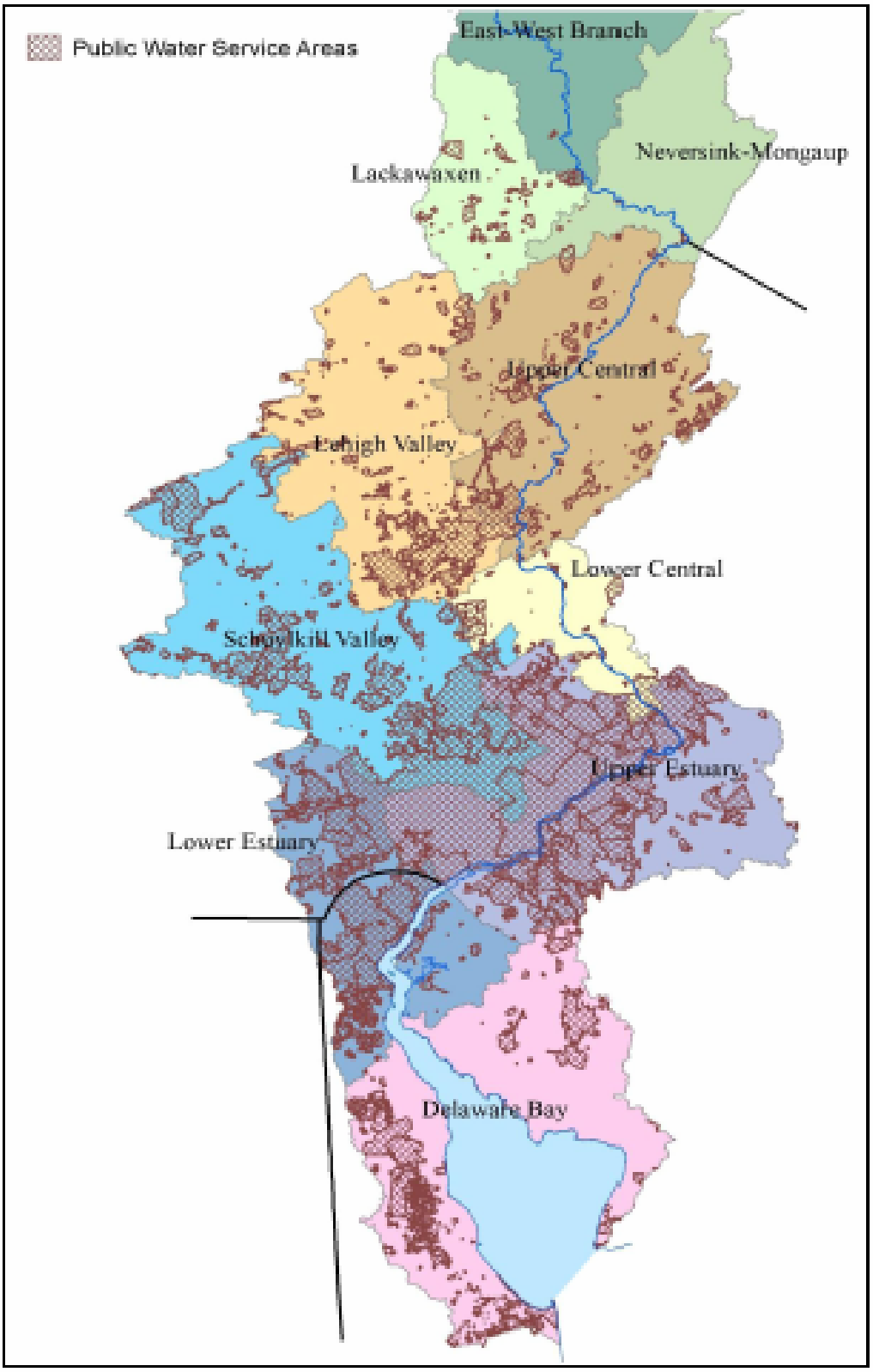


Figure 7. Public water supply service areas in the Delaware River Basin (DRBC 2011)

Irrigation Water Supply

Agricultural irrigation withdrawals allocated by DRBC total 36.5 mgd. The DRBC allocates groundwater withdrawals over 100,000 gpd therefore many small irrigation wells are not included in this total. Resources for the Future studied the economic value of freshwater in the U.S. estimated the median value of irrigation water withdrawals is $198/ac-ft in $1996 (Frederick et al. 1996) or $300/ac-ft ($0.92/1000 gal) in $2010 adjusting for 3% annually (Table 23). The value of irrigation withdrawals based on DRBC allocations is $33,630 per day or $12,275,000 per year (Table 24).

Table 23 Freshwater values in the United States by use

Use	2006 Median[1] ($/ac-ft)	2010 Median[2] ($/ac-ft)	2010 Median ($/1000 gal)
Hydropower	21	32	0.10
Industrial Process	132	200	0.61
Irrigation	198	300	0.92
Navigation	10	15	0.02
Thermoelectric Power	29	44	0.14

1. Frederick et al. 1996. 2. Adjusted to $2010 at 3% annually.

Table 24. Value of agricultural irrigation supply in the Delaware River Basin

Watershed	Withdrawal (mgd)	Irrigation Value/day ($0.92/1000 gal)	Irrigation Value/year ($0.92/1000 gal)
Upper Region	0.65	597	217,731
Upper Central	4.91	4,515	1,647,916
Lehigh Valley	0.20	184	67,118
Lower Central	1.51	1,389	507,084
Schuylkill Valley	0.02	23	8,358
Upper Estuary	4.15	3,819	1,394,036
Lower Estuary	7.58	6,976	2,546,164
Delaware Bay	17.53	16,128	5,886,540
Delaware Basin	**36.55**	**33,630**	**12,274,946**

Over 209,882 acres or 7% of cropland are irrigated in Delaware Basin counties (USDA 2009). About 1,926,524 acres or 24% of the basin is farmland, therefore, by proportion about 141,138 acres are irrigated (Table 25). Annual irrigation water needs from June - September are 9 inches for corn, soybeans, and grain (2,600 gpd/ac, 366 mgd). The economic value of water to irrigate 141,138 acres is $31.8 million, or $13.8 million in Del., $14.3 million in NJ, 0.9 million in NY, and $2.7 million in Pa.. The value of irrigation water demand = (9 in/12 in/ft)(141,138)($300/ac-ft) = $31,756,104/yr.

Table 25. Value of agriculture irrigation water demand in the Delaware River Basin

County	Cropland by county[1] (ac)	Irrigation by county[1] (ac)	Farmland in basin (ac)	Irrigated land in basin (ac)	Value of irrigation[2] @ $300/ac-ft
New Castle	51,913	2,711			
Kent	146,536	29,066			
Sussex	234,324	72,785			
Delaware	**432,773**	**104,562**	**254,143**	**61,403**	**$13,815,748**
Burlington	85,790	12,620			
Camden	8,760	2,647			
Cape May	7,976	2,342			
Cumberland	69,489	18,357			
Gloucester	46,662	12,891			
Hunterdon	100,027	1,501			
Mercer	21,736	1,028			
Monmouth	44,130	5,976			
Ocean	9,833	1,090			
Salem	96,530	18,001			
Sussex	65,242	454			
Warren	74,975	2,426			
New Jersey	**631,150**	**79,333**	**505,507**	**63,540**	**$14,296,541**
Broome	86,613	150			
Delaware	165,572	65			
Greene	44,328	735			
Orange	80,990	4,560			
Sullivan	50,443	75			
Ulster	75,205	4,707			
New York	**503,151**	**10,292**	**187,561**	**3,837**	**$863,230**
Berks	170,760	1,260			
Bucks	58,012	1,421			
Carbon	20,035	132			
Chester	117,145	1,659			
Delaware	1,646	36			
Lackawanna	39,756	258			
Lancaster	326,648	5,366			
Lebanon	89,566	1,276			
Lehigh	72,737	1,189			
Luzerne	66,577	60			
Monroe	29,165	97			
Montgomery	28,563	668			
Northampton	68,252	247			
Philadelphia	150	0			
Pike	27,569	12			
Schuylkill	81,276	1,896			
Wayne	99,939	118			
Pennsylvania	**1,297,796**	**15,695**	**979,313**	**11,843**	**$2,664,765**
Total	**2,864,870**	**209,882**	**1,926,524**	**141,138**	**$31,756,104**

1. Census of Agriculture 2007 (USDA 2009). 2. Frederick, VandenBerg, and Hansen 1996.

Thermoelectric Power Water Supply

Cooling water withdrawals for thermoelectric power plants in the Delaware Basin provide significant economic value. Over 89% of the energy in the United States is produced by thermoelectric power plants which evaporate water during cooling of condensate. The Delaware Basin provides 5,809

mgd of cooling water to run nuclear, coal, and gas fired power plants to generate 13,458 megawatts of electricity along the Delaware, Schuylkill, and Lehigh. About 95% of the cooling water returns to the river or bay (nonconsumptive use) and 5% evaporates (consumptive use). Table 26 lists power plants and associated cooling water withdrawals within the Delaware Basin obtained from U. S. Energy Information Administration (2002) and U.S. National Energy Technology Laboratory (2009) inventories of electric utility power plants and DRBC water allocation dockets.

Resources for the Future in a study of the economic value of freshwater in the United States estimated the median $1996 value of thermoelectric power water withdrawals is $29/ac-ft ($0.09/1000 gal) with a range of $9 to $63/ac-ft (Frederick et al. 1996). Adjusting for 3% annually, the median $2010 value of thermoelectric plant water withdrawals is $44 per ac-ft or $0.14/1000 gal. At $0.14/1000 gal, the value of thermoelectric water withdrawals in the Delaware Basin is $297 million/yr or $24 million/yr in Delaware, $196 million/yr in New Jersey, and $77 million/yr in Pennsylvania (Table 27).

Table 26. Thermoelectric power plant water withdrawals in the Delaware River Basin

State/Power Plant	Type	Capacity[1] (megawatts)	Withdrawal (mgd)	Value/day[2] ($0.14/1000 gal)[1]	Value/year ($0.14/1000 gal)
Delaware		**1,009**	**479**	**67,060**	**24,476,900**
Delmarva Delaware City		9	9		
Conectiv Edgemoor	Coal/Gas	1,000	470		
New Jersey		**4,920**	**3,830**	**536,200**	**195,713,000**
PSEG Salem 1 and 2	Nuclear	2,275	2,643		
PSEG Hope Creek	Nuclear	1,268	52		
Chambers Cogen. Salem	Coal	285			
Deepwater Station	Coal	82	219		
Logan Generating	Coal	242	38		
PSEG Mercer Trenton	Coal	768			
Pennsylvania		**7,529**	**1,500**	**210,000**	**76,650,000**
PECO Chester	Coal	56			
PECO Cromby	Coal	417			
PECO Croyden	Coal	546			
PECO Delaware (Phila.)	Coal	392			
PECO Eddystone	Coal	1,448			
PECO Fairless Hills	Coal	75			
PECO Falls	Coal	64			
PECO Limerick	Nuclear	2,230			
PECO Moser	Coal	64			
PECO Richmond (Phila.)	Coal	132			
PECO Schuylkill (Phila.)	Oil	233			
PECO Southwark (Phila.)	Coal	74			
PGE Northamp. Lehigh	Coal	134			
PPL Martins Creek	Coal	1,664	Shut 2007		
Delaware Basin		**13,458**	**5,809**	**813,260**	**296,839,900**

1. EIA 2002, NETL 2009, and DRBC. 2. Frederick et al. 1996 adjusted to $2010 at 3% annually.

Table 27. Value of thermoelectric power withdrawals in the Delaware River Basin

Watershed	Withdrawal [1] (mgd)	Value/day[2] ($0.14/1000 gal)	Value/year ($0.14/1000 gal)
Upper Region	0	0	0
Upper Central	394	55,160	20,133,400
Lehigh Valley	2	280	102,200
Lower Central	24	3,360	1,226,400
Schuylkill Valley	232	32,480	11,855,200
Upper Estuary	1,461	204,540	74,657,100
Lower Estuary	3,696	517,440	188,865,600
Delaware Bay	0	0	0
Delaware Basin	**5,809**	**813,260**	**296,839,900**

1. DRBC. 2. Frederick et al. 1996 adjusted to $2010 at 3% annually)

Industrial Water Supply

Industrial water withdrawals allocated by DRBC total 804 mgd in the Delaware River Basin (Table 28). A study of the economic value of freshwater in the U.S. indicates the median value of industrial withdrawals is $132/ac-ft in $1996 (Frederick et al. 1996) or $200/ac-ft ($0.61/1000 gal) in $2010 adjusting for 3% annually. The value of industrial withdrawals based on DRBC allocated supplies is $490,684 per day or $179,099,660 per year.

Table 28. Value of industry process water withdrawals in the Delaware River Basin

Watershed	Withdrawal [1] (mgd)	Industry Value/day[2] ($0.61/1000 gal)	Industry Value/year ($0.61/1000 gal)
Upper Region	0	0	0
Upper Central	31	18,727	6,835,355
Lehigh Valley	73	44,591	16,275,715
Lower Central	71	43,188	15,763,620
Schuylkill Valley	40	24,583	8,972,795
Upper Estuary	132	80,703	29,456,595
Lower Estuary	446	271,877	99,235,105
Delaware Bay	12	7,015	2,560,475
Delaware Basin	**804**	**$490,684**	**$179,099,660**

1. DRBC water allocations. 2. Frederick et al. 1996 adjusted to $2010 at 3% annually

Hydropower Water Supply

Hydropower water supply withdrawals allocated by DRBC total 539 mgd in the upper Delaware Basin at the Delaware Water Gap at Yards Creek and above Pt. Jervis (Table 29). A study of the economic value of freshwater in the U.S. indicates the median value of hydropower withdrawals is $21/ac-ft in $1996 (Frederick et al. 1996) or $32/ac-ft ($0.10/1000 gal) in $2010 adjusting for 3%

annually. The value of hydropower water withdrawals based on DRBC allocated supplies is $53,879 per day or $19,662,550 per year.

Table 29. Value of hydroelectric water supplies in the Delaware River Basin

Watershed	Withdrawal[1] (mgd)	Hydropower Value/day[2] ($0.10/1000 gal)	Hydropower Value/year ($0.10/1000 gal)
Upper Region	393	39,330	14,355,450
Upper Central	145	14,540	5,307,100
Lehigh Valley	0	0	0
Lower Central	0	0	0
Schuylkill Valley	0	0	0
Upper Estuary	0	0	0
Lower Estuary	0	0	0
Delaware Bay	0	0	0
Delaware Basin	**539**	**53,870**	**19,662,550**

1. DRBC water allocations. 2. Frederick et al. 1996 adjusted to $2010 at 3% annually

Fish/Wildlife

Fish Landings

The annual value of fish landings (Table 30) in the tidal Delaware River and Bay is $25.4 million in $2000 or $34.1 million in $2010 as reported to the National Marine Fisheries Service and tabulated by the National Ocean Economics Program (2007). Table 31 ranks the most lucrative fisheries in the Delaware Estuary as blue crab ($14.4 million/yr), summer flounder ($5.3 million/yr), Atlantic menhaden ($4.3 million/yr), eastern oyster ($3.7 million/yr), striped bass ($2.3 million/yr), and American eel ($0.8 million/yr). Figure 8 charts fish landings for Delaware Estuary species.

Table 30. Fish landings and landed value in the Delaware Estuary in $2000

	Delaware		New Jersey		Pennsylvania		Delaware Estuary	
Delaware Estuary Species[1]	**Pounds**	**Value ($2000)**	**Pounds**	**Value ($2000)**	**Pounds**	**Value ($2000)**	**Pounds[2]**	**Value[2] ($2000)**
Bass, Striped	188,671	$429,994	564,000	$1,287,000	211	$378	752,882	$1,717,372
Bluefish	19,565	$8,075	1,403,717	$500,053			1,423,282	$508,128
Carp. Common	3,764	$865			6,724	$26,805	10,488	$27,670
Catfish, Channel	6,922	$3,929					6,922	$3,929
Crab, Blue	3,799,820	$5,329,182	4,636,368	$5,471,115			8,436,188	$10,800,297
Crab, Horseshoe	229,602	$48,978					229,602	$48,978
Drum, Black	37,712	$21,867	1,518	$444			39,230	$22,311
Eel, American	139,648	$315,094	159,292	$310,417			298,940	$625,511
Flounder, Summer	5,464	$11,119	1,697,513	$3,988,869			1,702,977	$3,999,988
Herring, Blueback	1,434	$609					1,434	$609
Herring, Atlantic			6,039,473	$563,083			6,039,473	$563,083
Menhaden, Atlantic	85,080	$6,635	37,634,929	$3,193,724			37,720,009	$3,200,359
Oyster, Eastern	79,933	$490,465	444,227	$2,230,835			524,160	$2,721,300
Perch, White	55,973	$46,865	27,527	$29,654	4,560	$7,981	88,060	$84,500
Perch, Yellow					20,527	$71,847	20,527	$71,847
Shad, American	71,445	$42,408	58,981	$77,015			130,426	$119,423
Shellfish	30,130	$76,119					30,130	$76,119
Snails (Conchs)			30,250	$59,016			30,250	$59,016
Weakfish	24,604	$36,177	164,506	$225,051			189,110	$261,228
Whelk,Chan'd/Knob	277,217	$511,172					277,217	$511,172
Total	**5,056,984**	**$7,379,553**	**52,862,301**	**$17,936,276**	**32,022**	**$107,011**	**57,951,307**	**$25,422,840**

1. Dove and Nyman 1995. 2. NMFS and National Ocean Economics Program 2007.

Table 31. Fish landings and value in the Delaware Estuary in $2010

Delaware Estuary Species[1]	Value ($2000)[2]	Value ($2010)[3]
Crab, Blue	$10,800,297	$14,472,398
Flounder, Summer	$3,999,988	$5,359,984
Menhaden, Atlantic	$3,200,359	$4,288,481
Oyster, Eastern	$2,721,300	$3,646,542
Bass, Striped	$1,717,372	$2,301,278
Eel, American	$625,511	$838,185
Herring, Atlantic	$563,083	$754,531
Bluefish	$508,128	$680,892
Whelk,Chan'd/Knob	$511,172	$684,970
Weakfish	$261,228	$350,046
Shad, American	$119,423	$160,027
Perch, White	$84,500	$113,230
Shellfish	$76,119	$101,999
Perch, Yellow	$71,847	$96,275
Snails (Conchs)	$59,016	$79,081
Crab, Horseshoe	$48,978	$65,631
Carp. Common	$27,670	$37,078
Drum, Black	$22,311	$29,897
Catfish, Channel	$3,929	$5,265
Herring, Blueback	$609	$816
Total	**$25,422,840**	**$34,066,606**

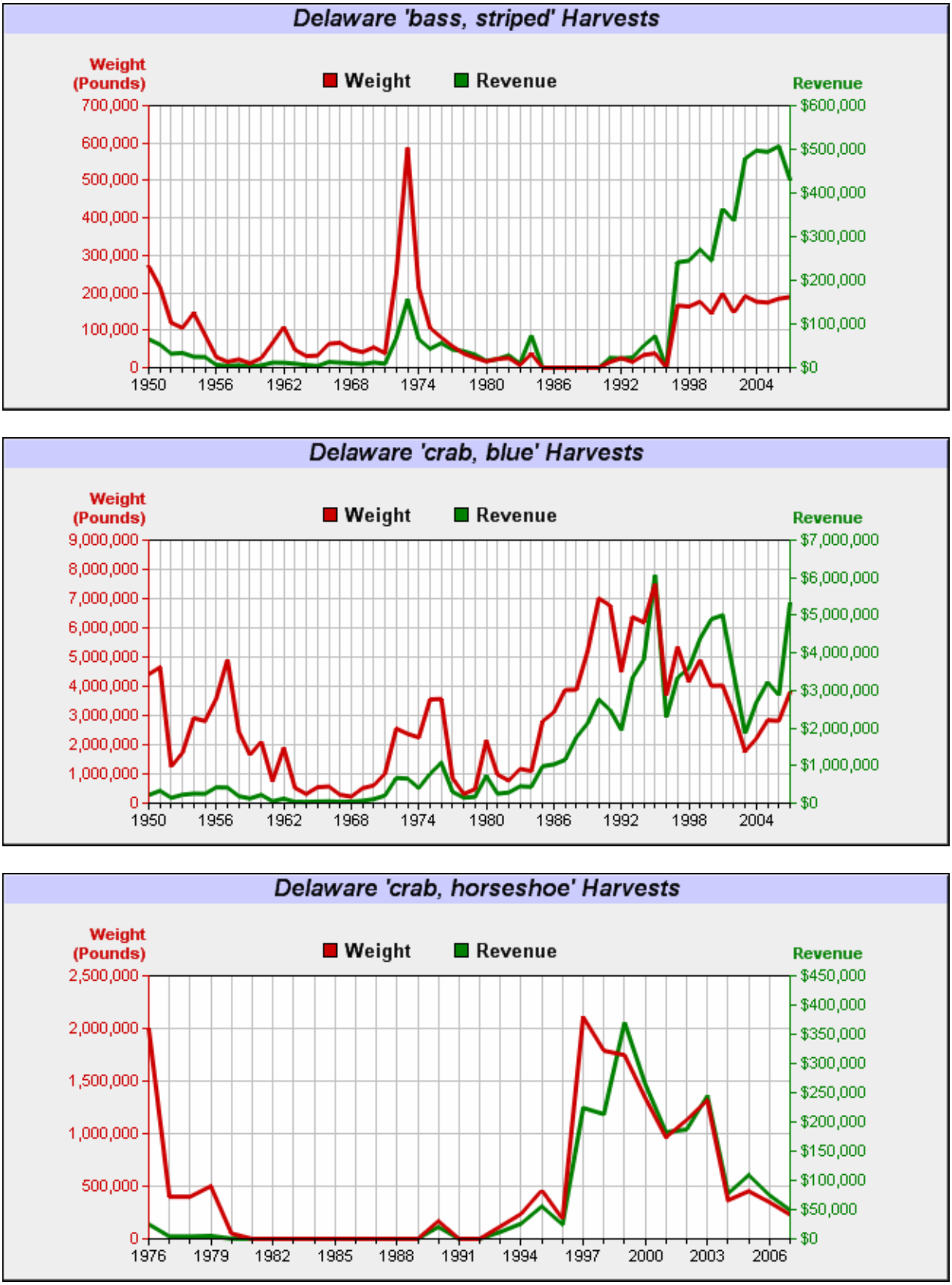
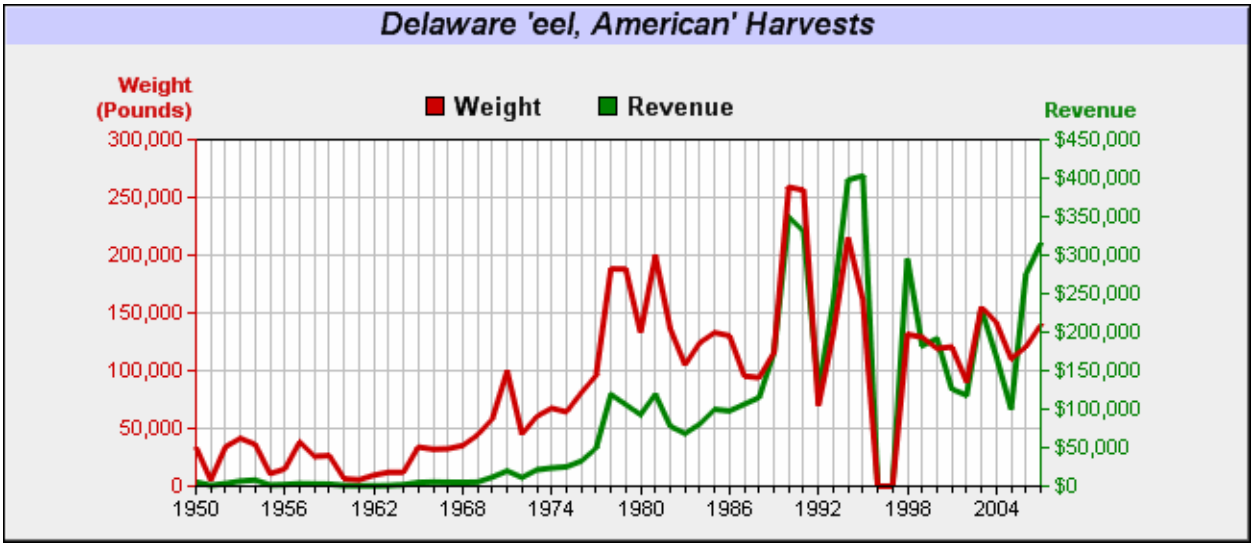
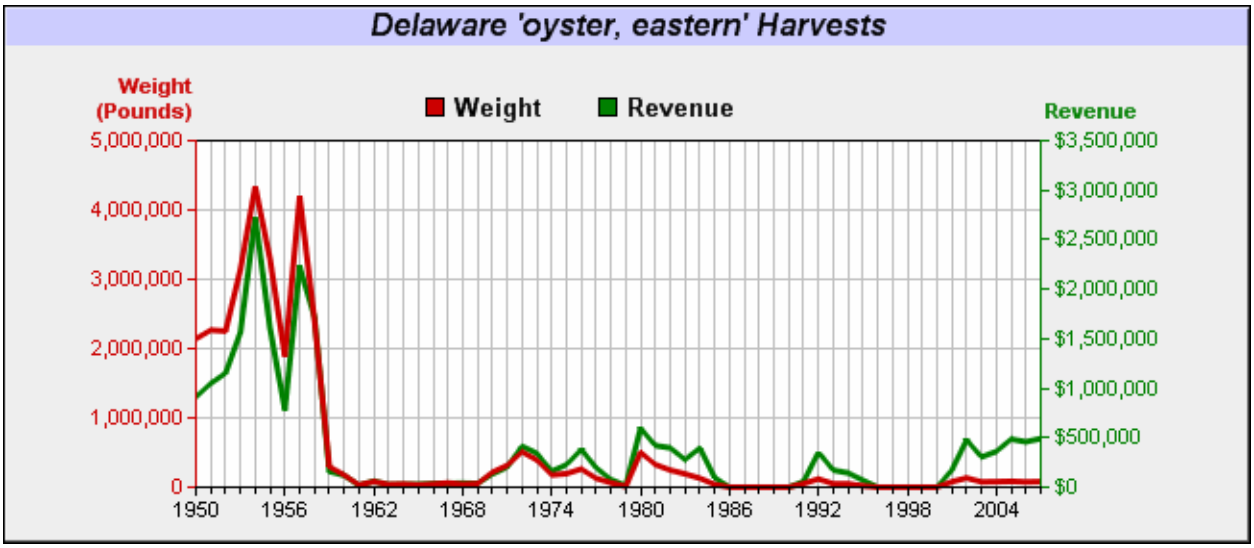


