Review Article

PFAS Binding Properties and Impact on Human Health: Physiochemical Properties, Impact of Multiple PFAS Compounds, Variation Among Species, and Fluoride Interaction.

ABSTRACT

PFAS are chemical compounds that are found in consumer goods and are known contaminates found in both the environment and within the body. Research has begun to help better understand these compounds and how they impact human health and the environment. While there is vast research surrounding these compounds, they have many different characteristics that make it difficult to understand their full impact. These compounds are even more complex because they can bio transform and interact with each other exhibiting competitive behavior. It is suggested that the properties of these chemicals have a significant impact on the ability to act in the body. Another interaction that can have human and environmental implications is that there is evidence of interactions between fluoride in the water with the PFAS contaminates. Studies also suggest that there are differences among species when exposed to PFAS and the physiological pathways impacted. The purpose of this review paper is to provide public health professionals the risk assessment tools to understand the properties of these compounds, not addressed to date and to better understand how PFAS react in the body, interact with one another, and interact with other inorganic substances.

Keywords: environmental contamination, Polyfluorinated substances, precursor molecules, kidneys, competitive binding

1. INTRODUCTION

Per and Poly fluorinated substances are a large complex group of over 6000 chemicals that are made up of carbon chains and surrounded by fluorine atoms. PFAS are very stable compounds that are difficult to remove from the environment and their chemical structures result in them being hydrophobic while lowering the surface tension of water. PFAS are commonly used in consumer goods due to their unique properties, which allow them to create nonstick surfaces (Glüge et al., 2020) [1]. The two compounds that were produced at the highest quantity historically were PFOA and PFOS and production of these compounds has since been halted. These chemicals have been found in all major environmental media such as soil, groundwater, surface water, and air (*Public Health Statement: Perfluoroalkyls* 2015) [2]. Over time, research has begun to show that these chemicals are significant environmental contaminants that are found in drinking water and are absorbed by plants and animals which have led to the contamination of food across the globe. Continued research has indicated that the consumption

of these chemicals is linked to various health concerns and further research is needed to understand more about the possible health risk (FDA, 2022) [3]. A large amount of uncertainty surrounds these compounds leading global regulators such as the USEPA to propose extremely conservative assumptions to risk assessments in recent months resulting in proposed drinking water regulations of 0ppt for PFOS and PFOA despite limited evidence in their distinct part in the complex mixtures and exposure patterns of this chemical group.

Research associated with the exposure to PFAS has indicated risks such as disruption of thyroid hormones, liver cancer, kidney disease, as well as difficulty with lipid and insulin regulation (Fenton et al., 2021) [4]. Melzer et al., 2010 [5] performed a representative population study that determined there was an association between higher PFOA concentration and thyroid disease however, other studies came to a different conclusion (Melzer et al., 2010) [5]. When looking at the impact of these compounds on the liver there are multiple studies that research not only the effect but the mode of how these chemicals cause damage (Fenton et al., 2021) [4]. Filgo et al., 2015 [6] performed a study that looked at rodents and how exposure to PFAS could have contributed to the presence of adenomas in the liver. This study specifically looked at peroxisome-activated receptors as the mechanism of tumor formation. The results of this study did indicate a correlation between high PFAS exposure and the number of tumor incidences however, the study indicated differences between the rat and human model and how there is still extensive research needed to fully understand the various modes of action (Filgo et al., 2015) [6].

Most of the research that has been done on PFAS have been done as single compound exposures which limits the knowledge researchers have on how these various compounds interact with one another. One researcher observed PFAS and how sorption can be used as a method of removing these chemicals. In this study it discussed how many of these chemicals are exposed as a mixture of contaminants with other organic substances or other PFAS. This research concluded that the interaction of PFAS indicates that these chemicals can strongly compete with one another for sorption sites (Kah et al., 2021) [7]. The results indicate the importance of understanding the interaction of PFAS with one another and the need to further examine the research considering the mechanisms of interaction/s with one another before and after exposure by humans. Another important aspect to consider when looking at the current research is how these animal studies translate to humans. Research has begun to surface that these chemicals can compete and displace one another (both in and outside human tissues), which would have a significant impact on how they react in the human body.

