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PFAS Chemicals and Their Effects on Cholesterol and Cardiovascular Disease

By

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An abstract of

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Abstract

PFAS Chemicals and Their Effects on Cholesterol and Cardiovascular Disease

By Andrew R. Marino

PFAS chemicals are known endocrine disruptors that can increase risk and affect health outcomes related to cardiovascular disease. PFAS can affect lipid panels, blood chemistry, adipose tissue, and inflammation. This suggests cholesterol production in the liver is an affected process that so happens to be an indicator of cardiovascular disease. However, more evidence is needed to determine the mechanism routes.

The study analyzes nine different PFAS compounds with Simple Linear Regressions, Logistic Regression, and One- Way ANOVA to determine correlation of PFAS effects on Low-Density Lipid, Total Cholesterol, and overall high cholesterol levels. The sample population collected is secondary data available through the National Health and Nutrition Examination Survey (NHANES) during 2017-2018 data collection. A 95% Confidence Interval range was used to determine significant values. Findings for Perfluorohexanesulphonic acid (PFHxS) and Low-Density Lipid suggested significant correlation. It was concluded that more in-depth analyses would need to be performed to accumulate more information on PFAS' effects on the endocrine system and cardiovascular disease. Results from this study suggested significance between Low Density Lipid and PFAS PFHxS. In conclusion, further analyses will need to be collected to determine the relationship of PFAS and Cardiovascular disease.

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Background: DuPont

In June 2023, a major chemical manufacturer, 3M, was held liable and required to pay $10.3 billion to compensate multiple U.S. cities for contaminating their water supplies and subsequently, their citizens with perfluoroalkyl and polyfluoroalkyl substances (PFAS) (Friedman and Giang,2023). Just prior to 3M's settlement, DuPont, another major chemical manufacturer, was held liable for a similar lawsuit; the settlement was $1.19 billion.

Although these settlements appear substantial by their dollar amounts, taxpayers from these affected communities will still pay much of the damages to their health inflicted by these companies (Friedman and Giang,2023). PFAS has been routinely linked with a variety of diseases from cancer to developmental defects in newborns. The EPA and researchers have attempted to put a cost value on associated health effects. This was estimated to be $62.6 billion, which is likely to be an underestimation of exposure and damages.

Lawsuits related to contaminated drinking water and PFAS are not new. In the early 2000s, after a corporate lawyer investigated complaints and compiled records from a local farmer in West Virginia near the Ohio River. Livestock, domestic animals, and wildlife began to appear diseased or unexpectedly die (Billott,2019). Over time, more communities and their residents living along the Ohio River began to voice similar observations and concerns. It was suspected that a DuPont chemical plant located on the Ohio River in Parkersburg, West Virginia was responsible for the uptick in disease and sickness in the surrounding areas (Billott,2019). The speculations became more evident when DuPont's plant employees reported similar illnesses affecting communities (Rich,2016).

DuPont was originally established in 1802 as a gun powder developer (DiGiannantonio,2022). Over time, the company evolved into a chemical acquirer and developer (DiGiannantonio,2022).

In addition to acquiring chemicals, it acquired other companies. In 1951, the company acquired the patent for PFAS from 3M (DiGiannantonio,2022). Teflon, a nonstick material, is one of the products created at DuPont and is a PFAS-based chemical (DiGiannantonio,2022). Scientists at DuPont as early as 1961 observed laboratory animals with enlarged livers when they ingested PFAS (DiGiannantonio,2022). Scientists considered the chemical questionable for its associated health implications. A decade later, employees at DuPont had high concentrations of PFAS in their blood (DiGiannantonio,2022). Ten years after that 3M discovered PFAS contributed to birth defects in lab rats (DiGiannantonio,2022). By the 1990s, it was discovered PFAS caused cancer (DiGiannantonio,2022). Despite all these discoveries, EPA testing did not happen throughout 50 years of production of a questionable compound. As a result of the company's negligence, they contaminated the Ohio River (DiGiannantonio,2022); created problematic health outcomes for their employees; polluted surrounding water tables that supplied drinking water to nearby communities; and obliterated the ecosystem surrounding the neighboring towns and wildlife near the plant and downstream (DiGiannantonio,2022). The EPA eventually became involved and brought on a lawsuit in 2004 as DuPont withheld information regarding the dangers of PFAS.

PFAS Structure

PFAS are characterized by an extremely stable fluorinated carbon chain with a functional group. In attempts to reduce the use of PFAS or to degrade faster, variations of PFAS were developed, too. These are characterized by shorter carbon fluorine chains and sometimes variation to the function group.

The chains' fluorine- carbon bonds stabilize and complement each other by completing their electron clouds. Fluorine's electron cloud has seven electrons; to complete an electron cloud, eight electrons are needed. Carbon has four electrons in its cloud; therefore, this can readily bond with other atoms to achieve a stable, neutral charge (Klein,2013). However, the tail of these fluorine-carbon chains are completed by a carboxylic acid function group that can readily bind to water or salts (NIH,n.d.). Furthermore, carboxylic acid function group naturally occur in the body and the same function group found in vinegar (chemistrytalk.org, n.d.).

What is unique about PFAS molecular structure and behavior is that they look like a lipid chain, and they are hydrophobic; however, PFAS molecules are lipophobic, too (Lind and Lind,2020). There are multiple PFAS compounds that are typically characterized as either long or short fluorine-carbon chains.