Figure 8. Fish landings in the Delaware Estuary (NMFS and NOEP 2007)





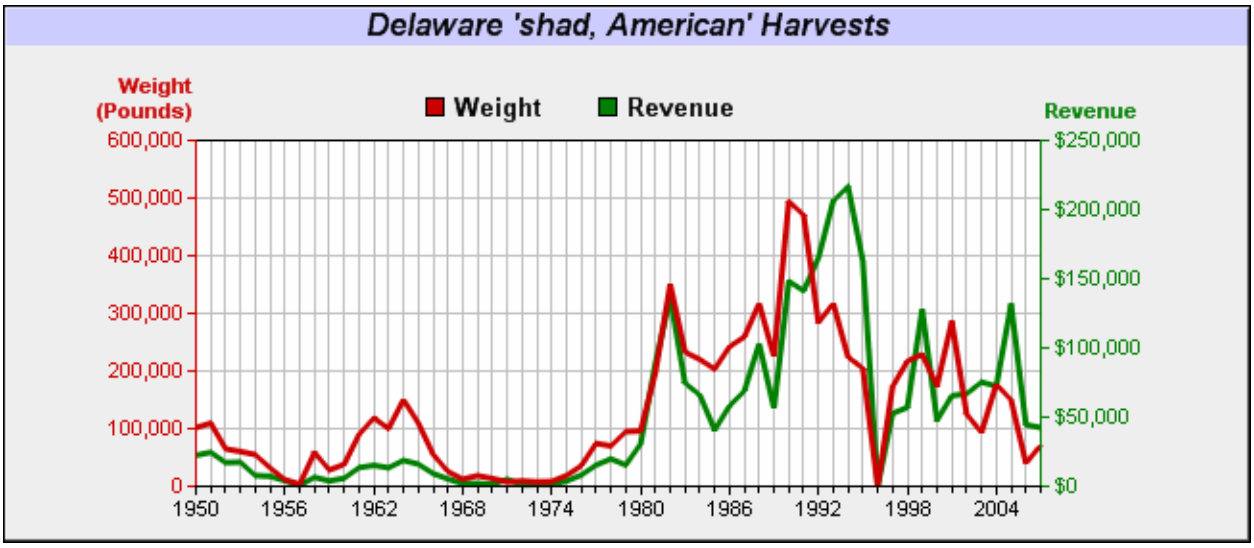
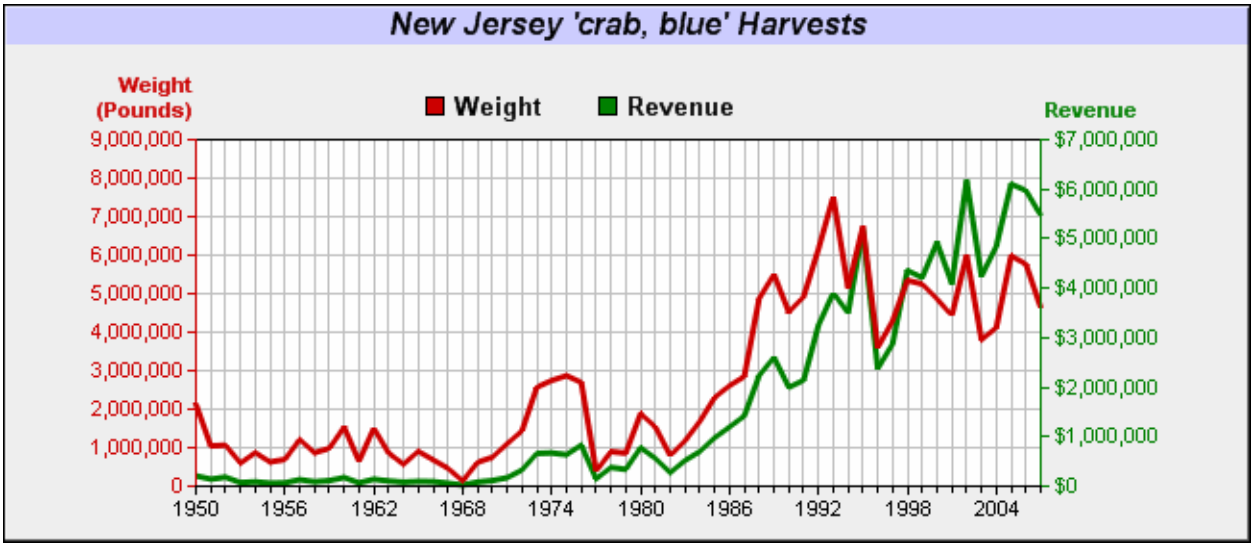
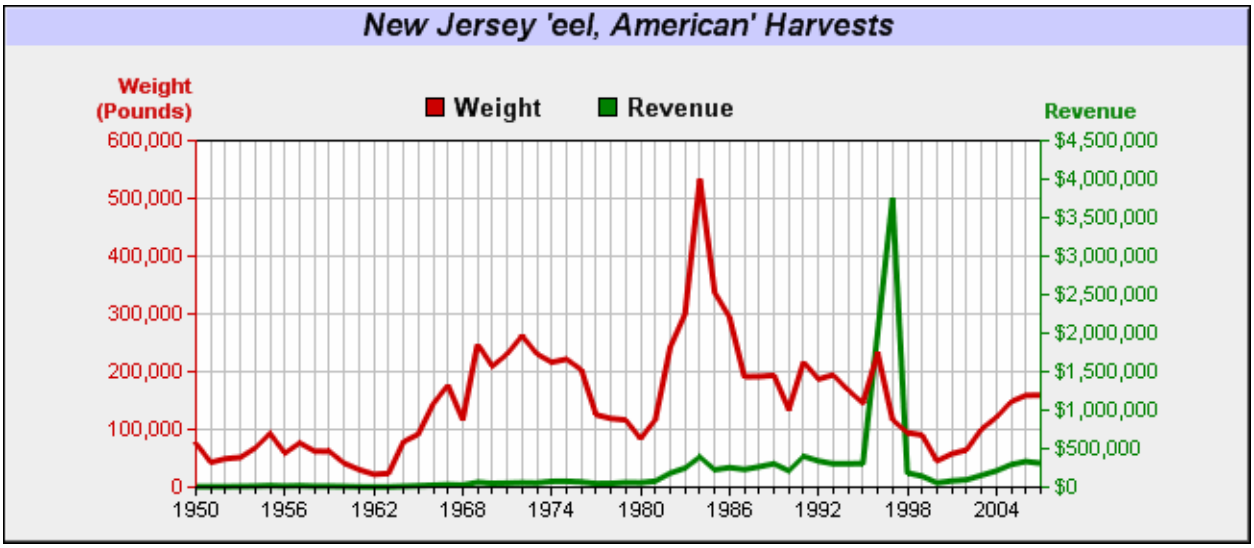


Figure 8. Fish landings in the Delaware Estuary, con't. (NMFS and NOEP 2007)





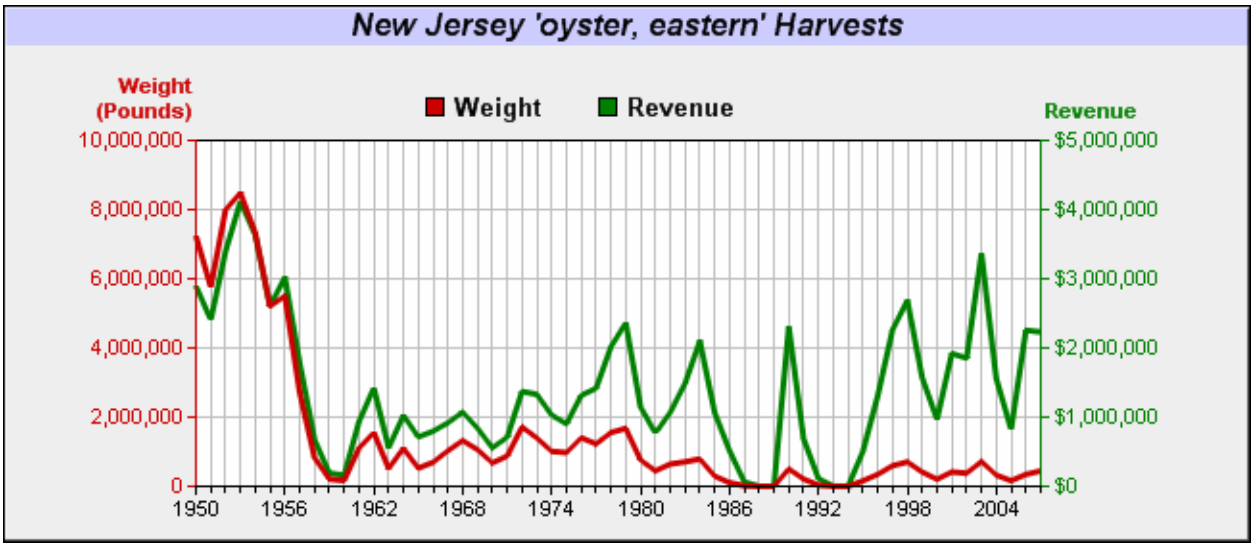
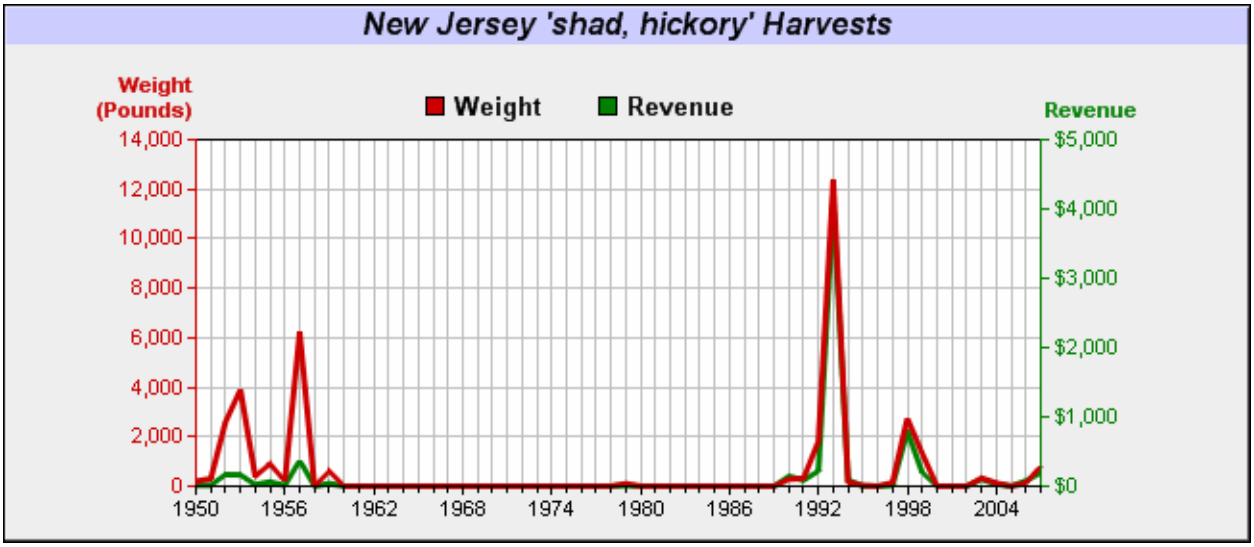
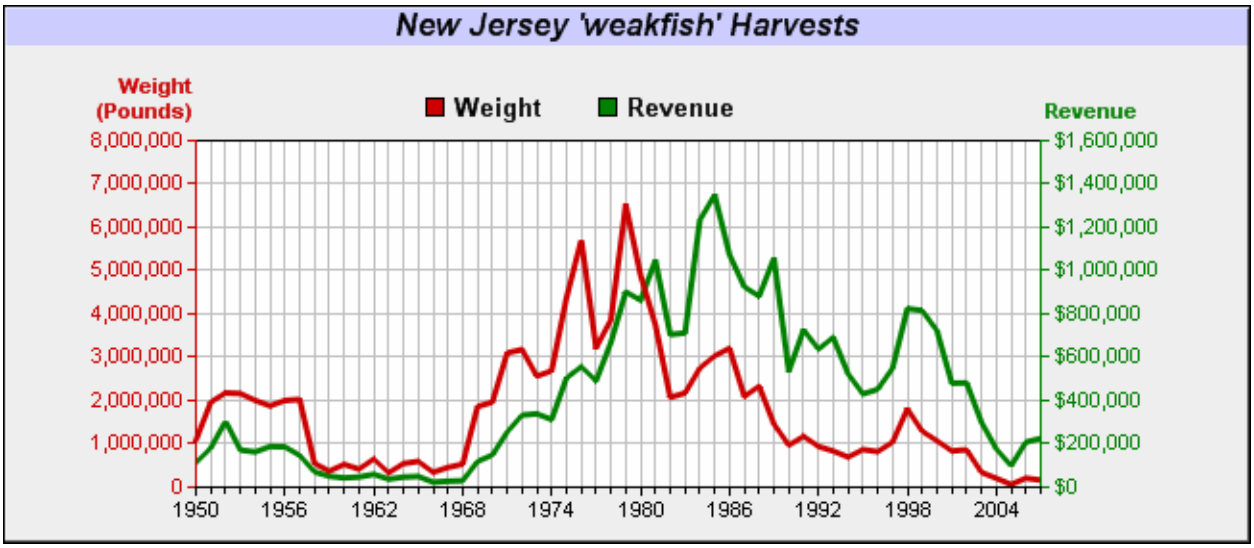


Figure 8. Fish landings in the Delaware Estuary, con't. (NMFS and NOEP 2007)





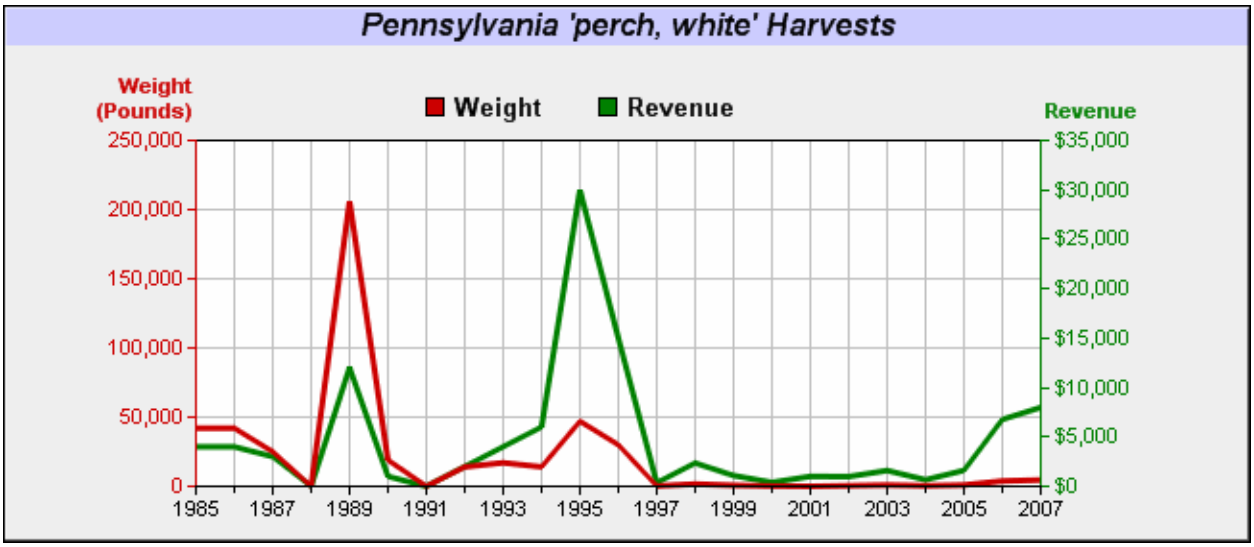


Figure 8. Fish landings in the Delaware Estuary, con't. (NMFS and NOEP 2007)

Fishing, Hunting, and Bird/Wild-life Watching

In Delaware, New Jersey, New York, and Pennsylvania, the U. S. Fish and Wildlife Service (2008) estimated the annual economic value of fishing, hunting, birding and wild-life/bird watching recreation was $9.2 billion in $2006. Trip-related expenditures include food and lodging, transportation, and hunting, fishing, and wildlife watching equipment. Most fishing, hunting, and birding/wildlife recreation occurs on farm, forest, wetlands, and open water ecosystems such as the Prime Hook and Bombay Hook National Wildlife Refuges in Delaware, the Cape May National Wildlife Refuge and Pine Barrens National Reserve in New Jersey, the Catskill Mountain Preserve in New York, the Delaware Water Gap National Recreation Area in Pennsylvania, and on the Delaware River and Bay and tributaries as well.

The Delaware Basin includes 50% of Delaware's land area, 40% of New Jersey's land area, 5% of New York State's land area, 14% of Pennsylvania's land area. Prorating based on the ratio of the area of the state within the basin to total state area, estimated economic value of fishing, hunting, and wild-life associated recreation in the Delaware Basin is $1,477 million/yr in $2006 or $134 million/yr in Delaware, $574 million/yr in New Jersey, $160 million/yr in New York, and $608 million/yr in Pennsylvania (Table 32).

Table 32. Value of fishing, hunting, and wildlife recreation in the Delaware River Basin

Recreation Activity	DE by state[1] ($M)	NJ by state[1] ($M)	NY by state[1] ($M)	PA by state[1] ($M)	DE in basin[2] ($M)	NJ in basin[2] ($M)	NY in basin[2] ($M)	PA in basin[2] ($M)	Del. Basin ($M)
Fishing	**97**	**752**	**926**	**1,291**	**48**	**301**	**46**	**181**	**576**
Trip Related	**49**	**471**	**585**	**299**	**24**	**188**	**29**	**42**	**284**
Equipment/other	**48**	**281**	**341**	**993**	**24**	**112**	**17**	**139**	**293**
Hunting	**41**	**146**	**716**	**1,609**	**21**	**58**	**36**	**225**	**340**
Trip-related	14	73	202	274	7	29	10	38	84
Equipment/other	28	73	514	1,335	14	29	26	187	256
Wildlife/Bird-watching	**131**	**537**	**1,568**	**1,443**	**65**	**215**	**78**	**202**	**561**
Trip Related	13	146	696	325	7	59	35	46	145
Equipment/other	118	391	872	1,118	59	156	44	156	415
Total	**269**	**1,436**	**3,209**	**4,343**	**134**	**574**	**160**	**608**	**1,477**

1. (USFWS 2008). Prorated by ratio of basin to state land area, Del. (50%), NJ (40%), NY (5%), and Pa. (14%).

Shad Fishing

The Pennsylvania Fish and Boat Commission (2011) published a fact sheet on the economic value of fishing and boating in Pennsylvania. A 1986 study of shad fishing on the Delaware River showed:

- Anglers spent an average of $25.40 per trip on gasoline, food, lodging, and tackle. Multiplied by 63,000 trips in 1986, anglers spent $1.6 million during a nine week season. Adjusting by 3% annually, the economic contribution by shad anglers would be about $3.2 million in $2010.
- The average shad angler was willing to pay $50 per day of shad fishing or $102 per day when adjusted to $2010 at 3% annually. Multiplied by 63,000 angler days, the annual economic value based on willingness to pay for the Delaware River shad fishery was $3.2 million in 1986 or $6.5 million adjusted to $2010.

Wild Trout Fishing

Releases from New York City reservoirs and excellent water quality in the forested Catskill watersheds contribute to a thriving cold water fishery in the upper Delaware Basin. Along the Beaverkill and East Branch, West Branch, and upper main stem of the Delaware River in New York, wild trout fishing contributes almost $18 million in annual business revenue, over $29 million in economic activity, and almost 350 jobs with $3.6 million in wages (Maharaj, McGurrin, and Carpenter, 1998).