The interaction of PFAS and inorganic ions clarifies the impact PFAS can have on both the environment and human health. The addition of fluoride in the water is a common practice and has caused a dramatic increase in fluoride concentration found in groundwater. The research surrounding this increase in fluoride concentration has raised health concerns that are associated with the exposure of fluoride including reduced kidney function in humans with increased residence time of toxins within the renal complex (Nordstrom & Smedley, 2022) [8]. The high concentration of these inorganic anions makes it likely that some form of interaction exists between fluoride and PFAS. The research suggest that this interaction is found through the photocatalytic process and indicates that both fluoride and PFAS have an impact on one another (Sansotera et al., 2014) [9]. This initial review looks to fully understand these PFAS by looking at their various properties and how that can influence their ability to bind, interact with each other, and the interaction they have with inorganic molecules. The purpose is to understand the various characteristics of PFAS and analyzes the health concerns associated with exposure and how this can differ across species.

LITERATURE REVIEW

1.1 PHYSIOCHEMICAL CHARACTERISTICS AND PFAS BINDING

An important step in researching PFAS and their impact on human health is to understand their physical and chemical properties and how they can bind to proteins in the body. When looking to understand the chemical properties of PFAS, studying their interaction in the soil can help researchers gain a better understanding of how they impact the environment and possible biological implications. A study conducted by Nguyen et al., 2020 [10] looked to understand the impact various soil and PFAS properties have on each other. To conduct this study soil samples that had varying types of soil were combined and exposed to various pH levels then analyzed. The Kd value which represents the soil-sorption coefficient was measured based on the concentration of PFAS in the aqueous phase compared to the soil phase. The results looked at the comparison of various factors and the Kd value that was found. The carbon chain length was one factor that was analyzed, and the results showed that compounds categorized as long chained had a positive correlation with the length of the chain and Kd value. These results suggest that hydrophobicity plays a significant role in the sorption in organic matrixes such as soil but also tissues.

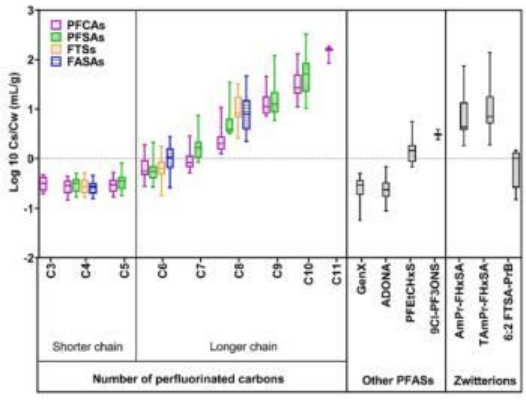

Figure 1: Kd value compared to carbon chain length.

Another aspect measured in this study was the impact molecular weight had on Kd value. The smaller molecules showed no correlation to MW and Kd value however, the larger molecules showed a positive correlation.

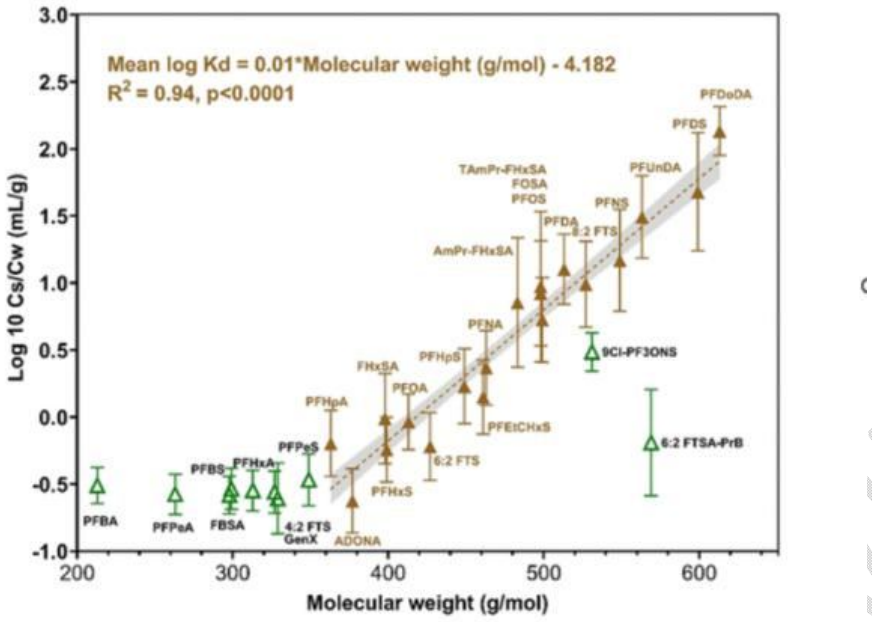

Figure 2: Kd value compared to molecular weight.