Perfluoroalkyl and polyfluoroalkyl (PFAS) chemicals are a family of chemicals that are characterized by their polyfluorene-carbon molecules with a function group (Department of Health Services Wisconsin, 2023). These chemicals include PFOS, PFOA, PFNA, PFDA, PFOSA, MeFOSAA, Et-FOSAA, and PFHxS to name a few (Department of Health Services Wisconsin, 2023). There are dozens more PFAS compounds than listed. Many of these were developed in response to PFAS to either alter the structure to degrade faster or to replace the chemical to reduce environmental accumulation.

PFAS Uses

PFAS are man-made chemicals that are frequently found in materials such as fabrics, food, packaging, non-stick surfaces, cleaning products, waterproofing materials, fire retardants, and other household products (Department of Health Services Wisconsin,2023). These chemicals

tend to be non-stick, grease, stain, or water resistant (Department of Health Services Wisconsin,2023) (Virginia Department of Health,2018). These chemicals are great at being hydrophobic and lipid resistant which are good for repelling grease, oil, and water (Virginia Department of Health,2018).

Contamination of the Environment and Human Exposure

PFAS has been known to contaminate groundwater and could potentially leach into drinking water and water tables (McMahon et al,2022). In a study from 2019, collected water samples from the northeastern United States, tested, and found that at least 47% of these samples had detectable PFAS levels (McMahon et al, 2022). It was noted that current water-carbon filtration methods may not be effective at removing PFAS from drinking water. The most common form of exposure to PFAS chemicals is through food or water supplies (McMahon et al, 2022).

PFAS-Associated Health Outcomes

Although the chemical is readily contaminating blood and urine, the long term PFAS exposure's health consequences are not fully understood (NIH, n.d.). It is known to have effects on metabolism, fertility, obesity, and fetal growth (NIH,n.d.). These chemicals have been associated with increased susceptibility to cancers such as testicular, prostate, and kidney cancers (Virginia Department of Health, 2018).

Furthermore, PFAS can influence cholesterol levels, thyroid, pancreatic, liver, and immune system functions (Virginia Department of Health, 2018). PFAS can contribute to dysfunction of the liver, metabolism, and lipid handling (Lind and Lind, 2020). PFAS can also contribute to an inflammatory response. Because the PFAS chain is not lipid soluble, it does not accumulate in

adipose tissues (Lind and Lind,2020). PFAS is transported through blood's plasma, a connective tissue, and deposited in the liver (Lind and Lind,2020). This can lead to altering liver function (Lind and Lind, 2020), resulting in abnormal lipid panels including increased total serum cholesterol levels (Lind and Lind,2020). PFAS is known as an endocrine disrupter and can contribute to metabolic disruptions, which could contribute to (thrombotic) cardiovascular disease and atherosclerosis (Lind and Lind,2020).

In a study analyzing epidemiological evidence of PFAS exposure with cardiovascular outcomes, Meneguzzi et al found positive correlations between increasing levels of PFAS and increasing cardiovascular events (Meneguzzi et al, 2021). Several of these studies utilized years of data collected from NHANES. Information pertaining to labs and self-reported cardiovascular events were collected, too (Meneguzzi et al, 2021). The results suggested PFAS could be remodeling cardiac tissue and increasing risks of arterial diseases through atherosclerosis (Meneguzzi et al, 2021).

In addition, there is some evidence that PFAS can be deposited in the brain. PFAS has been determined to make it through the blood-brain barrier (Starnes et al, 2022). Depending on the type of PFAS chain, it can accumulate in certain regions of the brain (Starnes et al, 2022). Currently, there is building evidence that PFAS can contribute to neurological disorders such as dementia and ADHD (Starnes et al, 2022).

Endocrine System Anatomy

The endocrine system involves multiple organs that regulate bodily functions and responses which contributes to the body's ability to maintain homeostasis. Much of the endocrine system begins in the brain, specifically the hypothalamus, then continues to the pituitary gland. Either

through neurological or chemical signaling, hormones reach endocrine organs to trigger a response, usually a cascade-effect. These are primarily made up of glands that are found extensively throughout the body. They lack ducts but are very vascular and can be described as branching. For example, this includes pancreas, gonads, thyroid, parathyroid, adrenal glands, pineal gland, and pituitary gland. Although hormonal signals go to endocrine – specific organs, hormones can involve organs that are not usually considered part of the endocrine system, such as the liver. Furthermore, these organs can produce hormones, too.

Endocrine System Physiology

Once a hormone is released from the hypothalamus and pituitary gland into the blood supply, it circulates throughout the body without exact control of where it goes. They will come into contact with multiple organ tissues. These signals travel through the system's blood supply to the vascular networks of endocrine organs and are released. Once hormones are released, there can be either a direct reaction or trigger a cascade reaction.

A single hormone can have a variety of effects on the body depending on the organ system (Marieb, 2015). What determines a hormone's responsiveness is if an organ's tissue has receptors for a hormone to bind to (Marieb, 2015)—the more receptors, the greater the response. Receptors are best described as clefts that receive chemicals. This reaction is best described as a lock and key model. The chemical molecule fits to the receptor which triggers a relay of biochemical signaling and cascades (Miller & Lappin, 2023). For a hormone to be responsive, the chemical can go through a series of receptors that will synthesize byproduct chemicals and trigger enzymes to catalyze a cascading effect which can release derivative hormones (Marieb, 2015).