Agriculture

In Delaware Basin counties, the USDA (2009) estimates the annual market value of agricultural products sold is $4.79 billion on 2,857,870 acres (4,465 sq mi) for crops (corn, wheat, oats, barley, soybeans, potatoes, and vegetables) and livestock and poultry (Table 33). On 1,926,524 acres (3,010 sq mi) of farmland within the Delaware Basin, the prorated annual market value of agricultural products sold is $3.37 billion or $1,750 per acre. The Delaware Basin covers 12,769 sq mi or just 13% of the combined land areas of Delaware (1,953 sq mi), New Jersey (7,417 sq mi), New York (47,214 sq mi), and Pennsylvania (44,816 sq mi) yet accounts for $3.37 billion or 27% of total annual farm products sold in the four states (Table 34).

Table 33. Farm products sold in the Delaware River Basin

State	State area (sq mi)	Area in Del. Basin (sq mi)	Ratio area basin/area state (%)	Farm products sold in state ($ million)	Farm products Del. Basin ($ million)	Products in basin/state (%)
Delaware	1,953	965	49%	1,083	636	59%
New Jersey	7,417	2,961	40%	987	603	61%
New York	47,214	2,555	5%	4,418	105	2%
Pennsylvania	44,816	6,280	14%	5,808	2,027	35%
Total	**101,400**	**12,761**	**13%**	**12,296**	**3,371**	**27%**

Table 34. Value of cropland and agriculture in the Delaware River Basin

County	Farmland by county[1] (ac)	Products sold by county[1] ($ million)	Products sold by county ($/ac)	Farmland in Del. Basin (ac)	Products sold in Del. Basin ($ million)
New Castle	51,913	45.7	880		
Kent	146,536	188.4	1,286		
Sussex	234,324	848.9	3,623		
Delaware	**432,773**	**1,083.0**	**2,502**	**254,143**	**636**
Burlington	85,790	86.3	1,006		
Camden	8,760	18.6	2,123		
Cape May	7,976	14.6	1,830		
Cumberland	69,489	156.9	2,258		
Gloucester	46,662	93.9	2,012		
Hunterdon	100,027	69.7	697		
Mercer	21,736	18.6	856		
Monmouth	44,130	105.4	2,388		
Ocean	9,833	11.5	1,170		
Salem	96,530	80.0	829		
Sussex	65,242	21.2	325		
Warren	74,975	75.5	1,007		
New Jersey	**631,150**	**752.2**	**1,192**	**505,507**	**602**
Broome	86,613	29.9	345		
Delaware	165,572	55.1	333		
Greene	44,328	16.4	370		
Orange	80,990	73.7	910		
Sullivan	50,443	42.1	835		
Ulster	75,205	65.6	872		
New York	**503,151**	**282.8**	**562**	**187,561**	**105**
Berks	170,760	367.8	2,154		
Bucks	58,012	70.6	1,217		
Carbon	20,035	8.9	444		
Chester	117,145	553.3	4,723		
Delaware	1,646	9.4	5,711		
Lackawanna	39,756	16.2	407		
Lancaster	326,648	1,072.1	3,282		
Lebanon	89,566	257.1	2,871		
Lehigh	72,737	72.1	991		
Luzerne	66,577	18.1	272		
Monroe	29,165	7.8	267		
Montgomery	28,563	30.0	1,050		
Northampton	68,252	31.8	466		
Philadelphia	150	0.5	3,333		
Pike	27,569	2.5	91		
Schuylkill	81,276	124.7	1,534		
Wayne	92,939	29.4	316		
Pennsylvania	**1,290,796**	**2,672.3**	**2,070**	**979,313**	**2,027**
Delaware Basin	**2,857,870**	**4,790.3**	**1,676**	**1,926,524**	**3,371**

1. Census of Agriculture 2007 (USDA 2009)

Forests

The U. S. Forest Service and Delaware Center for Horticulture (Nowak et al. 2008) estimated 7,137 acres of forests in New Castle County have a carbon storage benefit of $5.9 million ($827/ac) and air pollution removal of $1.9 million ($266/ac/yr). Applying these multipliers, Tables 35 and 36 indicate 4,343,190 (6,786 sq mi) of forests in the Delaware Basin have economic benefits from carbon storage ($3,591 million), air pollution removal ($1,155 million), building energy savings ($243 million), and carbon sequestration ($126 million).

Table 35. Economic benefits of forests in the Delaware River Basin

Forest Benefits	New Castle County.[1] ($/ac)	Delaware. Basin[2] ($ mil.)
Carbon storage	827	3,592
Carbon Sequestration	29	126
Air Pollution Removal	266	1,155
Building Energy Savings	56	243
Avoided Carbon Emissions	3	13

1. Nowak et al. 2008.
2. Computed for Delaware Basin forests (4,343,190 ac).

Table 36. Economic benefits of forests in the Delaware River Basin by state

Forest Benefits	Del. ($ mil.)	NJ ($ mil.)	NY ($ mil.)	Pa. ($ mil.)	Del. Basin ($ mil.)
Carbon Storage	78.8	564.8	1,147.5	1,800.8	3,592
Carbon Sequest.	2.8	19.8	40.2	63.1	126
Air Pollution Contr.	25.4	181.7	369.1	579.2	1,155
Energy Savings	5.4	38.2	77.7	121.9	243
Avoid Carbon Emiss.	0.3	2.0	4.2	6.5	13

Open Space

Public Parks

The Trust for Public Land (2009) found the 444-acre City of Wilmington park and recreation system provides annual economic value and savings to the public from health benefits from exercise in the parks ($9,734/ac), community cohesion benefit from people socializing in the parks ($2,383/ac), water pollution benefit from parks in treating stormwater ($921/ac), air pollution mitigation value from tree and shrub absorption ($88/ac).

Using value transfer from the data gathered for the City of Wilmington study, Table 37 indicates public parks (169 sq mi) within the Delaware Basin provide the following annual economic value:

- Health benefits from exercise in the parks ($1,283 million).
- Community cohesion benefit from people socializing in the parks ($314 million).
- Water pollution benefit from parks in treating stormwater ($121million).
- Air pollution mitigation value from tree and shrub absorption ($12 million).

Table 37. Value of public parks in the Delaware River Basin

State/county	Parks in Del. Basin (ac)	Health Benefits ($9,734/ac)	Community Cohesion ($2,383/ac)	Stormwater Benefit ($921/ac)	Air Pollution ($88/ac)
Kent	4,587	44,649,858	10,930,821	4,224,627	403,656
New Castle	12,440	121,090,960	29,644,520	11,457,240	1,094,720
Sussex	1,389	13,520,526	3,309,987	1,279,269	122,232
Delaware[1]	**18,416[1]**	**179,261,344**	**43,885,328**	**16,961,136**	**1,620,608**
Burlington	7,970	77,579,980	18,992,510	7,340,370	701,360
Camden	2,985	29,055,990	7,113,255	2,749,185	262,680
Cape May	2,911	28,335,674	6,936,913	2,681,031	256,168
Cumberland	2,640	25,697,760	6,291,120	2,431,440	232,320
Gloucester	4,868	47,385,112	11,600,444	4,483,428	428,384
Hunterdon	3,170	30,856,780	7,554,110	2,919,570	278,960
Mercer	8,283	80,626,722	19,738,389	7,628,643	728,904
Monmouth	105	1,022,070	250,215	96,705	9,240
Ocean	199	1,937,066	474,217	183,279	17,512
Salem	2,144	20,869,696	5,109,152	1,974,624	188,672
Sussex	2,961	28,822,374	7,056,063	2,727,081	260,568
Warren	5,563	54,150,242	13,256,629	5,123,523	489,544
New Jersey[2]	**31,800[2]**	**426,339,466**	**104,373,017**	**40,338,879**	**3,854,312**
Broome	389	3,786,526	926,987	358,269	34,232
Delaware	546	5,314,764	1,301,118	502,866	48,048
Orange	413	4,020,142	984,179	380,373	36,344
Sullivan	1,570	15,282,380	3,741,310	1,445,970	138,160
Ulster	50	486,700	119,150	46,050	4,400
New York[3]		**28,890,512**	**7,072,744**	**2,733,528**	**261,184**
Berks	3,979	38,731,586	9,481,957	3,664,659	350,152
Bucks	11,402	110,987,068	27,170,966	10,501,242	1,003,376
Carbon	2,820	27,449,880	6,720,060	2,597,220	248,160
Chester	12,020	117,002,680	28,643,660	11,070,420	1,057,760
Delaware	6,274	61,071,116	14,950,942	5,778,354	552,112
Lehigh	2,500	24,335,000	5,957,500	2,302,500	220,000
Luzerne	195	1,898,130	464,685	179,595	17,160
Monroe	875	8,517,250	2,085,125	805,875	77,000
Montgomery	14,138	137,619,292	33,690,854	13,021,098	1,244,144
Northampton	1,393	13,559,462	3,319,519	1,282,953	122,584
Philadelphia	9,689	94,312,726	23,088,887	8,923,569	852,632
Pike	125	1,216,750	297,875	115,125	11,000
Schuylkill	829	8,069,486	1,975,507	763,509	72,952
Wayne	350	3,406,900	834,050	322,350	30,800
Pennsylvania[4]	**58,331[3]**	**648,177,326**	**158,681,587**	**61,328,469**	**5,859,832**
Total	**108,547**	**1,282,668,648**	**314,012,676**	**121,362,012**	**11,595,936**

1. State, county, and municipal park land in Delaware from county and local comprehensive plans.
2. County and municipal park land from New Jersey State Comprehensive Outdoor Recreation Plan (SCORP).
3. County/municipal parks in New York from county and local comprehensive plans.
4. County/municipal parks in Pennsylvania from DVRPC 2007 & Berks/Schuylkill counties comprehensive plans.

Delaware Water Gap National Recreation Area

The Delaware Water Gap National Recreation Area (DWGNRA) preserves almost 109 square miles of forest and floodplain along 40 miles of the upper Delaware River and 29 miles of the Appalachian Trail. Stynes and Sun (2002) estimated the DWGNRA had 4,867,272 recreation visits in 2001 including 487,727 local day trips, 3,650,455 non-local day trips, 486,727 motel visits, and

243,364 camping overnights. Total visitor spending in the DWGNRA in 2001 was $100 million including $12.4 million for local day trips, $46.5 million for non-local day trips, $30.9 million for motels, and $10.3 million for camping overnights. In 2001, the DWGNRA generated $106 million in sales, and 7,563 direct/indirect jobs with $100 million in wages.

Marcellus Shale Natural Gas

The U.S. Geological Survey concluded that the Marcellus Shale Formation is a voluminous economic resource that lies under 4,700 square miles or 36% of the Delaware River Basin. Drilling for natural gas through the hydraulic fracturing process requires large quantities of water and has the potential to consume sizable tracts of land in the forested headwaters of the Delaware Basin (Figure 9). Hydraulic fracturing requires pumping water under high pressure to open fractures in the shale to allow natural gas to flow to the well. The hydrofracturing water must be recovered and treated before disposal to surface and ground waters. In forests, natural gas well drilling can require clearing of pads that range from 3 to 5 acres in area.

The DRBC is considering revisions to Article 7 of the Water Quality Regulations to protect the water resources of the Delaware Basin during construction and operation of natural gas projects with the following considerations:

- Gas drilling projects in the Marcellus Shale may reduce the flow in streams and aquifers.
- On-site drilling operations may potentially release pollutants into ground or surface water.
- The recovered hydrofracturing water must be treated and disposed of properly.

The Marcellus Shale Formation covers 54,000 square miles and lies up to a mile and a half below parts of Kentucky, New Jersey, New York, Ohio, Pennsylvania, and West Virginia (Figure 10). The Marcellus Shale lies under 4,700 square miles or 36% of the Delaware River Basin in New York, Pennsylvania, and a small tip of New Jersey (Figure 11). About 8.7% of the Marcellus Shale Formation lies within the Delaware River Basin (4,700 sq mi/54,000 sq mi).

The U.S. Geological Survey (Coleman et al. 2011) estimated the entire 54,000 square-mile Marcellus Shale Formation potentially contains a mean volume of 84 trillion cubic feet of recoverable natural gas with a range of 43 tcf (95 percentile) to 144 tcf (5 percentile). If the Delaware River Basin covers 4,700 sq mi or 8.7% of the Marcellus Shale, then by proportion approximately 7.3 trillion cubic feet of natural gas is potentially recoverable within the basin boundary (0.087 x 54,000). These estimates can vary as the thickness of Marcellus Shale in the Delaware Basin generally increases to the north toward the New York/Pennsylvania border and may range from 50 feet thick near Stroudsburg to more than 250 feet thick at Lackawaxen in Wayne County, Pennsylvania (Figure 12).

The U.S. Energy Information Administration (2011) reported the 2010 mean natural gas wellhead price was $4.16/1000 cf. The price of natural gas for residential customers was $11.21/1000 cf. At these unit prices, the estimated value of natural gas from the Marcellus Shale Formation within the Delaware River Basin is $30.4 billion at the wellhead and $81.8 billion when sold to residential customers (Tables 38 and 39).

Environmental economists classify natural gas as a nonrenewable resource with finite stock value over a defined time frame (say 25 or 50 years). Assuming the natural gas can be recovered within 25

years, the annual value of the Marcellus Shale gas recoverable from within the Delaware Basin is $1.2 billion/year at the wellhead and $3.3 billion/year when sold to residential customers.

Table 38. Wellhead value of Marcellus shale natural gas within the Delaware River Basin

State/Basin	Area Marcellus Shale (sq mi)	Wellhead Natural Gas Price[1] ($/1000 cf)	Volume Natural Gas[2] (tcf)	Wellhead Natural Gas Value ($ billion)	Wellhead Natural Gas Value[3] ($ billion/yr)
Pennsylvania	2,338	$4.16	3.6	$15.0	$0.6
New York	2,362	$4.16	3.7	$15.4	$0.6
Delaware Basin	**4,700**	**$4.16**	**7.3**	**$30.4**	**$1.2**

1. EIA 2010. 2. USGS 2011. 3. Assumes 25 year natural gas recovery period.

Table 39. Residential value of Marcellus shale natural gas within the Delaware River Basin

State/Basin	Area Marcellus Shale (sq mi)	Residential Natural Gas Price[1] ($/1000 cf)	Volume Natural Gas[2] (tcf)	Residential Natural Gas Value ($ billion)	Wellhead Natural Gas Value[3] ($ billion/yr)
Pennsylvania	2,338	$11.21	3.6	$40.4	$1.6
New York	2,362	$11.21	3.7	$41.5	$1.7
Delaware Basin	**4,700**	**$11.21**	**7.3**	**$81.8**	**$3.3**

1. EIA 2010. USGS 2011. 3. Assumes 25 year natural gas recovery period.

On a per volume basis, the value of untreated drinking water in streams and wells (at $7.48/1000 cf or $1.00/1000 gal) exceeds the value of natural gas at the wellhead (at $4.16/1000 cf) in the Delaware Basin. The total value of untreated drinking water from streams/wells (1,803 mgd) in the Delaware Basin is $0.7 billion/year, less than the estimated value of natural gas recoverable at the wellhead ($1.2 billion/year). The value of treated drinking water in the basin (at $35.70/1000 cf or $4.78/1000 gal) is $3.1 billion/year which is comparable to the total natural gas value sold to residential customers or $3.3 billion/year (Table 40).

Table 40. Value of Marcellus shale gas compared to drinking water in the Delaware River Basin

Price/ Value	Natural Gas	Drinking Water
Quantity	7.3 trillion cf	1,803 mgd
Unit Price Wellhead Gas or Untreated Drinking Water	$4.16/1000 cf	$7.48/1000 cf
Total Value Wellhead Gas or Untreated DrinkingWater	$1.2 billion/yr	$0.7 billion/yr
Unit Price Residential Gas or Treated Drinking Water	$11.21/1000 cf	$35.70/1000 cf
Total Value Residential Gas or Treated Drinking Water	$3.3 billion/yr	$3.1 billion/yr

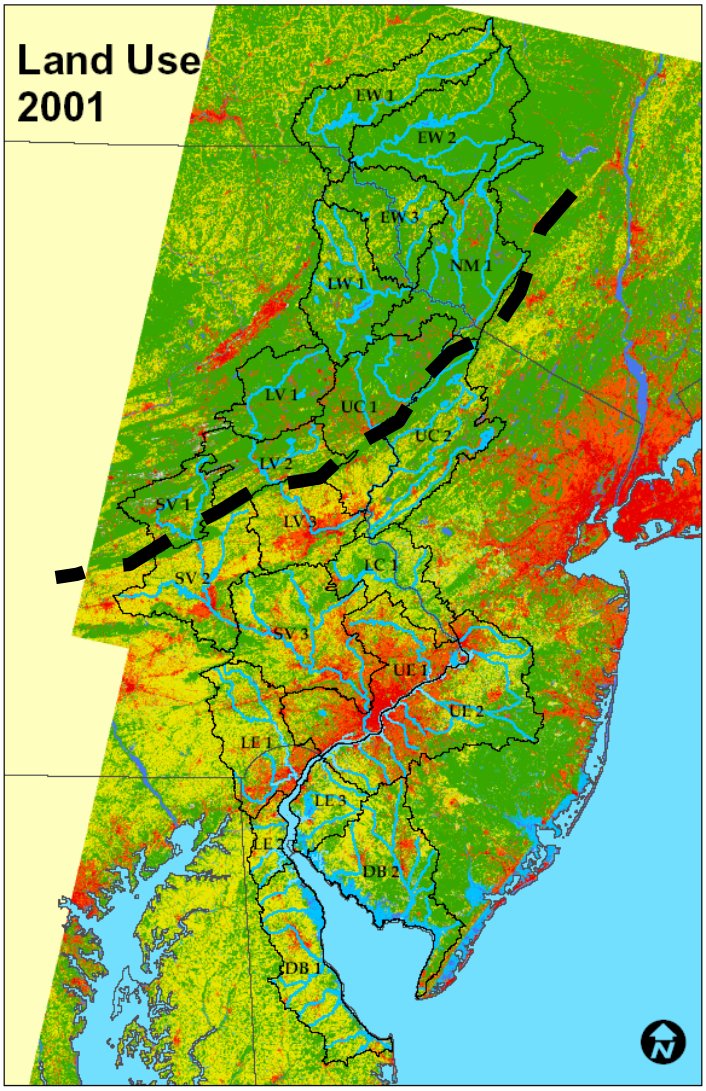


Figure 9. Land use including forested headwaters in the Delaware Basin (Marcellus Shale southerly boundary delineated as dashed line).

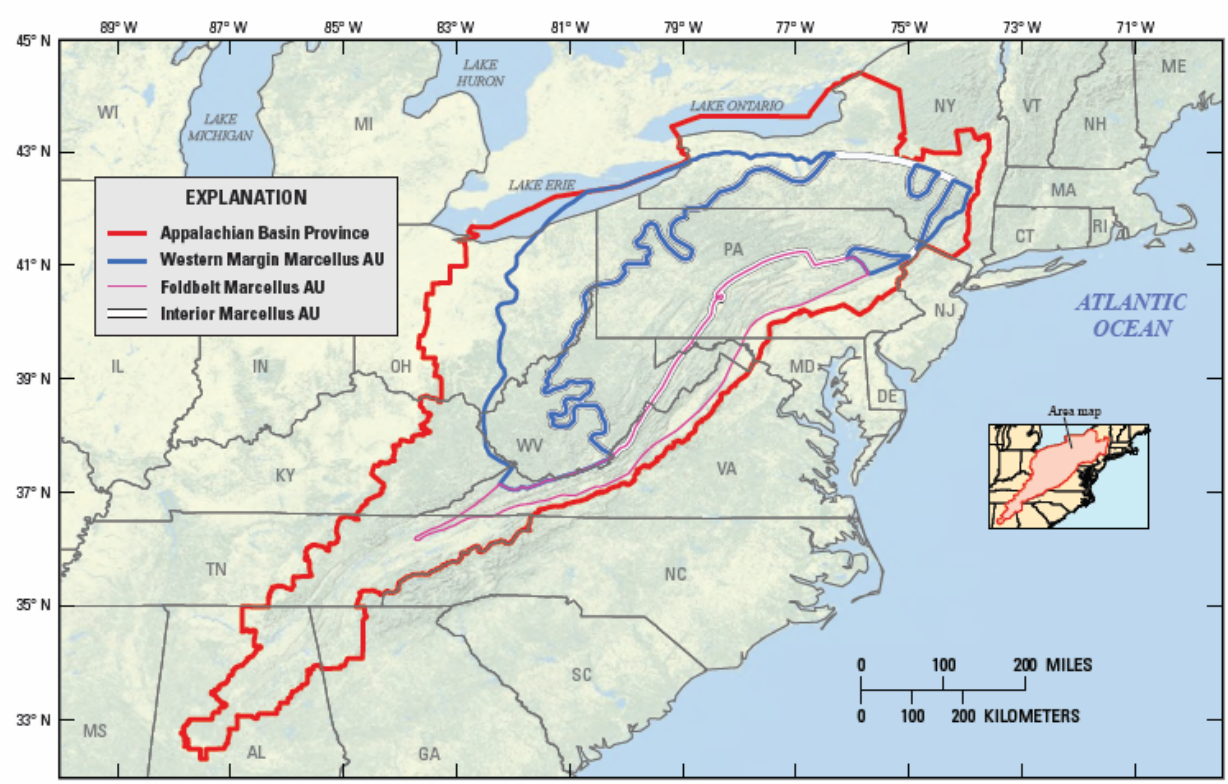


Figure 10. Marcellus Shale Formation in the Appalachian Basin Province (USGS 2011)

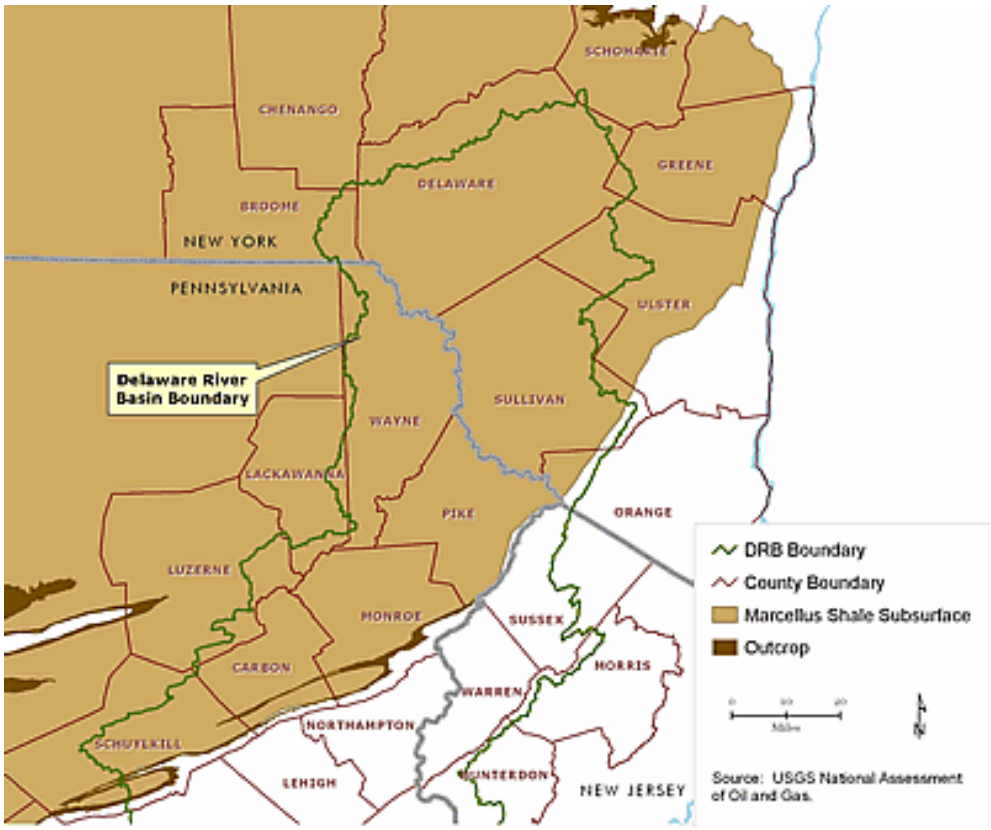


Figure 11. Marcellus Shale Formation within the Delaware River Basin (USGS)