The study believed this was due to hydrophobicity and more contact points due to their size. This result is consistent with previous studies that indicate with an increase in molecular weight there is a decrease in motility (Evich et al., 2022) [11]. The results showed that all PFAS Kd values decreased with an increase in solution pH. Overall, this data suggests that longer-chained PFAS compounds are more likely to be absorbed to the soil phase, where shorter chained are more readily found in the aqueous phase**.**

Sheng et al., 2018 [12] performed a study that tested the difference in PFAS and alternative PFAS compounds to analyze their differing structures and understand how that could influence their ability to bind and their level of toxicity. To conduct this experiment researchers tested the differences in 2 PFAS legacies and 6 different alternative compounds (6:2 Cl-PFESA, 6:2FTCA, 6:2 FTSA, HFPO-DA, HFPO-TA, and HFPO-TeA). Tests were completed to determine the effect each compound had on cell viability, changes in gene expression, and their ability to bind. To test the cell viability, human liver cells were exposed to each of these chemicals, then a MTT and a neutral red assay was performed to understand the activity of the liver cells. Half of the exposed cells were then analyzed using flow cytometry to understand the amount of cell proliferation expressed among the groups, while a PCR was performed on the other half of cells to analyze changes in gene expression. A CD spectroscopy was used to analyze the impact the chemicals had on the structure of (human liver-fatty acid binding protein) hL-FABP. To determine the ability of these different chemicals to bind, a florescent placement assay was performed as well as the use of software to analyze these molecules and their process of molecular docking**.**

The results of the MTT and neutral red assay was recorded on concentration response curves that compared the dose concentration and the inhibitory effect the compound had on liver cells. The recorded data from these tests indicate that at low concentrations these chemicals have a stimulatory effect on the cells, while at high concentrations they have inhibitory effects. The flow cytometry results showed the effects that these chemicals have on the cell cycle and suggested that four of the groups (6:2 FTCA, HFPO-TA, HFPO-TeA, 6:2 FTSA) promoted cells from the G2 phase into the S phase. Specifically, in the 6:2 FTSA group there was an increase in the cell population in the S phase from 20 percent to 21.27 percent.

The results of the PCR observed that the changes in gene expression varied among treatment groups, however overall, the groups showed no up-regulation in PPARa and other related genes but did show upregulation with high dosage in genes that are involved in the cell cycle such as Cdk2 and Cdk6. The data also showed an up-regulation of Ddit3 when cells were exposed to HFPO-TA and HFPO-TeA. This gene, when expressed, can result in cellular damage therefore the up regulation of this gene can indicate higher levels of toxicity.

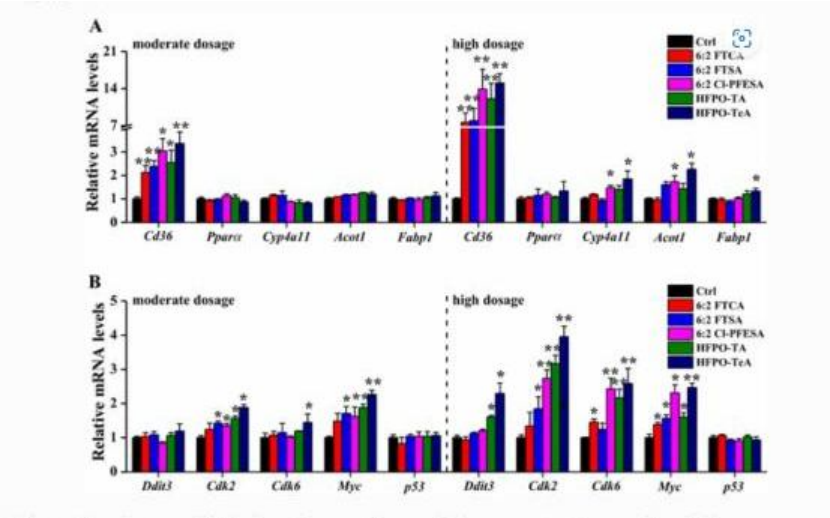

Figure 3: level of genes expressed after exposure.