Hormones are released by three types of stimuli: humoral, neural, and hormonal. Humoral occurs as a direct response from an endocrine organ to changes in the body (Marieb,2015), such as changes in glucose (Marieb,2015). Neural stimuli occur as a response to environmental change, such as stress (Marieb,2015). For instance, as a response to stress, neural impulses would trigger the sympathetic nervous system to send signals to responsive endocrine glands that will release stress-induced hormones (Maireb, 2015), which then circulate throughout the body (Maireb,2015). Lastly, hormonal stimuli occur as a response to other hormones released (Marieb,2015).

Hormones responses regulate through up- and down-regulation (Marieb,2015). Up regulation occurs when there is a significant number of receptors present (Marieb,2015). Therefore, decreasing a hormone's concentration and increasing bodily response (Marieb,2015). Down-regulation has less receptors present which maintain a higher concentration of hormone, and the body has less of a response to the hormone (Marieb,2015).

Hypothalamus- Pituitary Axis (HPA) Anatomy

The hypothalamus and pituitary gland are the initial regions of the endocrine system. Located in the diencephalon behind the optic chasm, the hypothalamus regulates the body's homeostasis. This organ controls and initiates processes involved with the autonomic nervous system; endocrine system; physical, emotional responses; body temperature regulation; food intake regulation; hydration regulation; and sleep-wake cycles. Particular to the hypothalamus' functions, it releases and inhibits functions through hormones and nerves; these processes are triggered by environmental and biochemical changes. These simple triggers can create catalyzing, systemic responses from the hypothalamus. For instance, the hypothalamus can affect

cardiac tissue and blood pressure by sending neural signals to the peripheral nervous system. Biochemical changes in blood can trigger feedback loops to regulate hormonal releasing/inhibiting secretions (Marieb, 2015). Besides being a part of the central nervous system, it is also a part of the limbic system as a main structure/organ. The limbic system relays inputs to the hypothalamus to then carry out visceral and emotional responses. Within the hypothalamus (the ganglia) there are eight specialized regions, nuclei. Each nucleus is specialized to produce hormones, regulate visceral processes, and initiate neural connections. These nuclei include preoptic nucleus, anterior hypothalamic nucleus, supraoptic nucleus, suprachiasmatic nucleus, dorsomedial nucleus, posterior hypothalamic nucleus, ventromedial nucleus, and arcuate nucleus.

The pituitary gland is situated below the third ventricle, outside of the brain stem and basal ganglia, stemming below the hypothalamus from the infundibulum (Marieb, 2015). It is surrounded by cerebral fluid and situated behind the nasal passage. The gland is made up of two regions: the posterior and the anterior. The anterior lobe of the pituitary gland is made up of blood vessel capillaries, which receive chemical signaling from the hypothalamus through blood (Marieb, 2015). The posterior lobe is made up of bundles of neurons that receive electrical, neural impulses from the hypothalamus (Marieb, 2015). The posterior is more part of the brain than endocrine system because of the neural bundle, the hypothalamic-hypophyseal tract (Marieb,2015). The pituitary gland releases signaling hormones into the body. Once these hormones reach their destinations, they trigger a cascade effect creating other organ-specific hormones (Marieb, 2015).

Hypothalamus- Pituitary Axis (HPA) Physiology

A part of maintaining homeostasis includes triggering bodily processes. The hypothalamus can do this by signaling to the pituitary gland with hormones (Marieb, 2015). There are two ways hypothalamic-derived hormones reach the pituitary gland. In the nuclei of the hypothalamus multiple types of hormones are produced: thyrotropin-releasing hormone (TRH), gonadotropin-releasing hormone (GnRH), growth hormone-releasing hormone (GHRH), corticotropin-releasing hormone (CRH), somatostatin, and oxytocin (Marieb, 2015). Although not a hormone, dopamine is produced from the hypothalamus, too. Many of the hormones listed are releasing hormones. The production of these chemicals in the hypothalamus are stimulated by neural impulses; dopamine is needed for production of some of them. These are then sent to the pituitary gland through two different pathways, either secretion into capillary blood vessels or neural impulses. Most of the releasing hormones will be sent to the anterior pituitary lobe to trigger production and release of hormones. Depending on the pathway, stimuli will be sent to one of the two pituitary lobes. Neural signaling will go to the posterior lobe which is more part of the brain, whereas chemical signals will transfer through blood vessels to the anterior lobe which is considered more part of the endocrine system. Both pathways will prompt more secretion from the hypothalamus to the pituitary gland. Once the pituitary gland releases hormones into the body, they will circulate and readily react with receptors on different organs (Marieb, 2015). The anterior lobe of the pituitary gland will produce and release into the body growth hormone (GH), thyroid stimulating hormone (TSH), adrenocorticotrophin hormone (ATCH), follicle stimulating hormone (FSH), luteinizing hormone (LH), and prolactin. The posterior lobe will produce and release into the body, antidiuretic hormone (ADH), and oxytocin.

There are two general types of hormones—amino acids and steroids. Amino acids are water soluble, whereas steroids are lipid soluble (Marieb, 2015). Steroid hormones are lipid soluble meaning that they can pass through a cell's wall (Marieb, 2015). All steroidal hormones are derivatives of cholesterol (Craig et al, 2023); it is a necessary precursor to steroid hormone production (Marieb, 2015). Once a steroid-based hormone is created, these are sent throughout the body and can pass through cell walls. The steroid binds with receptors to pass into the nucleus and bind to DNA regions to transcribe mRNA (Marieb, 2015). This initiates the process to synthesize new proteins (Marieb, 2015). This sequence is a common process in protein development or chemical/electrical communication (Marieb, 2015).