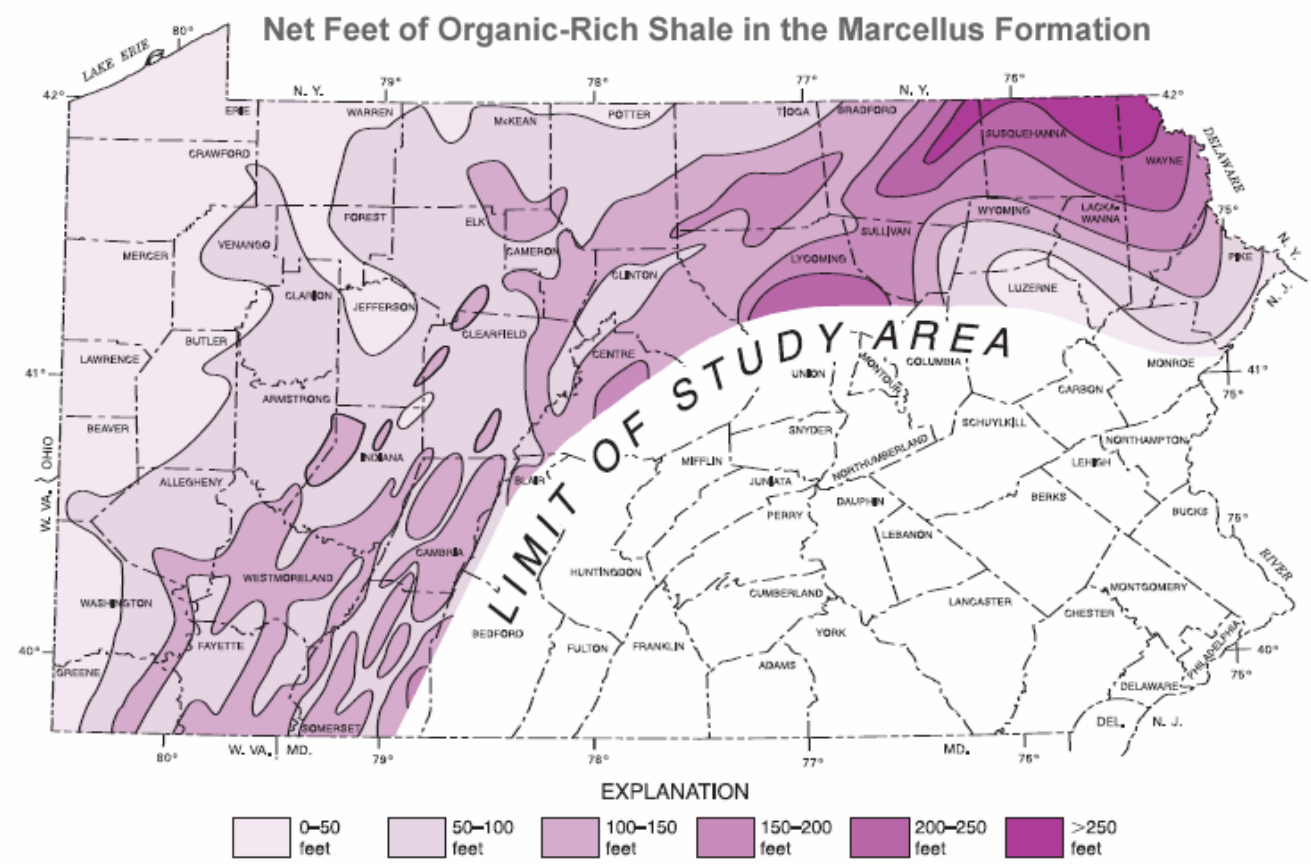


Figure 12. Thickness of Marcellus shale in Pennsylvania (Pennsylvania Geological Survey)

Maritime Transportation

Navigation

The 130-mile long Delaware River and Bay ship channel from Cape Henlopen to the head of navigation at Trenton has significant instream navigation use value. The Delaware River port from Wilmington to Chester, Paulsboro, Camden, and Philadelphia is the 6th largest port in the U.S. based on imports. The volume of the 720 square mile Delaware Estuary at mean depth of 32 feet is 14.7 million ac-ft or 4.8 trillion gallons. A study of the economic value of freshwater in the U.S. estimated the median value of instream navigation uses is $10/ac-ft in $1996 (Frederick et al. 1996) or $15/ac-ft in $2010 based on 3% annually. Accordingly, the instream navigation value of the Delaware River and Bay (14.7 million ac-ft) from the ocean to head of tide at Trenton is $220 million.

C&D Canal

The 35-feet deep Chesapeake & Delaware Canal is a valuable commercial conduit that flows through the Delaware Basin in Delaware and carries 40% of all ship traffic to/from the Port of Baltimore. The C&D Canal trims 300 miles from the trip for ships that would otherwise sail up the Chesapeake Bay to Baltimore from the ocean. Normally 6 to 35 ships per day sail through the C&D Canal.

The Port of Baltimore is responsible for 16,700 direct jobs and $3.7 billion in wages (Maryland Port Administration 2010). Of 360 ports in the U.S., Baltimore is No. 1 in forest product, gypsum, and sugar imports and No. 2 in automobile exports. In 2009, the Port of Baltimore was 11th among all U.S. port districts with $10.8 billion in exports after Seattle (9th) and San Francisco (10th). Baltimore was 12th in the U.S. with $19.4 billion in imports after Norfolk (10th) and Port Arthur, Texas (11th). If 40% of all Baltimore-bound ship traffic sails through the C&D Canal, then 40% of the economic activity generated by the port can be indirectly attributed to this avenue of commerce that cuts through Delaware River Basin in Delaware (Table 41).

Table 41. Economic activity generated by Port of Baltimore through the C&D Canal

Activity	Port of Baltimore[1]	C&D Canal[2]
Jobs	16,700	6,700
Wages	$3.7 billion	$1.5 billion
Imports	$19.4 billion	$7.8 billion
Exports	$10.8 billion	$4.3 billion

1. Maryland Port Authority 2010. 40% of Baltimore-bound shipping sails through C&D Canal.

Port Activity

For over 300 years since the time of William Penn, the Delaware River has been an economic engine that is now the largest freshwater port in the world. The Economy League of Greater Philadelphia (2008) concluded that Delaware River ports from Wilmington to Philadelphia to Trenton:

- Collectively is the largest freshwater port in the world with $2.4 billion in total economic output.
- Generate $81 million in tax revenues to Delaware, Pennsylvania, New Jersey (Table 42).
- Import 1/2 of the nation's cocoa beans, 1/3 of the bananas, and 1/4 of all fruit and nuts.
- Rank 5th among ports in the USA in import cargo value and 20th in export value.
- In Chester, Philadelphia, Wilmington, Camden and Paulsboro handled 16% of container trade in the U.S. and 51% of container trade value nationwide.
- Biggest commodity is petroleum that accounts for 65% of the port's imports while fruits and nuts account for 4%.

Table 42. Tax revenues from Delaware River ports, 2005
(Economy League of Greater Philadelphia 2008)

Type	DE	NJ	PA	Total
Individual Income Tax	$2,538,803	$6,679,380	$13,102,579	$22,320,762
Sales and Use Tax		5,326,255	13,851,735	$19,177,990
Corporate Income Tax	888,055	1,988,447	3,632,195	$6,508,697
Selective Tax	1,075,499	2,674,104	7,807,469	$11,557,072
Other State Tax, License, Fees	2,536,226	1,597,420	5,199,444	$9,333,090
Total State and Local Tax	**7,038,582**	**18,266,605**	**55,974,357**	**$81,279,544**

The Economy League reports that nearly 2,900 ships (8 per day) docked at Delaware River ports in 2006, up 10% from 1995. Most shipping traffic were tankers, containers, bulk, refrigerated (meat/fruits/vegetables) and auto transport vessels (Table 43).

Table 43. Delaware River port vessel calls, 1996-2000
(Economy League of Greater Philadelphia 2008)

Year	General	Container	Roll on	Refrg	Bulk	Tanker	Chem	Auto	Passengr	Total
1995	304	368	84	333	405	812	138	110	16	2,570
2006	248	581	78	373	402	861	144	121	39	2,847
change	-56	213	-6	40	-3	49	6	11	23	277
% change	-18%	-58%	-7%	12%	-1%	6%	4%	10%	144%	11%

Top Delaware River port exports (Table 44) are motor vehicles (31% and petroleum products (12%) and top imports are petroleum (65%) and iron and steel (7%).

Table 44. Top exports and imports at Delaware River ports (Economy League 2008)

Cargo	Exports	Imports
Motor Vehicles	31%	
Petroleum	12%	65%
Precious stones/Metals	7%	
Industrial Machinery	6%	2%
Plastics	6%	
Iron and Steel		7%
Fruits and Nuts		4%
Meat		3%

In 2005, Delaware River ports at Philadelphia, Chester, and Camden were the 6th, 35th, and 37th largest ports in the U.S. based on imports of goods and cargo (Table 45). The five ports along the Delaware River had combined imports of $41 billion, the 5th largest port in the U.S. after Los Angeles, Newark (NJ), Houston, and Long Beach (CA) and ahead of Seattle, Norfolk (VA), and Baltimore. The five ports along the Delaware had combined exports of $6.4 billion making it the 20th largest port in the USA after Oakland (CA) and Baltimore but ahead of Charleston (SC).

Table 45. Rank of Delaware River imports/exports in United States by value of goods, 2005

Imports Rank in U.S.	Port	Imports ($))
6	Philadelphia	$29,500,000,000
35	Chester	$5,700,000,000
37	Wilmington	$5,500,000,000
79	Paulsboro	$250,000,000
103	Camden	67,000,000
5	**Delaware R.**	**$41,017,000,000**
Exports Rank in U.S.	**Port**	**Exports ($)**
22	Philadelphia	$2,400,000,000
24	Wilmington	$2,200,000,000
32	Chester	$1,600,000,000
74	Camden, NJ	$150,000,000
84	Paulsboro, NJ	$89,000,000
20	**Delaware R.**	**$6,439,000,000**

4. Ecosystem Services

Other Studies

Data from the following studies were examined to estimate the value of ecosystem services in the Delaware River Basin in Delaware, New Jersey, New York, and Pennsylvania:

- Cecil County green infrastructure study by the Conservation Fund, Annapolis, Md (2007).
- New Jersey Department of Environmental Protection with the University of Vermont (2007)
- Ecosystem services value of forests by the Wilderness Society (2001)
- Ecosystem services value of Peconic Estuary watershed by University of Rhode Island (2002)
- U.S. National Wildlife Refuge System by Univ. of Maryland and Nature Conservancy (2008)
- Economic value of ecosystem services in Massachusetts by the Audubon Society (2003).

Ecosystem services include air filtration, water filtration, recycling nutrients, soil conservation, pollinating crops and plants, climate regulation, carbon sequestration, flood/stormwater control, and hydrologic cycle regulation. Ecological resources provide marketable goods and services such as timber, fish and wildlife recreation, hiking, and boating/kayaking. A Cecil County, Md. study by the Conservation Fund (Table 46) found the largest ecosystem services values result from stormwater/flood control, water supply, and clean water functions (Weber 2007).

Table 46. Ecosystem services values for Cecil County, Maryland (Weber 2007)

Ecosystem Service	Upland Forest ($/ac/yr)	Riparian Forest Wetlands ($/ac/yr)	Nonriparian Wetlands ($/ac/yr)	Tidal Marsh ($/ac/yr)
Carbon sequestration	31	65	65	65
Clean air	191	191	191	
Soil and peat formation	17	946	450	1,351
Stormwater/flood control	679	32,000	32,000	1,430
Water supply	8,630	8,630	8,630	
Clean water	1,100	1,925	1,100	11,000
Erosion/sediment control	151	3,418	151	12,700
Water temperature regulation		4,450		
Pest control	50	50	50	
Pollination	75	75	75	
Wood products	142			
Recreation, fish, wildlife habitat	486	534	534	544
Community services savings	439	439	439	439
Increase in property values	42	42		
Total	**12,033**	**52,765**	**43,685**	**28,146**

The New Jersey Department of Environmental Protection (2007) partnered with the University of Vermont and estimated the value of New Jersey's natural capital was $20 billion/year plus or minus

$9 billion/year in $2004 with a net present value of $681 billion based on a discount rate of 3% calculated in perpetuity (over 100 years in the future). Natural capital is the sum of goods (commodities like water, crops, and timber that can be sold) and services (functions like flood control, water filtration, and wildlife/fisheries habitat) provided by watershed ecosystems such as wetlands, forests, farms, and open water. In addition to these direct benefits, ecosystems also provide indirect benefits such as ecotourism by hunters, fishermen, boaters, and hikers who spend money to visit natural sites and realize value from improved water quality and habitat. Table 47 summarizes total ecosystem goods and services in New Jersey. Farm products, fish, minerals, and water supply provide the most ecosystem goods. Nutrient cycling, soil disturbance regulation, water regulation, habitat, aesthetic/recreational, waste treatment, and water supply provide the greatest ecosystem services.

Table 47. Ecosystem goods and services provided by New Jersey natural capital (NJDEP 2007)

Ecosystem	$ million/yr	%
Natural Goods	**$5,864**	**100%**
Farm products	3,676	63%
Commercial/recreational fish	958	16%
Minerals	587	10%
Raw Water	381	7%
Saw timber	147	3%
Fuelwood	95	2%
Game/fur animals	21	1%
Ecoservices	**$19,803**	**100%**
Nutrient cycling	5,074	26%
Disturbance regulation	3,383	17%
Water regulation	2,433	12%
Habitat	2,080	11%
Aesthetic/recreational	1,999	10%
Waste treatment	1,784	9%
Water supply	1,739	9%
Cultural//spiritual	778	4%
Gas/climate regulation	246	1%
Pollination	243	1%
Biological control	35	<1%
Soil formation	8	<1%

The Wilderness Society (Krieger 2001) concluded forest ecosystem services values from climate regulation, water supply, water quality, and recreation benefits totaled $392/ac in $1994 or $631/ac in $2010 at a 3% annual discount rate (Table 48).

Table 48. Forest ecosystem service values for U.S. temperate forests (Krieger 2001)

Ecosystem Good or Service	1994 Value ($/ac)	2010 Value[1] ($/ac)
Climate regulation	57.1	91.9
Disturbance regulation	0.8	1.3
Water regulation	0.8	1.3
Water supply	1.2	1.9
Erosion and sediment control	38.8	62.5
Soil formation	4.0	6.4
Nutrient cycling	146.1	235.2
Waste Treatment	35.2	56.7
Biological Control	0.8	1.3
Food Production	17.4	28.0
Raw Materials	55.8	89.8
Genetic Resources	6.5	10.5
Recreation	26.7	43.0
Cultural	0.8	1.3
Total	**392.1**	**631.3**

1. $2010 computed at 3% annually.

A contingent value study by University of Rhode Island economists found natural resources values in the Peconic Estuary watershed in Suffolk County on Long Island New York ranged from $6,560/ac for wetlands to $9,979/ac for farmland in $1995 (Johnston et al. 2002). The University of Maryland studied the National Wildlife Refuge System and determined ecosystem values of freshwater wetlands and forests were $6,268/ac and $845/ac, respectively (Ingraham and Foster 2008). The Audubon Society found the economic value of ecosystems in Massachusetts ranged from $984/ac for forests to $15,452/ac for saltwater wetlands (Breunig 2003).

According to the 2007 USDA Census of Agriculture (2009) the market value of agricultural crops, poultry, and livestock sold from 1,926,524 acres of farmland in the Delaware River Basin was $3.37 billion or $1,676/ac. The market value of agriculture from 254,143 acres of farmland in Delaware in the basin was $636 million or $2,502/ac. The market value of agriculture from 505,507 acres of farmland in New Jersey was $602 million or $1,192/ac. The market value of agriculture from 187,561 acres of farmland in New York in the basin was $105 million or $562/ac.
The market value of agriculture from 979,313 acres of farmland in Pennsylvania counties in the basin was $2.0 billion or $2,070/ac.

Table 49 compares ecosystem services values ($/acre) from other studies. Data from the NJDEP/University of Vermont study are used for value transfer since the Delaware Basin includes New Jersey ecosystems and two adjacent states in the watershed (Del. and Pa.) share a similar climate (humid continental) at 40 degrees north in latitude, similar physiographic provinces (Piedmont/Coastal Plain) and similar aquifers, soils, and ecosystems. Farmland natural goods values are estimated from market values from the 2007 USDA Census of Agriculture. Cecil County, Maryland occupies a small sliver of the Delaware Basin and utilized higher ecosystem values on a per acre basis for forests and wetlands than the other studies. The NJDEP ecosystem service estimates ($/ac) are lower than Cecil County values for wetlands/forests and Mass Audubon values for

wetlands but higher than Wilderness Society values for forests and U. S. Wildlife Refuge values for freshwater wetlands and forests. Values from previous studies were adjusted to $2010 based on 3% annually. Net present values were calculated based on an annual discount rate of 3% in perpetuity (over 100 years in the future).

Table 49. Comparison of ecosystem service value studies

Ecosystem	Cecil Co. Maryland 2006 ($/ac/yr)	New Jersey DEP 2004 ($/ac/yr)	Wilderness Society 2001 ($/ac/yr)	Peconic Estuary 1995 ($/ac/yr)	US Wildlife Refuge 2008 ($/ac/yr)	Mass Audubon 2003 ($/ac/yr)	USDA Census[1] 2007 ($/ac/yr)
Freshwater wetland	43,685	11,802			6,268	15,452	
Marine		8,670					
Farmland		6,229		9,979		1,387	1,676
Forest land	12,033	1,714	641		845	984	
Saltwater wetland	28,146	6,269		$6,560		12,580	
Undeveloped				$2,080			
Urban		296					
Beach/dune		42,149					
Open freshwater		1,686			217	983	
Riparian buffer	52,765	3,500					
Shellfish areas				$4,555			

1. Value of goods only as measured by agricultural crops, livestock, and poultry sold.

Delaware Basin

The estimated value of natural goods and services provided by ecosystems in the Delaware River Basin (12,742 sq mi) is $21 billion ($2010) with a net present value (NPV) of $683 billion (Table 50). The ecosystems services value of the Delaware portion of the Delaware Basin (965 sq mi) is $2.5 billion ($2010) with a NPV of $81.4 billion (Figure 13). The ecosystems services value of the New Jersey portion of the Delaware Basin (2,960 sq mi) is $6.6 billion ($2010) with a NPV of $213.4 billion. The ecosystems services value of the New York portion of the Delaware Basin (2,556 sq mi) is $3.5 billion ($2010) with a NPV of $113.6 billion. The ecosystems services value of the Pennsylvania portion of the basin (6,290 sq mi) is $8.6 billion ($2010) with a NPV of $279.6 billion. NPV is based on an annual discount rate of 3% over a perpetual life time (>100 years).

Natural goods are commodities that can be sold such as water supply, farm crops, fish, timber, and minerals). Natural services provide ecological benefits to society such as flood control by wetlands, water filtration by forests, and fishery habitat by beach and marine areas. Ecosystems within the Delaware Basin are comprised of forests (53%), farmland (24%), freshwater wetlands (5%), saltwater wetlands (2%), and open water/marine (1%). Over 15% of the Delaware Basin is urban (Figure 14).

Farms, freshwater wetlands, forests, and saltwater wetlands provide the highest total ecosystems goods and services values (Table 51 and Figures 15 and 16). Ecosystems that provided the highest natural good values are farmland ($3.2 billion or $1,676/ac/ yr), followed by forest ($1.2 billion or $275/ac), and freshwater wetlands ($114 million or $270/ac). The highest natural ecosystem services values are provided by forests ($7.4 billion or $1,703/ac) followed by freshwater wetlands

($5.6 billion or $13,351/ac), farmland ($1.6 billion or $827/ac), and saltwater wetlands ($1.0 billion or $7,076/ac).

The DB2 Delaware Bay ($2,497,635,761), UE2 New Jersey Coastal Plain ($2,093,235,974), DB1 Delaware Bay ($1,922,732,778), NM1 Neversink R. ($1,212,219,295), EW2 West Branch Del. R. ($1,137,547,038), UC1 Pocono Mt. ($1,106,108,992), UC2 NJ Highlands ($1,072,263,808), SV3 Schuylkill above Philadelphia ($1,098,758,690), and LW1 Lackawaxen R. ($1,006,865,455) watersheds each provide over $1 billion in annual ecosystem services value (Table 52 and Figure 17).

Watersheds with the highest value of annual ecosystem services per acre include the DB2 Delaware Bay ($4,991/ac), DB1 Delaware Bay ($4,797/ac), LE3 Salem River ($4,288/ac), LE2 C&D Canal ($3,941/ac), UE2 New Jersey Coastal Plain ($3,205/ac), LW1 Lackawaxen R. ($2,631/ac), NM1 Neversink R. ($2,321/ac), SV2 Schuylkill above Valley Forge ($2,276/ac), and LV1 Lehigh River above Lehighton ($2,263/ac) as these systems have high amounts (over 75%).of forests, wetlands, and farm habitat (Figure 18).

The above estimates do not include the ecosystem services value of open water (720 sq mi) in the tidal Delaware River and Bay between the shores of Delaware, Pennsylvania, and New Jersey. The ecosystem services value of open water habitat in the river and bay is $61 billion or $1,946/ac.