The data from these tests suggest that these chemicals could cause proliferation in cells by promoting the movement of cells into the S phase of the cell cycle. There was also an indication of up-regulation in Ddit3 indicating another means of toxicity through cell damage. The results showed the highest level of cytotoxicity was found in HFPO-TeA, which consist of a backbone atom number of 12, compared to the other compounds that exhibited lower levels of cytotoxicity and were associated with a lower backbone number. The results indicate that while all these compounds (excluding 6:2 FTCA) do have a level of cytotoxicity similar if not stronger than the two legacies, the extent of toxicity can vary among each compound due to their structure.

The results from the CD spectroscopy indicated that the exposure of PFAS to liver cells caused a change in the recombinant protein structure. The typical protein make up contained 15.7 percent alpha helix and 54.4 percent beta sheets, while after the exposure of PFAS the alpha helix percent increased while the beta sheet decreased. The extent of this change varied among the groups depending on several characteristics such as the backbone or functional group. The results suggested that HFPO-TeA was the group that caused the most change due to their structure of the 12-atom backbone and three side chains. The results of the molecular docking showed that all PFAS excluding FTSA directly interact with the liver cell binding protein. While they all showed direct interaction it was determined that this interaction could occur through various methods. PFOA, PFOS, and 6:2 FTCA showed the same method of carboxyl head interaction with the amino acids causing a hydrophobic reaction and forming a hydrogen bond. 6:2 CL-PFESA and HFPO illustrated a different type of reaction due to the oxygen found in their backbone. The data concluded that the compound HFPO-TeA had the greatest binding affinity and the greatest impact in the protein structure**.** These results support that the structural

differences among these compounds play a role in their ability to bind to proteins and indicate a correlation between the backbone length and ability to bind to proteins to cause potential harm.

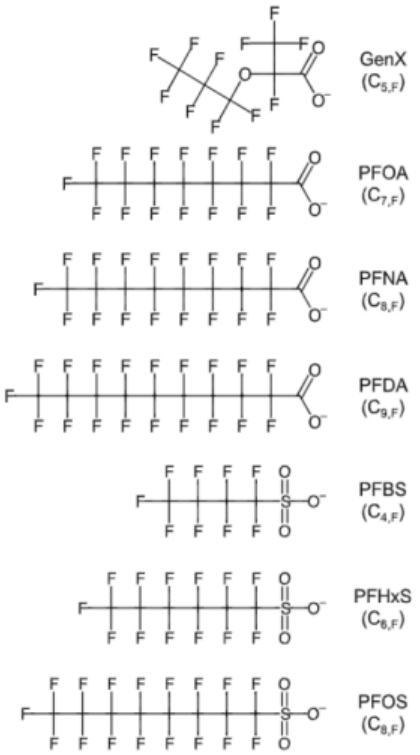

Figure 4: Chemical structure of PFAS compounds

Alesio et al,. 2022 [13] performed research that looked at the differing structures of PFAS and their ability to bind to albumin. To conduct their research florescent quenching and equilibrium dialysis were performed. Fluorescent quenching is a technique that can be used to measure the specific binding of organic molecules due to the amino acid residues. Equilibrium dialysis which measured all forms of binding using liquid chromatography/mass spectrometry. Seven various PFAS were used that had differing structures (GenX, PFOA, PFNA, PFDA, PFBS, PFHxs, PFOS).

The samples used in this experiment were created by combing serum albumin with each of the chemical compounds and with a phosphate buffer and the samples were then incubated. The results from the equilibrium dialysis were reported as a fraction of bound PFAS (*f)* which differed among the compounds. PFNA, PFDA, and PFO were the compounds that showed the highest *f* indicating a greater affinity to bind, whereas the compounds PFOA, PFBS, and PFHxS exhibited lower *f* showing a weaker binding affinity**.** The results suggest that the *f* increased with the number of carbons supporting the idea that compound backbone length increases their ability to bind.