The second type of hormones are amino acid-based; they commonly have a two-part receptor system (Marieb, 2015). Once a hormone is released into the blood supply, the molecule pairs and triggers the receptor in which a protein is released and relays to another receptor that releases an enzyme which creates a derivative hormone (Marieb, 2015). These can create more cascades that initiate more processes. An example of an amino acid-based hormone is Growth Hormone (GH) (Marieb, 2015).

Growth Hormone

Growth Hormone Releasing Hormone (GHRH), which is needed to release Growth Hormone (GH) from the anterior lobe of the pituitary gland, is produced in the arcuate nucleus of the hypothalamus (Marieb,2015), and regulated by dopamine (Patel et al, 2016). This initiates chemical signaling from the hypothalamus to the pituitary gland's anterior lobe. Once GHRH reaches the pituitary gland, this triggers the pituitary glands somatotropin cells to produce and release GH into the system.

Growth hormone (GH) is an amino acid-based hormone that is frequently used throughout the body (Marieb,2015). Many of the processes GH is involved with are directly related to metabolism and indirect to growth (Marieb,2015). This is secreted when triggered by low levels of GH, low levels of glucose, low levels of fatty acids, high levels of amino acids in the blood, or from exercise (Marieb,2015). Growth Hormone Inhibiting Hormone (GHIH) can inhibit the production of GH, which is produced through feedback loops (Marieb,2015).

GH is involved in multiple processes reaching a variety of organ tissues**.** The liver is one of many organs that is sensitive to GH and frequently involved in metabolic and growth processes (Marieb, 2015). GH can trigger insulin-like growth factors when it reaches the liver, bones, or muscle. This will promote growth protein production in organs (Marieb,2015). Particular to the liver, insulin-like growth factor will act similar to a hormone (Marieb,2015). Another process GH has on the liver is initiating the production of cholesterol (Marieb, 2015).

Cholesterol

Once growth hormone reaches the liver, cholesterol is created by synthesizing acetyl-CoA into HMG-CoA (Craig et al, 2023); then processes through several other byproducts and catalyzed by enzymes to create cholesterol (Craig et al, 2023). Then other proteins fold the cholesterol molecules before being sent into the blood supply to peripheral tissues (Craig et al, 2023). Cholesterol is a lipid that provides cells with their structure. It is also the scaffolding for all steroid hormones (Marieb, 2015). Therefore, all steroid hormones are derivatives of cholesterol (Craig et al, 2023). Cholesterol can derive either as dietary or de novo—meaning derived from the body, not a source (Craig et al, 2023). The liver synthesizes cholesterol, which occurs in the cytoplasm of hepatic cells (Craig et al, 2023). Once it is produced, it goes to other tissues to

provide structure or partake in the synthesis of steroid hormones, such as those secreted by gonads.

Methods

NHANES was decided upon as an ideal source for data analysis as it demonstrated efficiencies for multiple aspects of the analysis which benefited this learning process for new information. The objective of the analysis was to determine if a cardiovascular measure (cholesterol) was an indicator for PFAS exposure.

NHANES has archived multiple years of data sets dating back to the 1980s (NCHS,2023); the program makes it standard practice to continue updates if needed to maintain the completeness and accuracy of their data sets (NCHS,2023). The data is available to the public and maintained as de-identified secondary data sets. Furthermore, each year is complete with detailed guidebooks and data sets for multiple questionnaires and laboratory panels (NCHS,2023). These reflect nutritional, behavioral and health – wellbeing of thousands of participants. This reflects a significant sample size of the United States' overall wellbeing (NCHS,2023). Therefore, the data sets are comprehensive and extensive.

Because the comprehensiveness and completeness of this secondary data, the collection year for 2017-2018 was determined as ideal because this was the last data collection prior to the COVID-19 pandemic which started in 2019 and could contribute to incomplete data (NCHS,2023); therefore, the 2017-2018 sets balanced relevance and completeness (NCHS,2023). NHANES' regular updates and date range meant the data sets had accuracy and reliability without significant social change or disruption attributed from the pandemic (NCHS,2023). Furthermore, secondary data was an ideal source because the data was already de-identified which resulted as

cost and time efficient because IRB study-specific procedural approvals were not needed as subject recruitment and data aggregation was not needed.

NHANES' 2017-2018 data collections utilized included the following data sets: Total Cholesterol, Triglycerides and Low-Density-Levels, High-Density-Levels, PFAS, Additional PFAS, Smoking Status Questionnaire, Body-Mass-Index Questionnaire, and Demographics. The data sets were downloaded from a JMP format and converted to Excel XLS for user's computer ability. Another consideration was the SAS program. Already, NHANES data sets were ready to use for SAS in the JMP format; due to the code-programming features and data visualization, SAS Enterprise Guide was utilized throughout the process.

Once these files were downloaded from NHANES, code was generated for uploading, sequencing, and merging of all the data sets listed above; to create one record per de-identified participant in SAS Enterprise Guide.