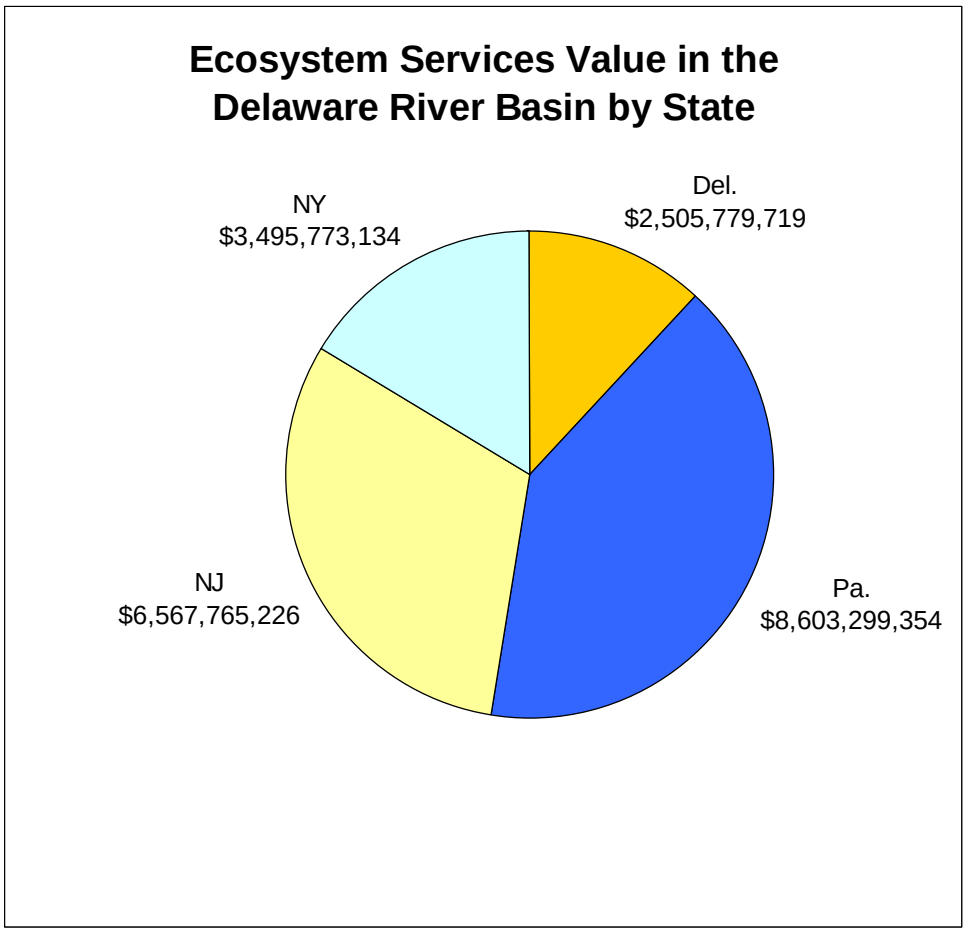


Figure 13. Ecosystem service value in the Delaware Basin by state

Table 50. Ecosystem services values in the Delaware River Basin by state

Ecosystem	Area (ac)	$/ac/yr 2010	PV 2010 $	NPV $
Delaware Basin				
Freshwater wetlands	422,838	13,621	5,759,329,048	187,178,194,067
Marine	16,588	10,006	165,982,947	5,394,445,767
Farmland	1,926,524	2,503	4,823,030,404	156,748,488,136
Forest land	4,343,190	1,978	8,591,367,360	279,219,439,184
Saltwater wetland	145,765	7,235	1,054,617,851	34,275,080,170
Urban	1,206,504	342	412,157,579	13,395,121,322
Beach/dune	900	48,644	43,758,633	1,422,155,566
Open water	92,615	1,946	180,210,703	5,856,847,857
Total	**8,154,924**		**$21,030,454,525**	**$683,489,772,069**
Delaware				
Freshwater wetlands	58,390	13,621	795,317,362	25,847,814,257
Marine	16,274	10,006	162,840,906	5,292,329,460
Farmland	254,143	3,329	846,164,877	27,500,358,509
Forest land	95,346	1,978	188,605,634	6,129,683,090
Saltwater wetland	61,617	7,235	445,802,585	14,488,584,028
Urban	123,048	342	42,034,778	1,366,130,274
Beach/dune	256	48,644	12,429,832	403,969,529
Open water	6,467	1,946	12,583,745	408,971,719
Total	**615,541**		**$2,505,779,719**	**$81,437,840,867**
New Jersey				
Freshwater wetlands	246,857	13,621	3,362,352,134	109,276,444,364
Marine	314	10,006	3,142,040	102,116,307
Farmland	505,507	2,019	1,020,866,015	33,178,145,495
Forest land	682,931	1,978	1,350,922,709	43,904,988,032
Saltwater wetland	83,563	7,235	604,583,594	19,648,966,813
Urban	321,090	342	109,688,612	3,564,879,893
Beach/dune	499	48,644	24,253,858	788,250,378
Open water	47,259	1,946	91,956,264	2,988,578,571
Total	**1,888,020**		**6,567,765,226**	**213,452,369,853**
New York				
Freshwater wetlands	34,792	13,621	473,886,107	15,401,298,475
Marine	0	10,006	0	0
Farmland	187,561	1,389	260,613,634	8,469,943,113
Forest land	1,387,514	1,978	2,744,673,732	89,201,896,298
Saltwater wetland	0	7,235	0	0
Urban	20,806	342	7,107,761	231,002,225
Beach/dune	0	48,644	0	0
Open water	4,878	1,946	9,491,900	308,486,749
Totalac	**1,635,551**		**3,495,773,134**	**113,612,626,859**
Pennsylvania				
Freshwater wetlands	82,799	13,621	1,127,773,445	36,652,636,971
Marine	0	10,006	0	0
Farmland	979,313	2,897	2,837,548,786	92,220,335,530
Forest land	2,177,399	1,978	4,307,165,285	139,982,871,763
Saltwater wetland	585	7,235	4,231,672	137,529,329
Urban	741,560	342	253,326,429	8,233,108,930
Beach/dune	145	48,644	7,074,943	229,935,659
Open freshwater	34,011	1,946	66,178,794	2,150,810,818
Total	**4,015,812**		**8,603,299,354**	**279,607,229,001**

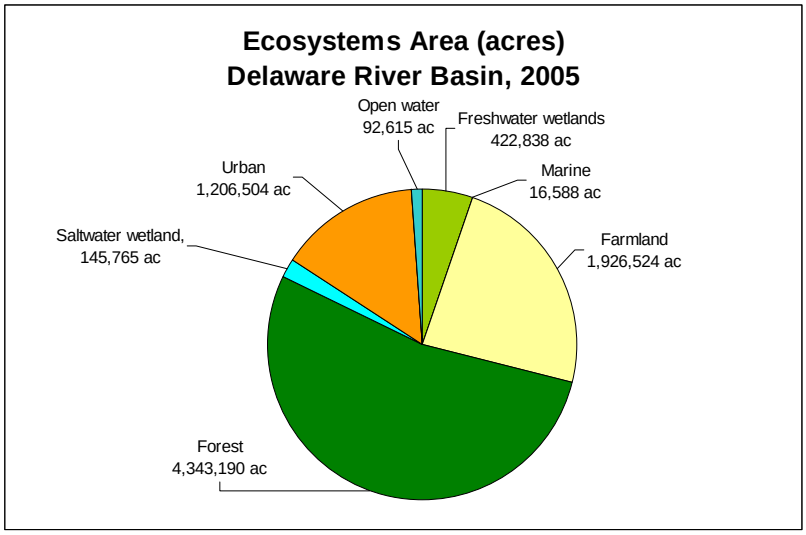


Figure 14. Ecosystem service areas within the Delaware River Basin

Table 51. Value of ecosystem goods and services in the Delaware River Basin

Natural Goods						
Ecosystem	**Area (ac)**	**$/ac/yr 2004**	**$/yr 2004**	**$/ac/yr 2010**	**$/yr 2010**	**NPV $**
Freshwater wetlands	422,838	234	98,943,997	270	114,191,069	3,711,209,745
Marine	16,588	1,125	18,661,829	1,298	21,537,580	699,971,336
Farmland	1,926,524	1,676	3,228,854,342	1,676	3,228,854,342	104,937,766,110
Forest land	4,343,190	238	1,033,679,112	275	1,192,966,996	38,771,427,378
Saltwater wetland	145,765	139	20,261,377	160	23,383,615	759,967,482
Urban	1,206,504	13	15,684,557	15	18,101,515	588,299,247
Beach/dune	900	0	0	0	0	0
Open water	92,615	921	85,298,217	1,063	98,442,502	3,199,381,302
Total	**8,154,924**		**4,501,383,431**		**4,697,477,618**	**152,668,022,601**
Natural Services						
Ecosystem	**Area (ac)**	**$/ac/yr 2004**	**$/yr 2004**	**$/ac/yr 2010**	**$/yr 2010**	**NPV $**
Freshwater wetlands	422,838	11,568	4,891,385,289	13,351	5,645,137,979	183,466,984,322
Marine	16,588	7,544	125,142,079	8,707	144,426,223	4,693,852,233
Farmland	1,926,524	717	1,381,317,758	827	1,594,176,062	51,810,722,026
Forest land	4,343,190	1,476	6,410,547,773	1,703	7,398,400,363	240,448,011,806
Saltwater wetland	145,765	6,131	893,687,073	7,076	1,031,402,464	33,520,580,080
Urban	1,206,504	283	341,440,730	327	394,056,064	12,806,822,075
Beach/dune	900	42,149	37,915,873	48,644	43,758,633	1,422,155,566
Open water	92,615	765	70,850,311	883	81,768,202	2,657,466,554
Total	**8,154,924**		**14,152,286,885**		**16,333,125,990**	**530,826,594,663**
Goods & Services						
Ecosystem	**Area (ac)**	**$/ac/yr 2004**	**$/yr 2004**	**$/ac/yr 2010**	**$/yr 2010**	**NPV $**
Freshwater wetlands	422,838	11,802	4,990,329,286	13,621	5,759,329,048	187,178,194,067
Marine	16,588	8,670	143,820,496	10,006	165,982,947	5,394,445,767
Farmland	1,926,524	2,503	4,823,030,404	2,503	4,823,030,404	156,748,488,136
Forest land	4,343,190	1,714	7,444,226,885	1,978	8,591,367,360	279,219,439,184
Saltwater wetland	145,765	6,269	913,802,685	7,235	1,054,617,851	34,275,080,170
Urban	1,206,504	296	357,125,287	342	412,157,579	13,395,121,322
Beach/dune	900	42,149	37,915,873	48,644	43,758,633	1,422,155,566
Open water	92,615	1,686	156,148,527	1,946	180,210,703	5,856,847,857
Total	**8,154,924**		**18,866,399,443**		**21,030,454,525**	**683,489,772,069**

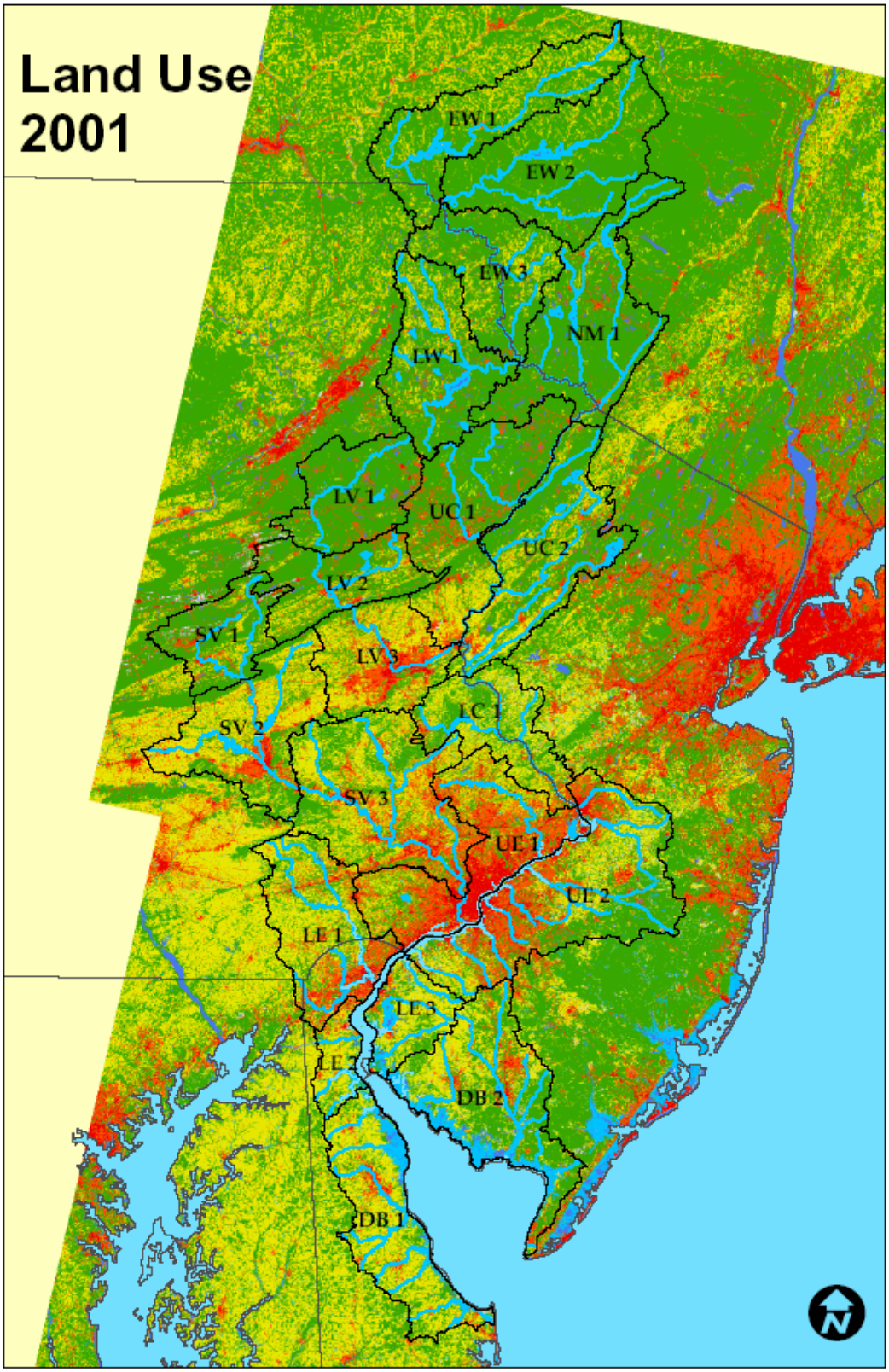


Figure 15. Land cover in the Delaware River Basin
(NOAA CSC 2001)

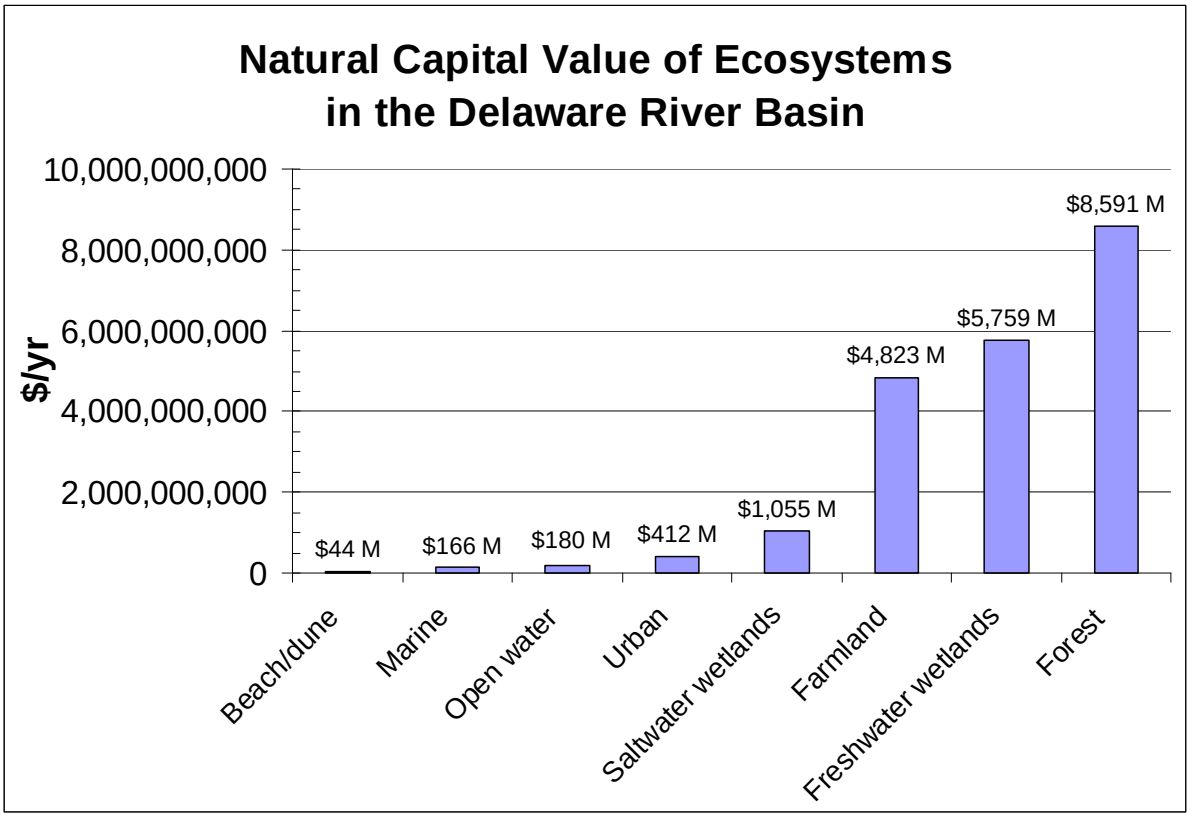


Figure 16. Ecosystem service value ($2010) of habitat within the Delaware River Basin

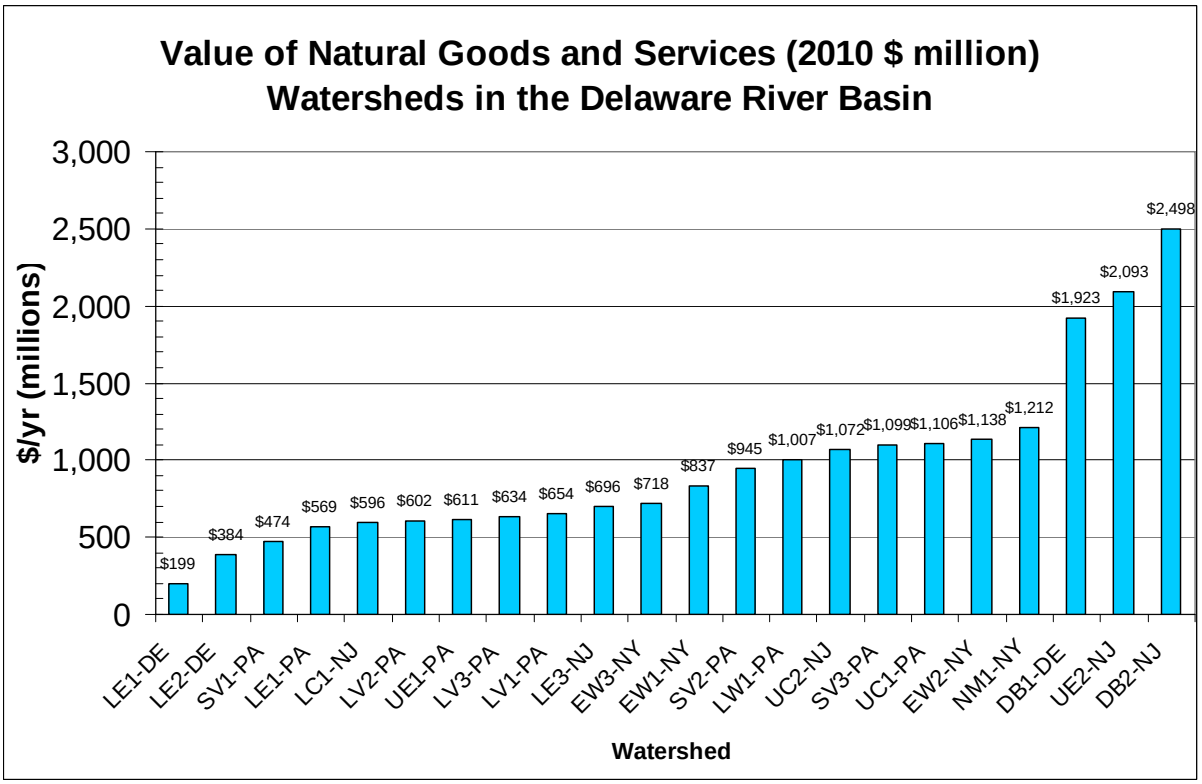


Figure 17. Ecosystem services values of watersheds within the Delaware River Basin

Table 52. Ecosystem services value of watersheds in the Delaware River Basin

Watershed	Area (sq mi)	2010 $/yr	2010 $/ac/yr
LE1 Brandywine/Christina	187	199,035,649	1,664
LE2 C&D Canal	152	384,011,292	3,941
DB1 Delaware Bay	626	1,922,732,778	4,797
Delaware	**962**	**2,505,779,719**	**4,071**
UC2 NJ Highlands	745	1,072,263,808	2,248
LC1 Del. R. above Trenton	159	208,902,978	2,053
UE2 New Jersey Coastal Plain	1,021	2,093,235,974	3,205
LE3 Salem River	254	695,858,091	4,288
DB2 Delaware Bay	782	2,497,635,761	4,991
New Jersey	**2,950**	**6,567,765,226**	**3,479**
EW1 East Branch Del. R.	666	836,579,484	1,963
EW2 West Branch Del. R.	841	1,137,547,038	2,114
EW3 Del. R. above Pt. Jervis	314	430,101,000	2,142
NM1 Neversink R.	734	1,076,794,000	2,321
New York	**2,556**	**3,495,773,134**	**2,137**
EW3 Del. R. above Pt. Jervis	210	287,647,100	2,142
NM1 Neversink R.	82	135,425,000	2,321
LW1 Lackawaxen R.	598	1,006,865,455	2,631
UC1 Pocono Mt.	779	1,106,108,992	2,219
LV1 Lehigh River above Lehighton	451	653,896,676	2,263
LV2 Lehigh River above Jim Thorpe	430	601,508,831	2,183
LV3 Lehigh River above Bethlehem	480	633,649,592	2,064
LC1 Del. R. above Trenton	295	387,587,286	2,053
SV1 Schuylkill above Reading	348	474,099,567	2,126
SV2 Schuylkill above Valley Forge	649	945,100,081	2,276
SV3 Schuylkill above Philadelphia	874	1,098,758,690	1,965
UE1 Penna Fall Line	693	611,041,618	1,377
LE1 Brandywine/Christina	401	568,524,810	2,216
Pennsylvania	**6,275**	**8,603,299,354**	**2,142**
Delaware Basin	**12,742**	**21,030,454,525**	**2,579**

Estimates of ecosystem services in the Delaware River Basin using the NJDEP/University of Vermont values coupled with market values from the USGS Census of Agriculture ($21.0 billion or $683.5 billion NPV) are conservative and in the lower end of the range. If lower per acre estimates of ecosystem services value from other studies were used instead of the NJDEP values, the total value of natural resources in the Delaware Basin would be $9.6 billion or NPV = $311 billion (Table 53). If higher per acre estimates of ecosystem services value from other studies were used, the total value of natural resources in the Delaware Basin would be $94.7 billion or NPV = $3.1 trillion (Table 54).

Estimate	PV $B	NPV $B
Low	9.6	311
NJDEP/USDA	21.0	683
High	94.7	3,100

Table 53. Low range estimate of ecosystem services in the Delaware River Basin

Ecosystem	Area (ac)	$/ac/yr	PV $	NPV $
Freshwater wetlands	422,838	6,268[5]	2,650,346,040	86,136,246,300
Marine	16,588	8,670[2]	143,820,496	4,674,166,116
Farmland	1,926,524	1,387[6]	2,672,088,886	86,842,888,779
Forest land	4,343,190	641[3]	2,783,984,500	90,479,496,255
Saltwater wetland	145,765	6,269[2]	913,802,685	29,698,587,269
Barren land	18,630	0	0	0
Urban	1,206,504	296[2]	357,125,287	11,606,571,818
Beach/dune	900	42,149[2]	37,915,873	1,232,265,862
Open water	92,615	217[5]	20,097,408	653,165,771
Total acres	**8,173,554**		**9,579,181,174**	**311,323,388,171**
sq mi	**12,771**			

Table 54. High range estimate of ecosystem services in the Delaware River Basin

Ecosystem	Area (ac)	$/ac/yr	PV $	NPV $
Freshwater wetlands	422,838	43,685[1]	18,471,660,300	600,328,959,736
Marine	16,588	8,670[2]	143,820,496	4,674,166,116
Farmland	1,926,524	9,979[4]	19,224,783,698	624,805,470,173
Forest land	4,343,190	12,033[1]	52,261,599,829	1,698,501,994,444
Saltwater wetland	145,765	28,146[1]	4,102,710,221	133,338,082,193
Barren land	18,630	0	0	0
Urban	1,206,504	296[2]	357,125,287	11,606,571,818
Beach/dune	900	42,149[2]	37,915,873	1,232,265,862
Open water	92,615	1,686[2]	156,148,527	5,074,827,144
Total acres	**8,173,554**		**94,755,764,230**	**3,079,562,337,486**
sq mi	**12,771**			

1. Cecil Co., Md. 2006. 2. NJDEP 2007. 3. Wilderness Society 2001. 4. Peconic Estuary 1995. 5. U. S. Nat'l. Wildlife Refuge 2008. 6. Mass Audubon Society 2003. 7. USDA Agric. Census 2007.