The florescent quenching results suggested that PFBS had the lowest ability to bind with a *Fa* value of 3. The article contributes this result as PFBS being the smallest and most water-soluble compound, meaning it did not contain the same hydrophobic properties that is seen in other chemicals that illustrated strong binding affinities. Another compound that exhibited low binding affinity was GenX, which was believed to be due to its nonlinear structure preventing the

compound from binding. PFNA had a higher association constant than PFOS even though those two compounds contain the same number of carbons. These results suggest that other factors influence binding affinity such as size, hydrophobicity, and reaction with proteins all play a role**.** Another result from the florescent quenching data shows that both PFBS and GenX did not show any shifts in wavelength, indicating that these compounds did not react with the hydrophobic pocket also contributing to their low binding affinity. Through both methods in this study research suggests that the physical and chemical makeup of these compounds greatly influence their ability to bind to proteins in the body.

Serum albumin is an important area to observe because PFAS binding can have a significant impact on human health. A study conducted by Maso et al., 2021 [14] looked specifically at PFOA and its mechanism to bind to serum albumin. The study looked at the structure of hSA-PFOA complex by crystalizing both albumin and PFOA with a fatty acid (Myr) to replicate physiological conditions. The results show the definite binding sites of both PFOA and Myr molecules. The four binding sites identified as PFOA binding sites were located at Sudlow's drug-binding site 1 and II. The mechanism of binding exhibited by PFOA is first the carboxylate head group of PFOA1 binding site forms a hydrogen bond and interacts with various side chains on the amino acids. The fluorine atoms then form a polar interaction with oxygen and nitrogen atoms on the side chains. Through non-polar and polar interactions, this bond is further stabilized forming the Myr-hSA complex. The data shows that the various binding sites had slightly different mechanisms and different affinities for different compounds. In the study researchers compared PFOS and PFOA and their interaction with hSA. The results of this study help show that hSA is a protein that PFAS can readily bind to, and researchers looked to study the mechanism of binding. The various binding mechanisms and affinity to bind can be influential when understand the negative impact these chemicals can have in the body.

1.2 PFAS COMPETITVE BINDING AND DISPLACEMENT

While research has shown that various PFAS when exposed together can interact with one another, the method in which they interact is important to understand. Displacement is one way these compounds can interact with one another, which makes it important to research and understand how this reaction can affect body systems. A review written by Kah et al., 2021 [7] begins to investigate the effects PFAS have on one another. Research articles have shown the competitive nature of these compounds and have even suggested short chain compounds can be displaced from sorption sites due to long chain compounds.

A study conducted by Yang et al., 2021 [15] looks to introduced one way these molecules can interact through PFOS transformation pathways and their degradation behaviors. To conduct this experiment GOTiO2 was used as an electrode and photoelectrical degradation experiments were performed. This study looked at multiple different aspects such as degradation pathway, level of toxicity, and the influences of other factors on PFOS degradation. The results of the study showed different transformations PFOS can make when going through reactions, one results of degradation were the byproducts PFSAs and PFCA's. These compounds were observed both individually and in a mixture system to understand their interaction with one another. The degradation rates were observed for these compounds individually and the results indicated that the compounds with shorter chains had lower degradation rates. These rates were then compared to degradation rates exhibited in the mixture system. The results in this mixture system concluded that PFAS with longer carbon chains saw no significant decrease in the rate of degradation compared to the individual system. However, those with shorter chain saw a significant decrease in their degradation rate suggesting competitive inhibition among the compounds. This study helps illustrate the idea that

PFAS when studied in a mixture system may interact with one another influencing their toxicity in the mammalian tissues.

Wang et al., 2019 [16] conducted a study to analyze the competitive nature of PFOS, PFBS, PFOA, and PFBA by testing their absorption behavior. Absorption experiments were done both with a single solute and multiple solutes to compare both single solute absorption and co-absorption. The study suggests that in the single solute system the absorption capacity should be the same for all the compounds but because it is not, it suggests that other factors such as backbone length and hydrophobic interaction influence their absorption capacity. When comparing the co absorption experiment the results show that at a higher concentration of exposure the removal of PFBA and PFBS decreased by 77.78% and 72.09, this data suggest that PFAS with shorter chain lengths could be replaced by compounds with longer chains.