The data was then coded to clean and control. This consisted of formatting and creating categories for Body Mass Index (BMI), smoking frequency, and indications for high cholesterol (Glover, 2022). BMI was categorized into four groupings: underweight (1), normal (2), overweight (3) and obese (4) (Glover, 2022). Smoking was grouped as smoker (1), non-smoker (2), and unknown (3) (Glover, 2022); this third group was included as the sample size was significant size for unknown status. Lastly, Total Cholesterol and Low-Density Levels were controlled as high cholesterol (1) indicated by levels above normal ranges or categorized as not high cholesterol (0) (Glover, 2022). PFAS data was already organized as either detectable or undetectable through NHANES.

Three analyses were performed in SAS Enterprise Guide to better understand the association and relationship between cholesterol and PFAS chemicals: Simple Linear Regression, Logistic

Regression, and ANOVA One Way. These tests were chosen to demonstrate correlation and verify significance. Simple Linear and Logistic Regressions were both performed to accommodate for continuous and categorical data on cholesterol measures. Continuous data for PFAS were utilized throughout analyses.

For each type of analysis, nine PFAS compounds were analyzed. For Simple Linear Regression, Three regressions were performed per chemical. This consisted of Low-Density Level (LDL) Cholesterol-- continuous data, Total Cholesterol—continuous data, and High Cholesterol present—categorized data, formatted in earlier code. Each looked at the correlation of PFAS and Cholesterol levels. For Logistic Regression, one regression per PFAS compound was performed. Each has multiple controls including BMI, smoking status, age, gender, ethnicity, head of household education, head of household marital status, and head of household income level. Lastly, ANOVA One-Way was performed once per PFAS compound, and controlled for BMI, smoking status, age, gender, ethnicity/race, head of household education, head of household marital status, and head of household income level.

Results

Each analysis was analyzed for significance with a 95% Confidence Interval range; therefore, p-values must be equal or less than 0.05 to meet 95% confidence.

ANOVA One-Way (Table 5.) and Logistic Regression (Table 6.) analyses for PFDeA, PFHxS, Me-PFOSA-AcOH, PFNA, PFUA, n-PFOA, Sb-PFOA, n-PFOS, and Sm-PFOS were greater than 0.05 and not in the 95% Confidence Interval range.

For Simple Regression PFDeA (Table 7a.), Me-PFOSA-AcOH (Table 7c.), PFNA (Table 7d.), PFUA (Table 7e.), n-PFOA (Table 7f.), Sb-PFOA (Table 7g.), n-PFOS (Table 7h.), and Sm-

PFOS (Table 7i.), and each analysis for Low-Density Lipid, Total Cholesterol, and High Cholesterol were greater than 0.05 and not in the 95% Confidence Interval range. For Simple Regression PFHxS (Table 7b.), Total Cholesterol had a p-value of 0.8323; High Cholesterol had a p-value of 0.5426. Low-Density Lipid had a p-value of 0.0127; therefore, PFHxS Low-Density Lipid's p-value of 0.0127 is less than 0.05 and within the 95% Confidence Interval range.

Demographics (Tables 1-4)		
Item	**Frequency**	**Percent (%)**
BMI		
Underweight	2861	31.14
Normal	1998	21.75
Overweight	1920	20.90
Obese	2408	26.21
Missing	67	
Smoking Status		
Smokers	805	34.12
Non-Smokers	216	9.16
Unknown	1338	56.72
Missing	6895	
High Cholesterol (LDL or Total)		
High Cholesterol	1882	20.34
No High Cholesterol	7372	79.66

Table 1.

Gender		
Male	4557	49.24357035
Female	4697	50.75642965
Missing	0	
Ethnicity & Race		
Mexican American	1367	14.77199

Other Hispanic	820	8.861033
Non-H. White	3150	34.03933
Non-H. Black	2115	22.85498
Non-H. Asian	1168	12.62157
Other Race- Including Multi R.	634	6.851091
Missing	0	
Education Level (head of household)		
Less than HS degree	1656	18.90
HS/GED/AA/some college	5007	57.13
College graduate/above	2101	23.97
Refused/ Don't Know	0	0
Missing	490	
Marital Status (head of household)		
Married	6006	66.27
Divorced/Widowed	1830	20.19
Never Married	1227	13.54
Refused/ Don't Know	0	0
Missing	191	

Table 2.

Income		
$ 0 to $ 4,999	282	3.218076001

$ 5,000 to $ 9,999	252	2.875727491
$10,000 to $14,999	399	4.553235193
$15,000 to $19,999	536	6.116626726
$20,000 to $24,999	529	6.036745407
$25,000 to $34,999	960	10.95515235
$35,000 to $44,999	893	10.190574
$45,000 to $54,999	607	6.926851535
$55,000 to $64,999	573	6.538856556
$65,000 to $74,999	441	5.032523109
$20,000 and Over	328	3.743010385
Under $20,000	120	1.369394043
$75,000 to $99,999	829	9.460230515
$100,000 and Over	1624	18.53246605
Refused	170	1.939974894
Don't know	220	2.510555746
Missing	491	

Table 3.

Age (at screening)		
0 to 79 y/o	8827	95.385779
80 y/o	427	4.614221
0	357	3.857791
1-9 y/o	1746	18.86752

10-19 y/o	1582	17.09531
20-29 y/o	828	8.947482
30-39 y/o	859	9.282472
40-49 y/o	813	8.78539
50-59 y/o	919	9.930841
60-69 y/o	1104	11.92998
70-79 y/o	619	6.688999
80+ y/o	427	4.614221

Table 4.