5. Jobs and Wages

The Delaware River Basin is a jobs engine that supports 600,000 direct and indirect jobs with $10 billion in annual wages in the coastal, farm, ecotourism, water/wastewater, recreation, and port industries (Table 55).

Table 55. Direct and indirect jobs and wages related to the Delaware River Basin

Sector	Jobs	Wages ($ million)	Data Source
Direct Basin Related	240,621	4,900	U.S. Bureau of Labor Statistics, 2009
Indirect Basin Related	288,745	4,000	U.S. Census Bureau, 2009
Coastal	44,658	947	National Coastal Economics Program, 2009
Farm	45,865	1,880	USDA Census of Agriculture, 2007
Fishing/Hunting/Birding	44,941	1,476	U.S. Fish and Wildlife Service, 2008
Water Supply Utilities	8,750	485	UDWRA and DRBC, 2010
Wastewater Utilities	1,298	61	UDWRA and DRBC, 2010
Watershed Organizations	201	10	UDWRA and DRBC, 2010
Ski Area Jobs	1,753	$88	Penna. Ski Areas Association
Paddling-based Recreation	4,226		Outdoor Industry Association (2006
River Recreation	448	$9	U. S. Forest Service/Nat'l. Park Service, 1990
Canoe/Kayak/Rafting	225		Canoe Liveries and UDWRA, 2010
Wild Trout Fishing	350	$4	Maharaj, McGurrin, and Carpenter, 1998
Del. Water Gap Nat'l. Rec. Area	7,563	101	Stynes and Sun, 2002
Port Jobs	12,121	772	Economy League of Greater Phila., 2008
Delaware Basin Total	**> 600,000**	**>$10 billion**	

Jobs and salaries in the Delaware Basin were obtained from U. S. Bureau of Labor Statistics (2009) and U. S. Census Bureau (2009) data bases for the following scenarios (Tables 56-58):

1. Total number of jobs in each county within the Delaware Basin with jobs determined by NAICS industry code (formerly SIC code) and then grouped by census tract.
2. Direct Delaware Basin-related jobs such as water and sewer construction, living resources, maritime, tourism/recreation, ports, environmental services, and water/wastewater management determined for each NAICS code by state and county within the basin boundary.
3. Indirect jobs/wages provided by purchases of goods and services by direct jobs earners within the Delaware Basin in the interlinked regional economy. Indirect jobs were estimated by a multiplier of 2.2 applied to direct jobs and 1.8 to direct wages (Latham and Stapleford 1990), i.e., 100 direct jobs fund 120 indirect jobs and direct wages of $1,000 provide $800 indirect wages.

Within the Delaware Basin are 3,480,483 jobs earning $172.6 billion in annual wages including:

- Delaware (316,014 jobs, $16.5 billion wages)
- New Jersey (823,294 jobs, $38.1 billion wages)
- New York (69,858 jobs, $2.5 billion wages)
- Pennsylvania (2,271,317 jobs, $115.5 billion wages)

Jobs directly associated with the Delaware River Basin (such as water/sewer construction, water utilities, fishing, recreation, tourism, and ports) employ 240,621 with $4.9 billion in wages including:

- Delaware (15,737 jobs, $340 million wages)
- New Jersey (62,349 jobs, $1.3 billion wages)
- New York (32,171 jobs, $550 million wages)
- Pennsylvania (130,364 jobs, $2.8 billion wages)

Jobs indirectly related to the waters of the Delaware Basin (based on multipliers of 2.2 for jobs and 1.8 for salaries) employ 288,745 people with $4.0 billion in wages including:

- Delaware (18,884 jobs, $270 million wages)
- New Jersey (74,819 jobs, $1.0 billion in wages)
- New York (38,605 jobs, $400 million in wages)
- Pennsylvania (156,437 jobs, $2.2 billion in wages)

National Coastal Economy Report

The National Ocean Economic Program (2009) published a report that summarized the coastal economy in the United States that includes 6 industrial sectors:

- Marine Transportation
- Tourism and Recreation
- Living Marine Resources
- Marine Construction
- Ship and Boat Building
- Mineral Extraction.

According to the National Ocean Economic program (2009), the coastal counties within the Delaware Basin boundary contribute 44,658 coastal jobs with $947 million in annual wages with contributions of $1.8 billion toward the GDP. Table 59 summarizes employment, wages, and employment within the Delaware Basin obtained by multiplying the 2009 NOEP report county-wide values by the ratios of coastal county area within the basin by total coastal county area within the state which are 80% for Delaware, 5% for New Jersey and 86% for Pennsylvania. Using these ratios, 80%, 5%, and 86% of the employment and wages for coastal counties in Delaware, New Jersey, and Pennsylvania from the NOEP report are within the Delaware Basin boundary.

Table 56. Direct basin-related jobs within the Delaware River Basin by state, 2009

Sector	Industry	1997 NAICS Code	DE Jobs	DE Wages x$1,000	NJ Jobs	NJ Wages $1,000	NY Jobs	NY Wages $1,000	PA Jobs	PA Wages x$1,000
Construction	Marine Related	237120			81	4,532			923	58,999
	Water and Sewer	23711	529	21,838	2,485	109,527	551	36,387	3,138	211,691
	Construction	237990	126	5,678	318	19,547			306	16,427
Living Resources	Fish Hatcheries	112511								
	Aquaculture	112512								
	Fishing/Forestry	11411			50	2,028	21	424	67	2,485
	Finfish Fishing	114111			111	5,591				
	Shellfish Fishing	114112			28	995				
	Seafood Markets	445220	39	1,447	81	1,550			283	6,348
	Seafood Process.	31171			97	6,734				
	Comm. Fisheries		0	0	0	0			0	0
Minerals	Sand & Gravel	212321			166	8,109				
		212322	0	0	81	3,865				
	Oil & Gas	541360	16	752					39	3,802
Ship/Boat Building	Boat Bldg. Repair	336612								
	Ship Bldg. Repair	336611								
	Shipbuilding		0	0	0	0			0	0
Tourism/Recreation	Recreation	487990			52	1,184				
		611620	64	513	305	5,301			675	12,270
		532292			50	774				
	Amusement	713990	250	4,102	2,426	35,967	11,537	162,246	2,008	31,251
	Misc. Recreation				0	0	1,100	16,574	0	0
	Boat Dealers	441222	198	7,489	157	5,945				
	Restaurants	722110	3,714	173,787	26,512	415,604	17,029	264,832	59,217	974,264
		722211	6,797	4,102	14,697	190,314			31,766	422,438
		722212	265	3,876	312	4,717			1,138	18,281
		722213	942	13,509	2,388	32,495			7,628	119,695
	Hotels & Lodging	721110	650	11,673	2,323	52,310			6,965	243,253
		721191			92	1,583				
	Marinas	713930			202	6,410				
	RV Park/Camps	721211	105	3,611	339	11,894			39	494
	Scenic Tours	487210	18	393	37	748				
	Sporting Good	339920	0	0	245	5,287	702	9,972	245	3,780
	Zoos, Aquaria	712130							55	1,959
		712190			58	3,411			466	28,459
Transportation	Deep Sea Freight	483111								
	Marine Transport.	483112	954	32,378	1,823	71,222			904	43,155
	Search/Navigation	334511	39	2,856					716	61,370
	Warehousing	493110	313	13,739	2,396	95,952			8,477	336,427
		493120			361	14,120			337	14,571
	Ports		0	0	0	0			0	0
	Dredging/Disposal		0	0	0	0			0	0
Education/Research	Environ.organizations	813312	83	2,976	61	2268	103	1,221	682	23,574
	Environ. consulting	54162	205	10,745	1,193	61,107	133	7,700	1,441	895
Water/Wastewater	Water/sewage systms	2213	267	20,004	679	8,169	23	1,101	203	774
	Waste management	562	146	6,028	1,928	92,495	882	41,649	2,372	113,437
	Septic tank services	562991	17	644	215	10,381	90	4,173	274	10,145
Total			**15,737**	**342,140**	**62,349**	**1,292,136**	**32,171**	**546,279**	**130,364**	**2,760,244**

Table 57. Jobs and wages directly and indirectly related to the Delaware River Basin, 2009

State/County	(1) Total Jobs	(2) Basin Jobs	(3) Direct Jobs	(4) Indirect Jobs	(1) Total Wages $ billion	(2) Basin Wages $ billion	(3) Direct Wages $ billion	(4) Indirect Wages $ billion
Delaware	**390,900**	**316,014**	**15,737**	**18,884**	**19.5**	**16.5**	**0.34**	**0.27**
Kent	60,100	50,412			2.4	2.0		
New Castle	264,600	252,534			14.7	14.1		
Sussex	66,200	13,068			2.4	0.5		
New Jersey	**1,362,200**	**823,294**	**62,349**	**74,819**	**61.6**	**38.1**	**1.3**	**1.0**
Burlington	194,500	187,758			9.1	8.8		
Camden	196,800	169,909			8.7	7.5		
Cape May	47,500	14,545			1.4	0.4		
Cumberland	62,000	61,868			2.5	2.5		
Gloucester	99,100	89,183			3.9	3.6		
Hunterdon	47,300	23,650			2.8	1.4		
Mercer	222,900	178,320			12.4	9.9		
Monmouth	246,600	9,864			11.4	0.5		
Ocean	149,900	7,495			5.5	0.3		
Salem	21,900	21,900			1.0	1.0		
Sussex	38,200	23,302			1.4	0.9		
Warren	35,500	35,500			1.5	1.5		
New York	**341,300**	**69,858**	**32,171**	**38,605**	**12.8**	**2.5**	**0.55**	**0.4**
Broome	94,100	11,292			3.4	0.4		
Delaware	16,000	14,240			0.6	0.5		
Greene	14,300	572			0.5	19.9		
Orange	130,700	10,456			5.2	0.4		
Sullivan	26,300	25,511			0.9	0.9		
Ulster	59,900	7,787			2.2	0.3		
Pennsylvania	**2,596,260**	**2,271,317**	**130,364**	**156,437**	**126.5**	**115.5**	**2.8**	**2.2**
Berks	159,106	150,665			6.2	5.9		
Bucks	244,453	244,453			10.6	10.6		
Carbon	16,730	16,730			0.5	0.5		
Chester	231,368	212,996			13.6	12.5		
Delaware	201,208	201,208			10.1	10.1		
Lackawanna	96,604	4,830			3.2	0.2		
Lebanon	45,826	2,750			1.5	0.1		
Lehigh	166,932	166,932			7.4	7.4		
Luzerne	134,574	8,074			4.6	0.3		
Monroe	56,025	56,025			2.1	2.1		
Montgomery	453,962	453,771			27.7	27.7		
Northampton	96,536	96,536			3.8	3.8		
Philadelphia	619,396	619,396			33.3	33.3		
Pike	9,874	9,874			0.3	0.3		
Schuylkill	49,116	27,077			1.6	0.9		
Wayne	14,550	14,114			0.5	0.4		
Delaware Basin	**4,690,660**	**3,480,483**	**240,621**	**288,745**	**220.3**	**172.6**	**4.9**	**4.0**

Table 58. Direct basin-related and indirect jobs within the Delaware River Basin, 2009

Sector	Industry	1997 NAICS Codes	Direct Jobs	Direct Wages (x$1,000)	Indirect Jobs[1]	Indirect Wages[2] (x$1,000)
Construction	Marine Related	237120	1,004	63,531	1,205	50,825
	Water and Sewer	23711	6,703	379,443	8,044	303,554
	Construction	237990	750	41,652	900	33,322
Living Resources	Fish Hatcheries	112511	0	0	0	0
	Aquaculture	112512	0	0	0	0
	Fishing/Foresty	11411	138	4,937	166	3,950
	Finfish Fishing	114111	111	5,591	133	4,473
	Shellfish Fishing	114112	28	995	34	796
	Seafood Markets	445220	403	9,345	484	7,476
	Seafood Process.	31171	97	6,734	116	5,387
	Comm. Fisheries		0	0	0	0
Minerals	Sand & Gravel	212321	166	8,109	199	6,487
		212322	81	3,865	97	3,092
	Oil & Gas	541360	55	4,554	66	3,643
Ship/Boat Building	Boat Bldg. Repair	336612	0	0	0	0
	Shipbuilding		0	0	0	0
Tourism/Recreation	Recreation	487990	52	1,184	62	947
		611620	1,044	18,084	1,253	14,467
		532292	50	774	60	619
	Amusement	713990	16,221	233,566	19,465	186,853
	Misc. Recreation		1,100	16,574	1,320	13,259
	Boat Dealers	441222	355	13,434	426	10,747
	Restaurants	722110	106,472	1,828,487	127,766	1,462,790
		722211	53,260	616,854	63,912	493,483
		722212	1,715	26,874	2,058	21,499
		722213	10,958	165,699	13,150	132,559
	Hotels & Lodging	721110	9,938	307,236	11,926	245,789
		721191	92	1,583	110	1,266
	Marinas	713930	202	6,410	242	5,128
	RV Park/Camps	721211	483	15,999	580	12,799
	Scenic Tours	487210	55	1,141	66	913
	Sporting Good	339920	1,192	19,039	1,430	15,231
	Zoos, Aquaria	712130	55	1,959	66	1,567
		712190	524	31,870	629	25,496
Transportation	Deep Sea Freight	483111	0	0	0	0
	Marine Transport.	483112	3,681	146,755	4,417	117,404
	Search/Navigation	334511	755	64,226	906	51,381
	Warehousing	493110	11,186	446,118	13,423	356,894
		493120	698	28,691	838	22,953
	Ports		0	0	0	0
	Dredging/Disposal		0	0	0	0
Education/Research	Environ.organizations	813312	929	30,039	1,115	24,032
	Environ. consulting	54162	2,972	80,447	3,566	64,357
Water/Wastewater	Water/sewage systms	2213	1,172	30,048	1,406	24,038
	Waste management	562	5,328	253,609	6,394	202,887
	Septic tank services	562991	596	25,343	715	20,275
Total			**240,621**	**4,940,799**	**288,745**	**3,952,639**

1. Direct jobs are directly related to the Delaware Basin. 2. Indirect jobs/salaries are derived from purchases of goods and services calculated by multipliers of 2.2 for jobs and 1.8 for wages.

Table 59. Coastal employment, wages, and GDP within the Delaware River Basin (National Ocean Economic Program 2009)

Sector	Employment	Wages ($ million)	GDP ($ million)
Delaware	**12,139**	**$214**	**$392**
Marine Construction			
Living Resources	354	$8	$15
Offshore Minerals			
Tourism & Recreation	10,398	$151	$299
Marine Transportation	1,744	$53	$72
Ship and Boat Building			
New Jersey	**4,423**	**$140**	**$235**
Marine Construction			$9
Living Resources			$7
Offshore Minerals			$1
Tourism & Recreation	2,939		$110
Marine Transportation			$104
Ship and Boat Building			$4
Pennsylvania	**28,096**	**$593**	**$1,204**
Marine Construction			$4
Living Resources			$172
Offshore Minerals			$13
Tourism & Recreation	**20,093**		$538
Marine Transportation			$383
Ship and Boat Building			$68
Delaware Basin	**44,658**	**$947**	**$1,831**
Marine Construction			$12
Living Resources	354	$8	$195
Offshore Minerals			$14
Tourism & Recreation	33,430	$151	$947
Marine Transportation	1,744	$53	$560
Ship and Boat Building			$72

Farm Jobs

In 2007 there were 30,455 farms in Delaware Basin counties or 21,840 farms within the basin boundary (30,455 x 0.67 = 21,840). The USDA estimates each farm employs 2.1 full time equivalent jobs. Farming provides 45,865 jobs with $1.9 billion in wages in the Delaware Basin (Table 60).

Table 60. Farm jobs in the Delaware River Basin

County	Farmland by County[1] (ac)	Farmland in Del. Basin (ac)	Ratio Farmland County/Basin	Farms in County[1]	No. of Farms in Basin	Farm jobs in Basin (2.1 jobs/farm)
New Castle	51,913			825		
Kent	146,536			347		
Sussex	234,324			1,374		
Delaware	**432,773**	**254,143**	**59%**	**2,546**	**1,495**	**3,140**
Burlington	85,790			922		
Camden	8,760			225		
Cape May	7,976			201		
Cumberland	69,489			615		
Gloucester	46,662			669		
Hunterdon	100,027			1,623		
Mercer	21,736			311		
Monmouth	44,130			932		
Ocean	9,833			255		
Salem	96,530			759		
Sussex	65,242			1,060		
Warren	74,975			933		
New Jersey	**631,150**	**505,507**	**80%**	**8,505**	**6,812**	**14,305**
Broome	86,613			580		
Delaware	165,572			747		
Greene	44,328			286		
Orange	80,990			642		
Sullivan	50,443			323		
Ulster	75,205			501		
New York	**503,151**	**187,561**	**37%**	**3,079**	**1,148**	**2,410**
Berks	170,760			1,980		
Bucks	58,012			934		
Carbon	20,035			207		
Chester	117,145			1,733		
Delaware	1,646			79		
Lackawanna	39,756			417		
Lancaster	326,648			5,462		
Lebanon	89,566			1,193		
Lehigh	72,737			516		
Luzerne	66,577			610		
Monroe	29,165			349		
Montgomery	28,563			719		
Northampton	68,252			486		
Philadelphia	150			17		
Pike	27,569			54		
Schuylkill	81,276			966		
Wayne	92,939			603		
Pennsylvania	**1,290,796**	**979,313**	**76%**	**16,325**	**12,386**	**26,010**
Total	**2,857,870**	**1,926,524**	**67%**	**30,455**	**21,840**	**45,865**

Census of Agriculture 2007 (USDA 2009)

Fishing/Hunting/Bird and Wildlife Recreation Jobs

The 2007 NJDEP study estimates the average annual salary per ecotourism job is $32,843 using figures from the U.S. Fish and Wildlife Service (2001) report on fishing, hunting, and wildlife associated recreation. If fishing, hunting, and bird/wildlife associated recreation in the Delaware

River Basin accounts for $1.5 billion in annual economic activity ($2006), then ecotourism provides for 44,941 jobs (Table 61).

Table 61. Jobs from fishing, hunting, and wildlife recreation in the Delaware River Basin

Recreation Activity[1]	DE in Basin[2] (2006 $M)	NJ in Basin[2] (2006 $M)	NY in Basin[2] (2006 $M)	PA in Basin[2] (2006 $M)	Delaware Basin (2006 $M)
Fishing	48	301	46	181	576
Hunting	21	58	36	225	340
Wildlife/Bird-watching	65	215	78	202	560
Total	**134**	**574**	**160**	**608**	**1,476**
	DE Jobs @ $32,843	**NJ Jobs @** $32,843	**NY Jobs @** $32,843	**PA Jobs @** $32,843	**Del. Basin Jobs @** $32,843
Fishing	1,461	9,165	1,401	5,511	17,538
Hunting	639	1,766	1,096	6,851	10,352
Wildlife/Bird-watching	1,979	6,546	2,375	6,150	17,051
Total	**4,080**	**17,477**	**4,872**	**18,512**	**44,941**

1. (USFWS 2008). 2. Prorated by ratio of basin area within state to state land area: Delaware (50%), New Jersey (40%), New York (5%) and Pennsylvania (14%).

Water Utility Jobs

Over 300 public and private water utilities (including the City of New York with 5,600 employees and the City of Philadelphia with over 800 water system employees) withdraw up to 1,800 mgd of drinking water from surface water and groundwater supplies in the Delaware River Basin. According to the American Water Works Association, the average salary of a water system employee is $55,407. Therefore, water utilities in the Delaware River Basin employ at least 8,750 jobs with annual wages of $485 million (Table 62).

Wastewater Utility Jobs

Over 60 wastewater utilities discharge almost 1.2 billion gallons per day of treated wastewater to the Delaware River Basin. These wastewater utilities employ 1,298 employees who earn $61 million in annual wages (Table 63).