ANOVA One-Way		
PFAS Compound		**p-value**
PFDeA		0.7641
PFHxS		0.8849
Me-PFOSA-AcOH		0.1599
PFNA		0.3303
PFUA		0.7014
n-PFOA		0.6292
Sb-PFOA		0.7148
n-PFOS		0.5907
Sm-PFOS		0.4465

Table 5.

Logistic Regression		
PFAS Compound		**p-value**
PFDeA		0.8613
PFHxS		0.5484
Me-PFOSA-AcOH		0.1955
PFNA		0.3565
PFUA		0.8280
n-PFOA		0.7749
Sb-PFOA		0.8270
n-PFOS		0.7117
Sm-PFOS		0.7135

Table 6.

Simple Linear Regression PFDeA		
Cholesterol Value		**p-value**
Low- Density Lipid		0.4472
Total Cholesterol		0.8549
High Cholesterol		0.9401

Table 7a.

Simple Linear Regression PFHxS		
Cholesterol Value		**p-value**
Low- Density Lipid		0.0127
Total Cholesterol		0.8323
High Cholesterol		0.5426

Table 7b.

Simple Linear Regression Me-PFOSA-AcOH		
Cholesterol Value		**p-value**
Low- Density Lipid		0.2262
Total Cholesterol		0.3126
High Cholesterol		0.2252

Table 7c.

Simple Linear Regression PFNA		
Cholesterol Value		**p-value**
Low- Density Lipid		0.4354
Total Cholesterol		0.5952

High Cholesterol		0.3339

Table 7d.

Simple Linear Regression PFUA		
Cholesterol Value		**p-value**
Low- Density Lipid		0.3833
Total Cholesterol		0.9065
High Cholesterol		0.8470

Table 7e.

Simple Linear Regression n-PFOA		
Cholesterol Value		**p-value**
Low- Density Lipid		0.4900
Total Cholesterol		0.4805
High Cholesterol		0.7571

Table 7f.

Simple Linear Regression Sb-PFOA		
Cholesterol Value		**p-value**
Low- Density Lipid		0.2059
Total Cholesterol		0.3858
High Cholesterol		0.7348

Table 7g.

Simple Linear Regression n-PFOS		
Cholesterol Value		**p-value**
Low- Density Lipid		0.9073

Total Cholesterol		0.9514
High Cholesterol		0.7502

Table 7h.

Simple Linear Regression Sm-PFOS		
Cholesterol Value		**p-value**
Low- Density Lipid		0.7410
Total Cholesterol		0.6822
High Cholesterol		0.7227

Table 7i.

Discussion

Like PFAS, organophosphates are evidenced to contributing to irregularities in blood pressure control in the hypothalamus (Glover,2023); this can lead to hypertension and suggesting an attribution to cardiovascular dysregulation originating from the hypothalamus (Glover,2023). PFAS is known as an endocrine disruptor and accumulates in the blood and non-adipose tissues such as the liver and brain (Starnes et al,2022). PFAS has the potential to permeate the hypothalamus and potentially interfere with production and signaling of GHRH and GH because the chemical can penetrate the blood brain barrier and can be present in spinal fluid (Starnes et al,2022).

Furthermore, PFAS and its carboxyl acid function group are reactive with $Ca2^{+}$(calcium) (Brown-Leung and Cannon,2022); creating an increase in calcium release, which can induce cell apoptosis (Brown-Leung and Cannon,2022). Calcium is utilized in dopamine production and PFAS can alter dopamine receptors (Brown-Leung and Cannon,2022). This can impact potency of neural signals that can impact metabolism, mood, reproduction, and memory to name a few. This can impact up- and downstream signaling pathways and feedback loops (Piekarski et al, 2020). Also, there are links to the development of dementia or ADHD (Patel et al, 2016). Therefore, this change in dopamine could have an influence on growth hormone production. The type of PFAS could affect various regions of the brain differently; however, there has yet to be much data on this (Piekarski et al, 2020).

Literature suggests PFAS could significantly impact cardiovascular health, including atherosclerosis, liver fats, and lipid metabolism (Lind and Lind, 2020). In a specific PFAS, PFOA, there was an association between high plasma concentrations of PFOA in the body and self-reported cardiovascular disease, particularly myocardial infarction (Meneguzzi et al,2021).

The heart has a strong correlation between cardiovascular disease or liver damage with high cholesterol as an indicator of either of these diseases (Meneguzzi et al, 2021).

PFAS could potentially interact with the hypothalamic-pituitary axis and their production of growth hormone (GH) through dysregulation. In turn, this could impact cardiovascular disease. Because cholesterol production is initiated with GH, and serum total cholesterol has an association to cardiovascular disease, cholesterol is an ideal indicator for PFAS effect on cardiovascular disease.

According to the analyses, the majority of the regressions and variances did not provide significance with a 95% Confidence Interval range. Although cleaning and controlling data was performed for BMI, current smoking status, and categorization of high cholesterol, further cleaning could be performed for more in-depth analysis.

The only significant value was the simple- linear regression for perfluorohexanesulfonic acid (PFHxS) and Low-Density Lipid. PFHxS is unique because it is commonly found in the blood well after consuming water (Brown-Leung,2022). This is a molecule that is frequently found in the environment because it does not break down. It was phased out of production in 2002 by 3M (Minnesota Department of Health,2019). PFHxS is characterized by its long fluorine-carbon chain and has a sulfonic acid function group (Starnes et al, 2022). Therefore, functional groups could have a more significant role than the length of the fluorine-carbon chain and should be considered in future analyzes. An additional consideration is that the PFHxS may be influencing cholesterol through different routes within the body.

Furthermore, logistic regressions and ANOVA included education level, marital status, gender, age, and income as additional parameters. However, these did not provide significant results either. In future analyses, further cleaning, and more demographic analyses could be performed

as gender, age or generation may be more significant individual factors for health outcomes. Additional cleaning on triglyceride, HDLs, plasma, and diabetes could have been done, too (Lind and Lind, 2020).

Simple Linear Regressions used continuous data sets which include LDL levels and Total Cholesterol levels; whereas Logistic Regressions used categorical data of generalizable high cholesterol to determine if there were correlations between PFAS and cholesterol levels. Both utilized categorical data.

Again, nine NHANES PFAS captured values analyzing if cholesterol levels could be used to indicate PFAS' effects on cardiovascular health. Blood Pressure results could be considered as an indicator in future analyses as a potential indicator of PFAS' effects on cardiovascular health. However, the mechanism in the hypothalamus could be different as blood pressure is controlled in a separate nucleus of the hypothalamus that controls autonomic nervous system functions rather than an endocrine-based region.

Literature suggests that PFAS could significantly impact cardiovascular health—atherosclerosis, liver fats, and lipid metabolism (Lind and Lind, 2020). Rather than just analyzing cholesterol, liver function values such as ALT and AST could be a better predictor for growth hormone function and cardiovascular disease (Lind and Lind, 2020).

Considering the limitations of this study includes the region where the NHANES sample data is captured. Despite NHANES claims the sample is national representation of the United States, there could be confounders that are regionally based. This sample is taken from Los Angeles County, California. It is suspected that areas with known water PFAS-contamination could have higher levels of PFAS in the blood systems of residents. Furthermore, California has implemented policy movements restricting PFAS production—which could have had an impact

on the sample. As noted in the literature, in highly industrialized regions of the United States residents have an increased risk to PFAS exposure (Meneguzzi et al,2021). Another registry could be considered for analysis. Secondly, the pathways of how PFAS affects cardiovascular health have not been established. Also, there will always be a need for additional data regarding specific PFAS chemicals.

Conclusion

Although there has been an established relationship between the effects of PFAS on cardiovascular health since the 1980s, there is still a need to accumulate more knowledge of how PFAS chemicals influence the cardiovascular system.

This area of research could benefit further from more regression analyses by utilizing various registries to analyze correlations between PFAS levels and liver function, lipid, and plasma panels. An accumulation of this information may benefit from meta-analysis.

Considerations for future research should include further cleaning for more specific analyses. This would include analysis of PFAS based on functional group, in-depth analyses of significant-confidence interval valued PFAS chemicals like PFHxS, alternative demographic and health factors, and more cleaning on laboratory panels such as triglyceride, HDLs, plasma, and diabetes (Lind and Lind, 2020). In addition to analyzing cholesterol and lipid panels, liver function values such as ALT and AST could be a better predictor for cardiovascular disease (Lind and Lind, 2020). Comparison between liver and lipid values of high-detectable PFAS levels in blood chemistry could be analyzed. Another consideration would be the region where the NHANES sample data is captured. Although NHANES claims the sample is nationally representative of the entire United States, samples were collected from Los Angeles County, California. Analogous

registries to NHANES can be analyzed in comparison to NHANES results. It is suspected that areas with known water PFAS-contamination could have higher levels of PFAS in the blood systems of residents.

References

Carboxylic Acid Functional Group. Retrieved from https://chemistrytalk.org/carboxylic-acid-functional-group/

Functional groups. *AP®/College Biology.* Retrieved from https://www.khanacademy.org/science/ap-biology/chemistry-of-life/elements-of-life/a/functional-groups#:~:text=One%20example%20of%20a%20strongly,fatty%20acids%2C%20and%20other%20biomolecules.

PERFLUOROALKYL SUBSTANCES (PFAS). (2018). Retrieved from https://www.vdh.virginia.gov/epidemiology/epidemiology-fact-sheets/perfluoroalkyl-substances-pfas/#:~:text=What%20are%20Perfluoroalkyl%20Substances%20(PFAS,of%20household%20and%20industrial%20uses.

Anatomy and Physiology of the Endocrine System Notes. (2023). Retrieved from https://www.osmosis.org/notes/Anatomy_and_Physiology_of_the_Endocrine_System#page-3

Chemicals: Perfluoroalkyl and Polyfluoroalkyl (PFAS) Substances. (2023). *Environmental Health* Retrieved from https://www.dhs.wisconsin.gov/chemical/pfas.htm

Dopamine. (2023). *PubChem Compound Summary for CID 681.* Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/Dopamine

Hypothalamic dysfunction. (2023, 2023). Retrieved from https://www.mountsinai.org/health-library/diseases-conditions/hypothalamic-dysfunction

Per- and polyfluoroalkyl substances (PFAS). (2023). Retrieved from https://echa.europa.eu/hot-topics/perfluoroalkyl-chemicals-pfas

Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS). (2023). *Health & Education.* Retrieved from Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS)

PFAS Explained. (2023). *PFOA, PFOS and Other PFAS.* Retrieved from https://www.epa.gov/pfas/pfas-explained

Somatotropin (32-38). (2023). *National Center for Biotechnology Information.* Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/Somatotropin-_32-38

Barlow, C. A., Boyd, C.A., Kemp, M.J., Hoppe Par, K.A. (2019). PFAS Toxicology What is Driving the Variation in Drinking Water Standards?