Table 62. Public water supply jobs in the Delaware River Basin (DRBC and UDWRA 2010)

Water Purveyor	Jobs	Salaries
Delaware	**141**	**7,812,387**
United Water Delaware	55	3,047,385
City of Wilmington	31	1,717,617
City of Dover	14	775,698
City of Newark	7	387,849
City of Milford	6	332,442
Lewes Board of Public Works	5	277,035
Tidewater Utilities	5	277,035
Dover Air Force Base	1	55,407
New Castle Mun. Services Comm.	1	55,407
Town of Smyrna	1	55,407
Harrington	1	55,407
Camden-Wyoming Water Authority	1	55,407
Town of Milton	1	55,407
Other	12	664,884
New Jersey	**823**	**45,599,961**
Delaware and Raritan Canal	123	6,815,061
NJ American Water Co.	118	6,538,026
City of Trenton	78	4,321,746
City of Camden	33	1,828,431
City of Vineland	25	1,385,175
Aqua New Jersey	31	1,717,617
Merchantville-Pennsauken Water	18	997,326
Washington Twp. MUA	14	775,698
Willingboro Twp. MUA	14	775,698
Mount Holly Water	13	720,291
City of Bridgeton	11	609,477
City of Wildwood	11	609,477
Evesham Twp. MUA	8	443,256
Millville City Water Dept.	8	443,256
Evesham MUA	7	387,849
Hackettstown MUS	7	387,849
Millville Water Dept	8	443,256
Moorestown	8	443,256
Bordentown	7	387,849
Burlington Twp.	6	332,442
Mt. Laurel	6	332,442
Glassboro	6	332,442
Collingswood	6	332,442
Mapleshade	6	332,442
West Deptford	5	277,035
Woodbury	5	277,035
Burlington City	5	277,035
Pennsgrove	5	277,035
Deptford Twp.	5	277,035
Nesqehoning Boro Auth.	5	277,035
Medford Twp.	5	277,035
NJ American Mansfield/Oxford	5	277,035
Florence Twp.	5	277,035
Salem City	5	277,035
Other	201	11,136,807

New York	**5,600**	**310,279,200**
New York City	5,600	310,279,200
Pennsylvania	**2,186**	**121,119,702**
City of Philadelphia	863	47,816,241
Aqua Pennsylvania, Inc.	307	17,009,949
Forest Park/Point Pleasant Diversion	50	2,770,350
Bethlehem	46	2,548,722
Allentown	45	2,493,315
North Wales Water Authoriity	45	2,493,315
Bucks Co. Water and Sewer Auth.	45	2,493,315
Reading Area Water Authority	43	2,382,501
Bucks Co. Water and Sewer Auth.	41	2,271,687
Penna. American Water Co.	30	1,662,210
North Penn Water	26	1,440,582
Easton	24	1,329,768
Pennsylvania-American Water Co.	22	1,218,954
Schuylkill Co. Municipal. Authority	15	831,105
Pottstown Water Authority	14	775,698
Schuylkill Co. MUA	13	720,291
Muhlenberg Twp.	12	664,884
Lehigh County	12	664,884
PA American Nazareth	12	664,884
Hazelton	12	664,884
PA American Coatesville	12	664,884
Allentown City	12	664,884
Phoenixville Mun. Waterworks	12	664,884
Northampton Boro.	10	554,070
East Stroudsburg	10	554,070
PA American Yardley	10	554,070
Phoenixville	10	554,070
Morrisville	10	554,070
PA American Home District	10	554,070
PA American Penn District	10	554,070
Falls Twp.	10	554,070
Northampton Bucks Co. Auth.	10	554,070
Warminster Twp. MUA	10	554,070
Horsham Water and Sewer Auth.	10	554,070
Newtown Artesian Water	10	554,070
Milford	7	387,849
Tamaqua MWA	7	387,849
Lehighton MWA	7	387,849
Ambler Boro	7	387,849
Brodhead Creek Reg. Auth.	7	387,849
South Whitehall Twp. Auth.	7	387,849
Emmaus Munic. Water	7	387,849
Warrington Twp.	7	387,849
Wyomissing Boro	7	387,849
Schuylkill Haven Boro.	7	387,849
PA American Water Glen Alsace	7	387,849
Palmerton Mun. Auth.	7	387,849
Quakertown Mun. Water	6	332,442
Other	263	14,572,041
Delaware Basin	**8,750**	**484,811,250**

Table 63. Jobs and salaries at wastewater utilities in the Delaware River Basin

NPDES ID	Facility	Location	State	Jobs	Salaries
DE0020338	Kent Co. Levy Court WWTR	Frederica	DE	15	705,000
DE0021512	Lewes City POTW	Lewes	DE	3	141,000
DE0020320	Wilmington Wastewater Plant	Wilmington	DE	90	4,230,000
Delaware				**108**	**5,076,000**
NJ0027481	Beverly City Sewer Auth. STP	Beverly	NJ	3	141,000
NJ0024678	Bordentown Sewerage Auth.	Bordentown	NJ	5	235,000
NJ0024651	Cumberland Co. Utility Auth.	Bridgeton	NJ	7	329,000
NJ0024660	Burlington City STP	Burlington	NJ	5	235,000
NJ0021709	Burlington Twp. DPW	Burlington	NJ	4	188,000
NJ0026182	Camden County MUA	Camden	NJ	80	3,760,000
NJ0021601	Carneys Point Twp. Sewer Auth	Carneys Point	NJ	3	141,000
NJ0024007	Cinnaminson Sewerage Auth.	Cinnaminson	NJ	4	188,000
NJ0023701	Florence Twp. Sewer Auth.	Florence	NJ	5	235,000
NJ0026301	Hamilton Twp. DPW WWTP	Hamilton.	NJ	16	752,000
NJ0020915	Lambertville City Sewer Auth.	Lambertville	NJ	4	188,000
NJ0024759	Ewing Lawrence Sewer Auth.	Lawrenceville	NJ	16	752,000
NJ0069167	Maple Shade Util, Authority	Maple Shade	NJ	5	235,000
NJ0026832	Medford Twp. Sewer Auth. STP	Medford	NJ	2	94,000
NJ0029467	Millville City Sewer Auth.	Millville	NJ	7	329,000
NJ0024996	Moorestown Twp. Utilities Auth	Moorestown	NJ	6	282,000
NJ0024015	Mount Holly Twp. MUA	Mount Holly	NJ	8	376,000
NJ0020184	Newton Town DPW	Newton	NJ	4	188,000
NJ0024821	Pemberton Twp. MUA STP	Pemberton	NJ	5	235,000
NJ0024023	Penns Grove Sewerage Auth.	Penns Grove	NJ	3	141,000
NJ0021598	Pennsville Twp. Sewer Auth.	Pennsville	NJ	4	188,000
NJ0024716	Phillipsburg Town STP	Phillipsburg	NJ	5	235,000
NJ0022519	Riverside Twp. DPW	Riverside	NJ	3	141,000
NJ0024856	Salem WWTP Facility	Salem	NJ	3	141,000
NJ0024686	Gloucester Co. Util. Auth. STP	Thorofare	NJ	24	1,128,000
NJ0020923	Trenton City DPW Sewer Auth.	Trenton	NJ	20	940,000
NJ0023361	Willingboro Twp. MUA	Willingboro	NJ	6	282,000
New York				**257**	**12,079,000**
NY0020265	Delhi WWTP	Delhi	NY	4	188,000
NY0030074	Liberty WWTF	Liberty	NY	4	188,000
NY0022454	Monticello STP	Monticello	NY	6	282,000
NY0029271	Sidney WWTP	Sidney	NY	6	282,000
New Jersey				**20**	**940,000**
PA0026867	Abington Twp. STP	Abington	PA	6	282,000
PA0026000	Allentown City WWTP	Allentown	PA	45	2,115,000
PA0026042	Bethlehem City STP	Bethlehem	PA	95	4,465,000
PA0021181	Bristol Borough Water/Sewer	Bristol	PA	3	141,000
PA0027103	Delaware Co. Reg. Water Auth.	Chester	PA	44	2,068,000
PA0026859	Coatesville WWTP	Coatesville	PA	6	282,000
PA0026794	Conshohocken Borough Auth.	Conshohocken	PA	4	188,000
PA0026531	Downingtown Regional WPCC	Downingtown	PA	7	329,000
PA0026549	Borough of Doylestown WWTP	Doylestown	PA	29	1,363,000
PA0027235	Easton Area Joint Auth. WWTP	Easton, PA	PA	14	658,000
PA0029441	Upper Dublin Twp. MS4 UA	Ft. Washington	PA	3	141,000
PA0051985	Horsham Twp. STP	Horsham	PA	3	141,000
PA0024058	Kennett Square Borough WWTP	Kennett Sq.	PA	3	141,000

PA0026298	Whitemarsh STP	Lafayette Hill	PA	4	188,000
PA0026182	Lansdale Borough STP	Lansdale	PA	5	235,000
PA0039004	Upper Gwynedd Towam. STP	Lansdale	PA	7	329,000
PA0026468	Morrisville Mun. Auth. Water	Morrisville	PA	10	470,000
PA0027421	Norristown Borough WWTP	Norristown	PA	10	470,000
PA0020532	Upper Montgomery Joint Sewer	Pennsburg	PA	4	188,000
PA0026689	Northeast WPCP	Philadelphia	PA	210	9,870,000
PA0026662	Philadelphia Southeast POTW	Philadelphia	PA	112	5,264,000
PA0026671	SW Water Pollution Control	Philadelphia	PA	200	9,400,000
PA0020460	Quakertown WWTP	Quakertown	PA	10	470,000
PA0026549	Reading WWTP	Reading	PA	29	1,363,000
PA0020168	East Stroudsburg Filtration Plant	Stroudsburg	PA	10	470,000
PA0029289	Stroudsburg STP	Stroudsburg	PA	10	470,000
PA0027031	Goose Creek STP	West Chester	PA	4	188,000
PA0026018	West Chester Taylor Run STP	West Chester	PA	4	188,000
PA0028584	West Goshen STP	West Chester	PA	8	376,000
PA0023256	Upper Gwynedd Twp. WWTP	West Point	PA	7	329,000
PA0025976	Upper Moreland Hatboro Sewer	Willow Grove	PA	7	329,000
Pennsylvania				**913**	**42,911,000**
Del. Basin				**1,298**	**61,006,000**

Watershed Jobs

Over 100 nonprofit watershed and environmental organizations employ at least 200 staff who earn at least 9.5 million in wages on programs to restore the watersheds in the Delaware Basin (Table 64).

Table 64. Watershed organization jobs and salaries in the Delaware River Basin

Watershed Organization	Town	State	Jobs	Salaries
Christina Conservancy, Inc.	Wilmington	DE	1	48,000
Coalition for Natural Stream Valleys	Newark	DE		0
Delaware Audubon Society	Wilmington	DE	1	48,000
Delaware Nature Society	Hockessin	DE	20	960,000
Fairfield Watershed Association	Newark	DE		0
Friends of Bombay Hook	Smyrna	DE	1	48,000
Friends of White Clay Creek State Park	Newark	DE	1	48,000
Naamans Creek Watershed Association	Arden	DE		0
Nature Conservancy of Delaware	Wilmington	DE	2	96,000
Partnership for the Delaware Estuary, Inc.	Wilmington	DE	10	480,000
Save Wetlands and Bays	Millsboro	DE		0
St. Jones River Greenway Commission	Magnolia	DE		0
St. Jones River Watershed Association	Dover	DE	1	48,000
Waterfront Watch of Wilmington	Wilmington	DE	1	48,000
White Clay Creek Watershed Mgmt. Committee	Newark	DE	1	48,000
Delaware			**39**	**1,872,000**
Cape May County Watershed Area 16	Cape May Ct. Hse.	NJ	1	48,000
Citizens United to Protect the Maurice River	Millville	NJ	1	48,000
Cooper River Watershed Association	Haddonfield	NJ		0
Crafts Creek Spring Hill Brook Watershed	Bordentown	NJ		0
Crosswicks Creek Watershed Association	Yardville	NJ	1	48,000
Crosswicks-Doctors Creeks Watershed Association	New Egypt	NJ	1	48,000
Delaware River Greenway Partnership	Burlington	NJ	1	48,000
Fairview Lake & Watershed Conservation Foundation	West Caldwell	NJ		0
Friends Hamilton-Trenton-Bordentown Marsh	Robbinsville	NJ		0

Hunterdon Land Trust Alliance	Flemington	NJ	2	96,000
Mantua/Woodbury Creeks Watershed Association	Glassboro	NJ	1	48,000
Musconetcong Watershed Association	Asbury	NJ	1	48,000
New Jersey Coalition of Lake Associations	Sparta	NJ	1	48,000
Newton Creek Watershed Association	Collingswood	NJ	1	48,000
Oldmans Creek Watershed Association.	Mullica Hill	NJ	1	48,000
Paulinskill-Pequest Watershed Association	Blairstown	NJ	1	48,000
Phillipsburg Riverview Organization	Phillipsburg	NJ	3	144,000
Pinelands Preservation Alliance	Southampton	NJ	1	48,000
Pinelands Watershed Alliance	Tuckerton	NJ	1	48,000
Pohatcong Creek Watershed Association	Phillipsburg	NJ	1	48,000
Pompeston Creek Watershed Association	Cinnaminson	NJ	1	48,000
Raccoon Creek Watershed Association, Inc.	Mullica Hill	NJ	1	48,000
Rancocas Conservancy	Vincentown	NJ	2	96,000
Salem County Watershed Task Force	Woodstown	NJ		0
South Jersey Land and Water Trust	Glassboro	NJ	2	96,000
Upper Maurice River Watershed Association	Franklinville	NJ	1	48,000
New Jersey			**26**	**1,248,000**
Neversink River Program/The Nature Conservancy	Cuddebackville	NY	3	144,000
New York			3	
Aquashicola/Pohopoco Watershed Conservancy	Kresgeville	PA	1	48,000
Berks County Conservancy	Reading	PA	5	240,000
Bertsch-Hokendauqua-Catasauqua Watershed Assoc.	Bethlehem	PA	1	48,000
Brandywine Valley Association	West Chester	PA	8	384,000
Brodhead Forest & Stream Association	Stroudsburg	PA	1	48,000
Brodhead Watershed Association	Henryville	PA	1	48,000
Bushkill Stream Conservancy	Tatamy	PA	1	48,000
Chester Creek Watershed Association	Glen Mills	PA	1	48,000
Chester-Ridley-Crum Watersheds Association	Media	PA	5	240,000
Cooks Creek Watershed Association	Springtown	PA	1	48,000
Crum Creek Watershed Partnership	Swarthmoore	PA	1	48,000
Darby Cobbs Watershed Partnership	Philadelphia	PA	1	48,000
Darby Creek Valley Association	Drexel Hill	PA	1	48,000
Delaware River Shad Fishermen's Association	Bethlehem	PA	1	48,000
Delaware Riverkeeper Network	Bristol	PA	13	624,000
French and Pickering Creeks Conservation Trust	Valley Forge	PA	7	336,000
Friends of Cherry Valley	Stroudsburg	PA	1	48,000
Friends of Cobbs Creek Park	Philadelphia	PA	1	48,000
Friends of Crum Creek	Philadelphia	PA	1	48,000
Friends of Lake Afton	Yardley	PA	1	48,000
Friends of Mingo Creek	Royersford	PA	1	48,000
Friends of Poquessing Watershed, Inc.	Philadelphia	PA	1	48,000
Friends of Tacony Creek Park	Philadelphia	PA	1	48,000
Friends of the Del. Water Gap Nat'l. Recreation Area	Bushkill	PA	1	48,000
Friends of the Manayunk Canal	Philadelphia	PA	1	48,000
Friends of the Pennypack Park	Philadelphia	PA	1	48,000
Friends of the Wissahickon	Philadelphia	PA	1	48,000
Fry's Run Watershed Association	Easton	PA		0
Greater Pottstown Watershed Alliance	Pottstown	PA		0
Green Valleys Association	Pottstown	PA	3	144,000
Hay Creek Watershed Association	Geigertown	PA	1	48,000
Lackawaxen River Conservancy	Rowland PA	PA	1	48,000
Lake Wallenpaupack Watershed Association	Paupack	PA	2	96,000
Little Schuylkill Conservation Club	Delano	PA		0
Lower Merion Conservancy	Gladwyne	PA	6	288,000

Maiden Creek Watershed Association	Kempton	PA		0
Martins-Jacoby Watershed Association	Martins Creek	PA	1	48,000
Mid-Atlantic Council of Watershed Associations	West Chester	PA		0
Middle Anthracite Watershed Association	Sybertsville	PA	1	48,000
Mill Creek Council, Inc.	Philadelphia	PA	1	48,000
Monocacy Creek Watershed Association, Inc.	Bethlehem	PA	1	48,000
Neshaminy Creek Watershed Association	Rushland	PA	1	48,000
North Branch Watershed Association	Doylestown	PA	1	48,000
North Pocono CARE	Thornhurst	PA	2	96,000
Palisades Region Watershed Partnership	Pipersville	PA		0
Paunacussing Watershed Association	Carversville	PA		0
Pennsylvania Organization Watersheds and Rivers	Harrisburg	PA	3	144,000
Pennypack Ecological Restoration Trust	Huntington Valley	PA	8	384,000
Pennypack Watershed Partnership	Philadelphia	PA	1	48,000
Perkiomen Watershed Conservancy	Schwenksville	PA	4	192,000
Poquessing Watershed Partnership	Philadelphia	PA		0
Red Clay Valley Association	West Chester	PA	4	192,000
Saucon Creek Watershed Association	Bethlehem	PA	1	48,000
Schuylkill Action Network	Philadelphia	PA	2	96,000
Schuylkill Canal Association	Oaks	PA	1	48,000
Schuylkill Headwaters Association	Pottsville	PA	2	96,000
Schuylkill River Greenway Association	Pottstown	PA	1	48,000
Southampton Watershed Association	Southampton	PA	1	48,000
Springton Lake/Crum Creek Conservancy	Newtown Square	PA	1	48,000
Stony Creek Watershed Committee	Norristown	PA	1	48,000
Swarthmore College's Watershed Projects	Swarthmore	PA	2	96,000
Tinicum Conservancy	Erwinna	PA	4	192,000
Tinicum Creek Watershed Association	Upper Black Eddy	PA	2	96,000
Tobyhanna/Tunkhannock Creek Watershed Association	Pocono Lake	PA	1	48,000
Tohickon Creek Watershed Association	Pipersville	PA	1	48,000
Tookany/Tacony - Frankford Watershed Partnership	Philadelphia	PA	1	48,000
Upper Perkiomen Watershed Coalition	Palm	PA	1	48,000
Water Resources Association Delaware River Basin	Exton	PA	1	48,000
White Clay Watershed Association	Landenberg	PA	1	48,000
Wildlands Conservancy	Emmaus	PA	5	240,000
Wissahickon Restoration Volunteers	Philadelphia	PA	1	48,000
Wissahickon Valley Watershed Association	Ambler	PA	1	48,000
Wissahickon Watershed Partnership	Philadelphia	PA	1	48,000
Pennsylvania			**133**	**6,384,000**
Delaware Basin			**201**	**9,504,000**

Ski Area Jobs

In the Pocono Mountains of Pennsylvania, 9 ski resorts employ 1,753 direct jobs in the Delaware Basin from aggregate annual revenues of $87,655,063 from 1,908,228 skier visits based on an average mid-week lift ticket rate of $45/day.

Paddling-based Recreation

In the Mid-Atlantic census division (NY, NJ, PA), the Outdoor Industry Association (2006) estimates that paddling-based recreation is practiced by 11% of the population and is responsible for 3,356,000 participants and 22,844 jobs. Given the Delaware Basin is the home of 18.5% of the three

state's total population of 40,800,000 people, then the prorated paddling-based recreation in the basin is responsible for 620,860 participants and 4,226 jobs.

River Recreation

Cordel et al. (1990) from the U. S. Forest Service and U.S. National Park Service estimated river recreation along the Upper Delaware River and Delaware Water Gap was responsible for 448 jobs with wages of $8.8 million in $1986.

Canoe/Kayak/Rafting

The 37 canoe and kayak liveries along the Delaware, Lehigh, and Schuylkill, and Brandywine Rivers employ 225 people to lease watercraft to approximately 225,000 visitors with earnings of $9 million per year assuming a daily rental fee of $40 per person.

Wild Trout Fishing

Along the Beaverkill, East Branch, West Branch and upper main stem of the Delaware River in New York, wild trout fishing provides for 350 jobs with $3.6 million in wages.

Delaware Water Gap National Recreation Area

Stynes and Sun (2002) estimated the Delaware Water Gap Nat'l. Recreation Area recorded 4,867,272 visits in 2001 that generated $106 million in sales, 7,563 direct/indirect jobs, and $100 million wages.

Port Jobs

The Economy League of Greater Philadelphia (2008) reported that Delaware River ports:

- Employ 4,056 workers earning $326 million in wages (Table 65).
- Indirectly support an additional two jobs each in port activity and employee spending for a total of 12,121 port jobs with $772 million wages and $2.4 billion annual economic output.
- Most of the 4,056 direct port jobs are in cargo handling and warehousing with petroleum port jobs adding up to less than 10% of employment.
- Provide good jobs, the average salary of a port employee (with benefits) is over $80,000.

Table 65. Jobs at Delaware River ports
(Economy League of Greater Philadelphia 2008)

Employment Type	Jobs
Direct	**4056**
Cargo Handling	1,911
Warehousing	987
Federal Government	553
Construction	318
State/Local Government	152
Security	99
Wholesale	36
Indirect (Industry)	**4,655**
Induced (Worker Spending)	**3,410**
Total	**12,121**

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Appendix A

Economic Value (Potential) of Marcellus Shale Natural Gas in the Delaware River Basin

The U.S. Geological Survey (Coleman et al. 2011) estimated the entire 54,000 square-mile Marcellus Shale Formation from Kentucky and Ohio to Pennsylvania and New York potentially contains a mean volume of 84 trillion cubic feet of natural gas with a range of 43 tcf (95th percentile) to 144 tcf (5th percentile). If the Delaware River Basin covers 4,700 square miles or 8.7% of the Marcellus Shale, then by proportion a mean volume of 7.3 tcf of natural gas is potentially recoverable within the basin boundary (0.087 x 84 tcf) with a range of 3.7 tcf (95th percentile) to 12.5 tcf (5th percentile). These estimates may vary as the thickness of Marcellus Shale in the Delaware Basin increases to the northeast toward the New York/Pennsylvania border ranging from 50 feet thick near Stroudsburg to more than 250 feet thick under Lackawaxen in Wayne County, Pennsylvania.

In 2010, the U.S. Energy Information Administration reported the mean natural gas wellhead price was $4.16/1000 cf, down from a peak of $7.97/1000 cf in 2008. The residential customer price of natural gas was $11.21/1000 cf, down two dollars from the 2008 peak. Table A1 lists fluctuating annual wellhead and residential consumer prices of natural gas in the U.S. from 2006 through 2010.

Table A1. Wellhead and residential prices of natural gas in the United States, 2006-2010 (EIA)

Year	Wellhead Price ($/1000 cf)	Residential Price ($/1000 cf)
2006	6.39	13.73
2007	6.25	13.08
2008	7.97	13.89
2009	3.67	12.14
2010	4.16	11.21

At the 2010 wellhead unit price (Table A2), the mean value of potentially recoverable natural gas from the Marcellus Shale Formation within the Delaware River Basin is projected to be $30.4 billion with a range of $15.4 billion (95th percentile) to $52.0 billion (5th percentile). Assuming the natural gas can be recovered within 25 years, the mean annual wellhead value of Marcellus Shale gas within the Delaware Basin is potentially $1.2 billion/year with a range of $0.6 billion/year (95th percentile) to $2.0 billion/year (5th percentile). Figures A1 and A2 project total and annual wellhead value of natural gas recoverable from the Delaware Basin based on variable prices from 2006 to 2010.

At the 2010 residential consumer unit price (Table A3), the mean value of natural gas from the Marcellus Shale Formation within the Delaware River Basin is $81.8 billion with a range of $41.5 billion (95th percentile) to $140.1 billion (5th percentile). Assuming the natural gas can be recovered within 25 years, the mean annual residential consumer value of Marcellus Shale gas within the Delaware Basin is $3.3 billion/year with a range of $1.7 billion/year (95th percentile) to $5.6 billion/year (5th percentile). Figures A3 and A4 project total and annual residential consumer value of natural gas recoverable from the Delaware Basin based on prices from 2006 to 2010.