Bear, M. H., Reddy, V., & Bollu, P. C. (2023). Neuroanatomy, Hypothalamus. In *StatPearls*. Treasure Island (FL) ineligible companies. Disclosure: Vamsi Reddy declares no relevant financial relationships with ineligible companies. Disclosure: Pradeep Bollu declares no relevant financial relationships with ineligible companies.

Bilott, R. (October 8, 2019). *Exposure*.

Bookstaff, R. C., Kamel, F., Moore, R. W., Bjerke, D. L., & Peterson, R. E. (1990). Altered regulation of pituitary gonadotropin-releasing hormone (GnRH) receptor number and pituitary responsiveness to GnRH in 2,3,7,8-tetrachlorodibenzo-p-dioxin-treated male rats. *Toxicol Appl Pharmacol, 105*(1), 78-92. doi:10.1016/0041-008x(90)90360-7

Brennan, N. M., Evans, A. T., Fritz, M. K., Peak, S. A., & von Holst, H. E. (2021). Trends in the Regulation of Per- and Polyfluoroalkyl Substances (PFAS): A Scoping Review. *Int J Environ Res Public Health, 18*(20). doi:10.3390/ijerph182010900

Brian C. Crone, T. F. S., David G. Wahman, Samantha J. Smith, Gulizhaer Abulikemu, Eric J. Kleiner, Jonathan G. Pressman. (2019). Occurrence of Per- and Polyfluoroalkyl Substances (PFAS) in Source Water and Their Treatment in Drinking Wate. *Crit Rev Environ Sci Technology, 49*. doi:10.1080/10643389.2019.1614848

Brown-Leung, J. M., & Cannon, J. R. (2022). Neurotransmission Targets of Per- and Polyfluoroalkyl Substance Neurotoxicity: Mechanisms and Potential Implications for Adverse Neurological Outcomes. *Chem Res Toxicol, 35*(8), 1312-1333. doi:10.1021/acs.chemrestox.2c00072

Cao, Y., & Ng, C. (2021). Absorption, distribution, and toxicity of per- and polyfluoroalkyl substances (PFAS) in the brain: a review. *Environ Sci Process Impacts, 23*(11), 1623-1640. doi:10.1039/d1em00228g

Craig, M., Yarrarapu, S. N. S., & Dimri, M. (2023). Biochemistry, Cholesterol. In *StatPearls*. Treasure Island (FL) ineligible companies. Disclosure: Siva Naga Yarrarapu declares no relevant financial relationships with ineligible companies. Disclosure: Manjari Dimri declares no relevant financial relationships with ineligible companies.

Craig, M., Yarrarapu, S. N. S., & Dimri, M. (2023). Biochemistry, Cholesterol. In *StatPearls*. Treasure Island (FL) ineligible companies. Disclosure: Siva Naga Yarrarapu declares no relevant financial relationships with ineligible companies. Disclosure: Manjari Dimri declares no relevant financial relationships with ineligible companies.

DiGiannantonio, M. (2022). A Legal History of PFAS.

Elaine N. Marieb, K. H. (January 3, 2015). *Human Anatomy & Physiology* (10th ed.): Pearson.

Feng, X., Long, G., Zeng, G., Zhang, Q., Song, B., & Wu, K. H. (2022). Association of increased risk of cardiovascular diseases with higher levels of perfluoroalkylated substances in the

serum of adults. *Environ Sci Pollut Res Int, 29*(59), 89081-89092. doi:10.1007/s11356-022-22021-z

Friedman, L., Giang, V. (2023). 3M Reaches $10.3 Billion Settlement in 'Forever Chemicals' Suits. *The New York Times*.

Frisbee, S. J., Brooks, A. P., Jr., Maher, A., Flensborg, P., Arnold, S., Fletcher, T., . . . Ducatman, A. M. (2009). The C8 health project: design, methods, and participants. *Environ Health Perspect, 117*(12), 1873-1882. doi:10.1289/ehp.0800379

Glover, F. The Investigation of Chlorpyrifos Exposure on Hypertension and Metabolic Syndrome Prevalence and its Dysregulatory Effects within Hypothalamic Blood Pressure and Metabolism Pathways

Health Risk Assessment Unit, M. D. o. H. (2019). Health Risk Assessment Unit *Clean Water, Land, & Legacy Amendment*.

Jung, E., Kong, S. Y., Ro, Y. S., Ryu, H. H., & Shin, S. D. (2022). Serum Cholesterol Levels and Risk of Cardiovascular Death: A Systematic Review and a Dose-Response Meta-Analysis of Prospective Cohort Studies. *Int J Environ Res Public Health, 19*(14). doi:10.3390/ijerph19148272

Klein, D. R. (December 31, 2013). *Organic Chemistry* (2nd Edition ed.): Wiley.

Koskela, A., Ducatman, A., Schousboe, J. T., Nahhas, R. W., & Khalil, N. (2022). Perfluoroalkyl Substances and Abdominal Aortic Calcification. *J Occup Environ Med, 64*(4), 287-294. doi:10.1097/JOM.0000000000002479