Table A2. Wellhead value of Marcellus Shale natural gas within the Delaware River Basin

State/Basin	Area Marcellus Shale (sq mi)	Wellhead Natural Gas Price[1] ($/1000 cf)	Volume Natural Gas[2] (tcf)	Wellhead Natural Gas Value ($ billion)	Wellhead Natural Gas Value[3] ($ billion/yr)
Mean					
Pennsylvania	2,338	$4.16	3.6	$15.0	$0.6
New York	2,362	$4.16	3.7	$15.4	$0.6
Delaware Basin	4,700	$4.16	7.3	$30.4	$1.2
95th Percentile					
Pennsylvania	2,338	$4.16	1.8	$7.5	$0.3
New York	2,362	$4.16	1.9	$7.9	$0.3
Delaware Basin	4,700	$4.16	3.7	$15.4	$0.6
5th Percentile					
Pennsylvania	2,338	$4.16	6.2	$25.8	$1.0
New York	2,362	$4.16	6.3	$26.2	$1.0
Delaware Basin	4,700	$4.16	12.5	$52.0	$2.0

1. EIA 2010. 2. USGS 2011. 3. Assumes 25 year natural gas recovery period.

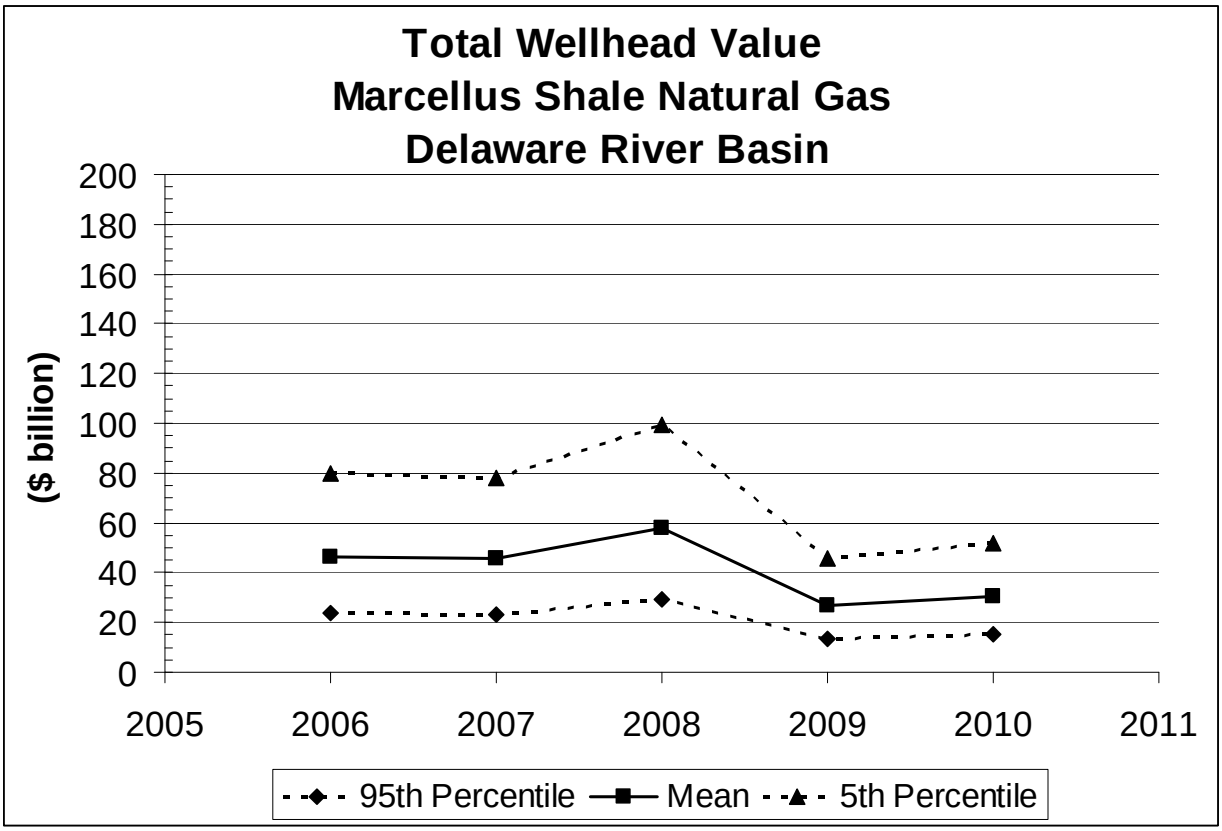

Figure A1. Total wellhead value of Marcellus shale natural gas in the Delaware River Basin Assumes mean volume of 7.3 tcf of natural gas potentially recoverable within basin boundary with a range of 3.7 tcf (95th percentile) to 12.5 tcf (5th percentile) as per Coleman et al. 2011 from the USGS. From EIA (2011), natural gas prices at wellhead ($/1000 cf): 2006 ($6.39), 2007 ($6.25), 2008 ($7.97), 2009 ($3.67), and 2010 ($4.16).

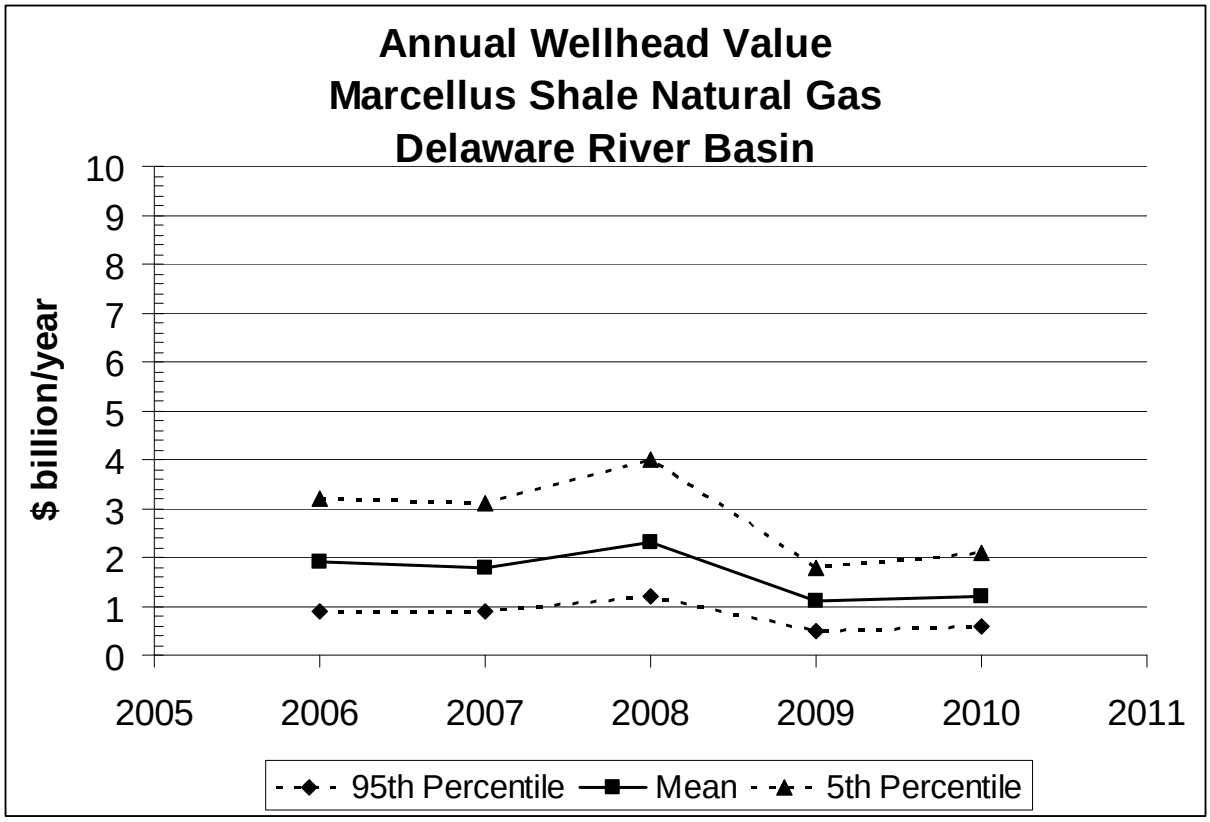

Figure A2. Total wellhead value of Marcellus shale natural gas in the Delaware River Basin Assumes mean volume of 7.3 tcf of natural gas potentially recoverable within basin boundary with a range of 3.7 tcf (95th percentile) to 12.5 tcf (5th percentile) as per Coleman et al. 2011 from the USGS. From EIA (2011), natural gas prices at wellhead ($/1000 cf): 2006 ($6.39), 2007 ($6.25), 2008 ($7.97), 2009 ($3.67), and 2010 ($4.16). Assumes 25 year natural gas recovery period.

Table A3. Residential value of Marcellus Shale natural gas within the Delaware River Basin

State/Basin	Area Marcellus Shale (sq mi)	Residential Natural Gas Price[1] ($/1000 cf)	Volume Natural Gas[2] (tcf)	Residential Natural Gas Value ($ billion)	Residential Natural Gas Value[3] ($ billion/yr)
Mean					
Pennsylvania	2,338	$11.21	3.6	$40.4	$1.6
New York	2,362	$11.21	3.7	$41.5	$1.7
Delaware Basin	4,700	$11.21	7.3	$81.8	$3.3
95th Percentile					
Pennsylvania	2,338	$11.21	1.8	$20.2	$0.8
New York	2,362	$11.21	1.9	$21.3	$0.9
Delaware Basin	4,700	$11.21	3.7	$41.5	$1.7
5th Percentile					
Pennsylvania	2,338	$11.21	6.2	$69.5	$2.8
New York	2,362	$11.21	6.3	$70.6	$2.8
Delaware Basin	4,700	$11.21	12.5	$140.1	$5.6

1. EIA 2010. 2. USGS 2011. 3. Assumes 25 year natural gas recovery period.

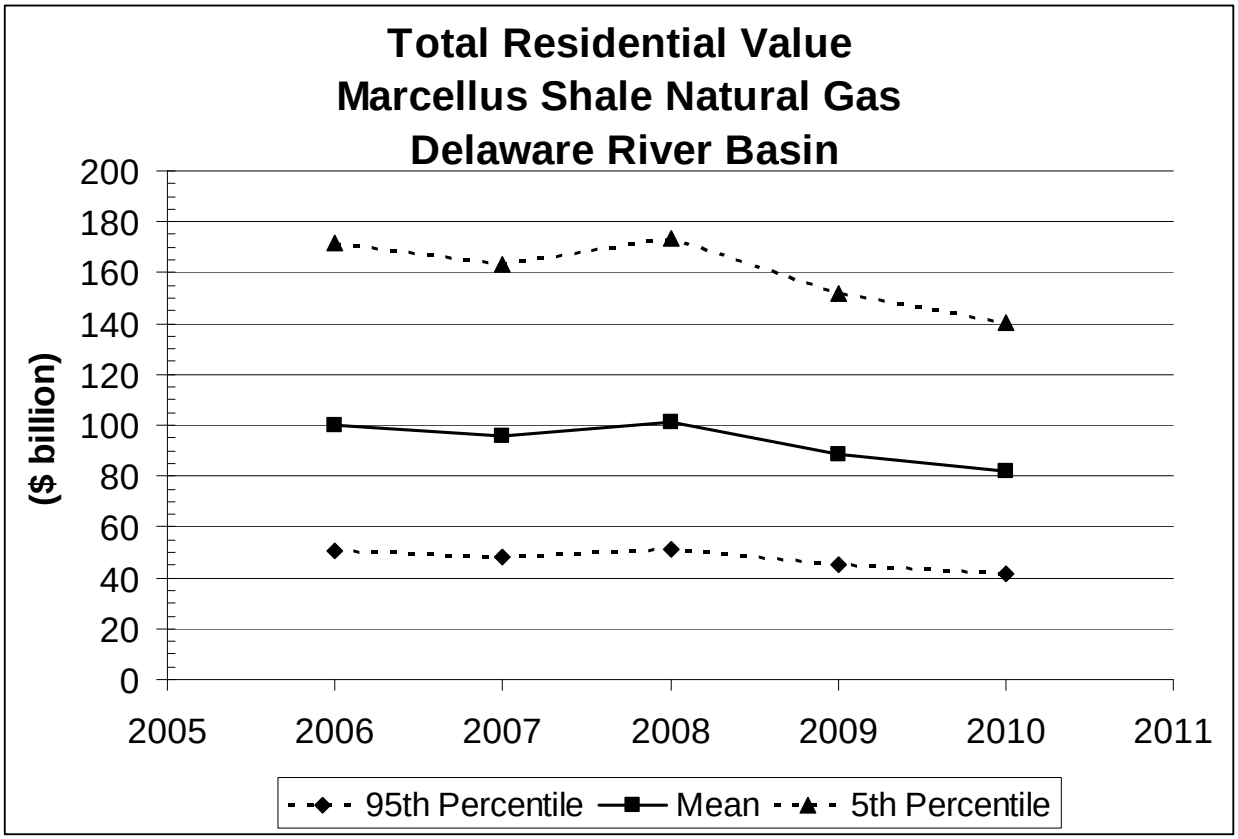

Figure A3. Total residential value of Marcellus shale natural gas in the Delaware River Basin Assumes mean volume of 7.3 tcf of natural gas potentially recoverable within basin boundary with a range of 3.7 tcf (95th percentile) to 12.5 tcf (5th percentile) from Coleman et al. 2011 (USGS). From EIA (2011), natural gas sold to residential consumers ($/1000 cf): 2006 ($13.73), 2007 ($13.08), 2008 ($13.89), 2009 ($12.14), and 2010 ($11.21).

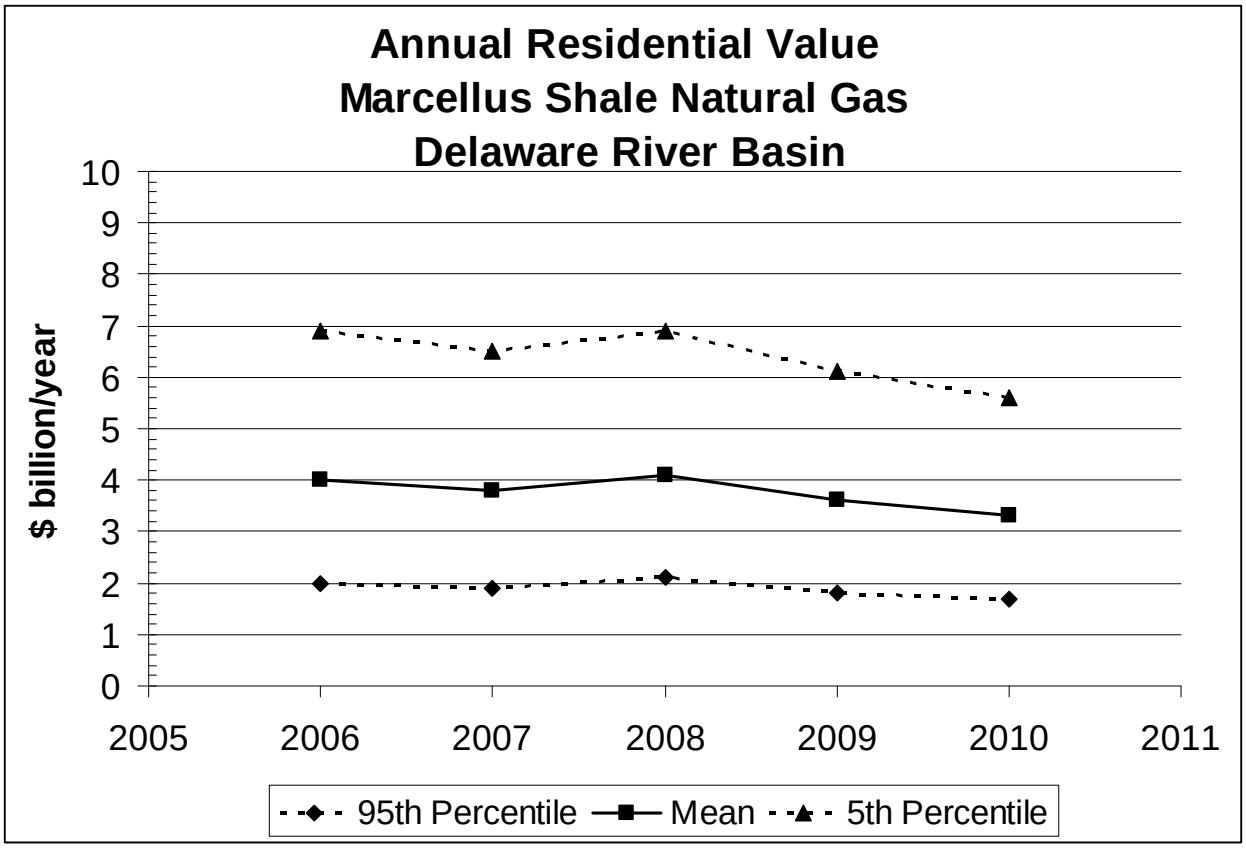

Figure A4. Annual residential value of Marcellus shale natural gas in the Delaware River Basin Assumes mean volume of 7.3 tcf of natural gas potentially recoverable within basin boundary with a range of 3.7 tcf (95th percentile) to 12.5 tcf (5th percentile) from Coleman et al. 2011 (USGS). From EIA (2011), natural gas sold to residential consumers ($/1000 cf): 2006 ($13.73), 2007 ($13.08), 2008 ($13.89), 2009 ($12.14), and 2010 ($11.21). Assumes 25 year natural gas recovery period.

Appendix B

Employment Codes by Industry, 2009

(U. S. Bureau of Labor Statistics)

Industry			NAICS Code
Agriculture, Forestry, Fishing and Hunting			11
	Crop Production		111
	Animal Production		112
		Aquaculture	1125
	Forestry and Logging		113
	Fishing, Hunting and Trapping		114
		Fishing	1141
	Support Activities for Agriculture and Forestry		115
Mining, Quarrying, and Oil and Gas Extraction			21
	Oil and Gas Extraction		211
	Mining (except Oil and Gas)		212
		Nonmetallic Mineral Mining and Quarrying	2123
	Support Activities for Mining		213
Utilities			22
	Utilities		221
		Electric Power Generation, Transmission and Distribution	2211
		Natural Gas Distribution	2212
		Water, Sewage and Other Systems	2213
Construction			23
	Construction of Buildings		236
		Residential Building Construction	2361
		Nonresidential Building Construction	2362
	Heavy and Civil Engineering Construction		237
		Land Subdivision	2372
		Highway, Street, and Bridge Construction	2373
		Other Heavy and Civil Engineering Construction	2379
	Specialty Trade Contractors		238
Manufacturing			31
	Food Manufacturing		311
		Seafood Product Preparation and Packaging	3117
	Beverage and Tobacco Product Manufacturing		312
	Textile Mills		313
	Textile Product Mills		314
	Apparel Manufacturing		315
		Apparel Knitting Mills	3151
	Leather and Allied Product Manufacturing		316
	Wood Product Manufacturing		321
	Paper Manufacturing		322
	Petroleum and Coal Products Manufacturing		324
	Chemical Manufacturing		325
		Basic Chemical Manufacturing	3251
		Resin, Synthetic Rubber, and Artificial Synthetic Fibers and Filaments Manufacturing	3252
		Pesticide, Fertilizer, and Other Agricultural Chemical Manufacturing	3253
		Pharmaceutical and Medicine Manufacturing	3254
		Paint, Coating, and Adhesive Manufacturing	3255
		Soap, Cleaning Compound, and Toilet Preparation Manufacturing	3256
		Other Chemical Product and Preparation Manufacturing	3259
	Plastics and Rubber Products Manufacturing		326

	Nonmetallic Mineral Product Manufacturing		327
		Cement and Concrete Product Manufacturing	3273
		Lime and Gypsum Product Manufacturing	3274
		Other Nonmetallic Mineral Product Manufacturing	3279
	Primary Metal Manufacturing		331
	Fabricated Metal Product Manufacturing		332
	Machinery Manufacturing		333
	Computer and Electronic Product Manufacturing		334
		Computer and Peripheral Equipment Manufacturing	3341
		Communications Equipment Manufacturing	3342
		Audio and Video Equipment Manufacturing	3343
		Semiconductor and Other Electronic Component Manufacturing	3344
		Navigational, Measuring, Electromedical, and Control Instruments Manufacturing	3345
		Manufacturing and Reproducing Magnetic and Optical Media	3346
	Electrical Equipment, Appliance, and Component Manufacturing		335
	Transportation Equipment Manufacturing		336
		Motor Vehicle Manufacturing	3361
		Motor Vehicle Body and Trailer Manufacturing	3362
		Motor Vehicle Parts Manufacturing	3363
		Aerospace Product and Parts Manufacturing	3364
		Railroad Rolling Stock Manufacturing	3365
		Ship and Boat Building	3366
		Other Transportation Equipment Manufacturing	3369
	Furniture and Related Product Manufacturing		337
	Miscellaneous Manufacturing		339
Wholesale Trade			42
	Merchant Wholesalers, Durable Goods		423
	Merchant Wholesalers, Nondurable Goods		
	Wholesale Electronic Markets and Agents and Brokers		425
Retail Trade			44
	Motor Vehicle and Parts Dealers		441
	Furniture and Home Furnishings Stores		442
	Electronics and Appliance Stores		443
		Electronics and Appliance Stores	4431
	Building Material and Garden Equipment and Supplies Dealers		444
	Food and Beverage Stores		445
	Health and Personal Care Stores		446
	Gasoline Stations		447
	Clothing and Clothing Accessories Stores		448
	Sporting Goods, Hobby, Book, and Music Stores		451
	General Merchandise Stores		452
	Miscellaneous Store Retailers		453
	Nonstore Retailers		454
Transportation and Warehousing			48
	Air Transportation		481
		Scheduled Air Transportation	4811
		Nonscheduled Air Transportation	4812
	Rail Transportation		482
		Rail Transportation	4821
	Water Transportation		483
		Deep Sea, Coastal, and Great Lakes Water Transportation	4831
		Inland Water Transportation	4832
			4883
	Truck Transportation		484
		General Freight Trucking	4841

		Specialized Freight Trucking	4842
	Transit and Ground Passenger Transportation		485
		Urban Transit Systems	4851
		Interurban and Rural Bus Transportation	4852
		Taxi and Limousine Service	4853
		School and Employee Bus Transportation	4854
		Charter Bus Industry	4855
		Other Transit and Ground Passenger Transportation	4859
	Pipeline Transportation		486
		Pipeline Transportation of Crude Oil	4861
Information			51
	Publishing Industries (except Internet)		511
	Motion Picture and Sound Recording Industries		512
	Broadcasting (except Internet)		515
	Telecommunications		517
	Data Processing, Hosting, and Related Services		518
	Other Information Services		519
Finance and Insurance			52
	Monetary Authorities-Central Bank		521
	Credit Intermediation and Related Activities		522
	Securities, Commodity Contracts, and Other Financial Investments and Related Activities		523
	Insurance Carriers and Related Activities		524
	Funds, Trusts, and Other Financial Vehicles		525
Real Estate and Rental and Leasing			53
	Real Estate		531
	Rental and Leasing Services		532
	Lessors of Nonfinancial Intangible Assets (except Copyrighted Works)		533
Professional, Scientific, and Technical Services			54
	Professional, Scientific, and Technical Services		541
		Management, Scientific, and Technical Consulting Services	5416
		Scientific Research and Development Services	5417
Management of Companies and Enterprises			55
	Management of Companies and Enterprises		551
Administrative and Support and Waste Management and Remediation Services			56
	Administrative and Support Services		561
		Travel Arrangement and Reservation Services	5615
	Waste Management and Remediation Services		562
Educational Services			61
	Educational Services		611
		Colleges, Universities, and Professional Schools	6113
		Technical and Trade Schools	6115
		Educational Support Services	6117
Health Care and Social Assistance			62
	Ambulatory Health Care Services		621
	Hospitals		622
	Nursing and Residential Care Facilities		623
	Social Assistance		624
Arts, Entertainment, and Recreation			71
	Performing Arts, Spectator Sports, and Related Industries		711
	Museums, Historical Sites, and Similar Institutions		712
	Amusement, Gambling, and Recreation Industries		713
		Other Amusement and Recreation Industries	7139
Accommodation and Food Services			72
	Accommodation		721
		Traveler Accommodation	7211

		RV (Recreational Vehicle) Parks and Recreational Camps	7212
		Rooming and Boarding Houses	7213
	Food Services and Drinking Places		722
Other Services (except Public Administration)			81
	Repair and Maintenance		811
	Personal and Laundry Services		812
	Religious, Grantmaking, Civic, Professional, and Similar Organizations		813
		Social Advocacy Organizations	8133
		Business, Professional, Labor, Political, and Similar Organizations	8139
	Private Households		814
Public Administration			92
	Executive, Legislative, and Other General Government Support		921
	Justice, Public Order, and Safety Activities		922
	Administration of Human Resource Programs		923
	Administration of Environmental Quality Programs		924
	Administration of Housing Programs, Urban Planning, Community Development		925
	Administration of Economic Programs		926
	Space Research and Technology		927
	National Security and International Affairs		928