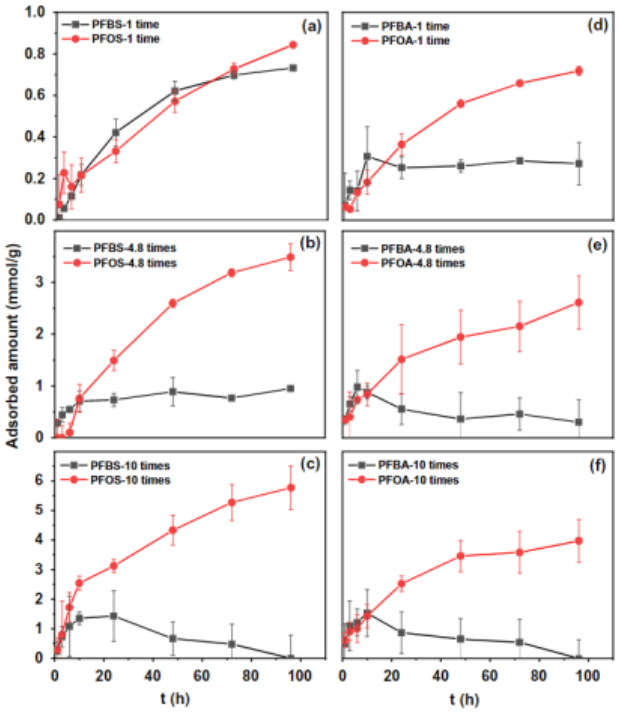

Figure 5: Amount of each chemical absorbed over time.

When looking at the various characteristics one important aspect to study is the ability for these chemicals to bioaccumulate and biomagnify**.** A study conducted by Chen et al., 2018 [17] looked to analyze various PFAS and their concentrations found in water, sediment, and wildlife using samples from Tahiu lake. When examining the concentration of these chemicals found in various organisms it was determined that there was large variation between the compounds and their ability to accumulate in organisms. PFOS was one compound that was found in high concentrations in all organisms indicating high ability to accumulate and possible precursor transformation in these organisms. It is important to note that up to 80-90% of PFOS can convert to PFOA in organic matrixes such as human tissue and organic soils. The concentrations of these chemicals in the water, soil, and organisms is seen in the figure below.

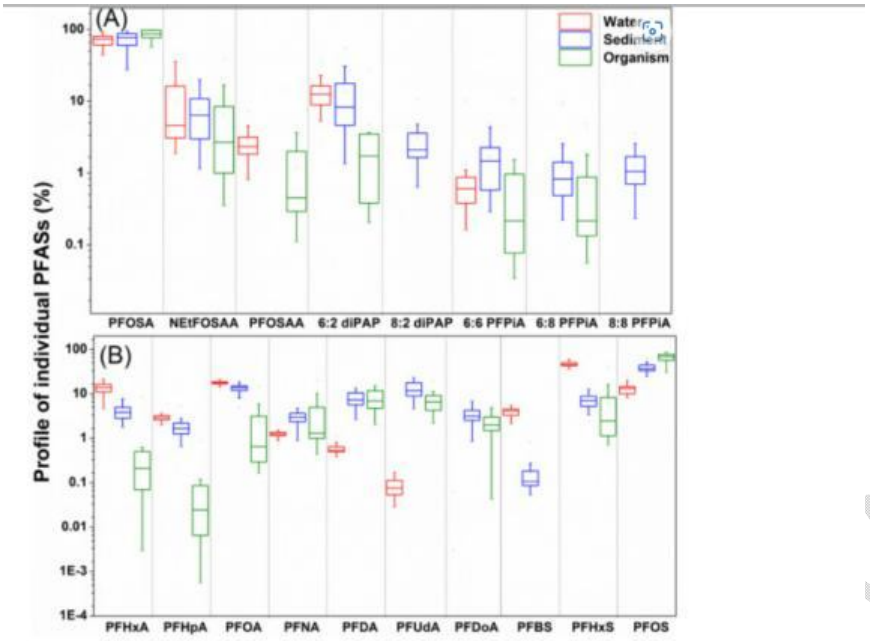

Figure 6: Composition of PFASs in various samples

The study also looked at the magnification of these chemicals by monitoring the concentration and comparing the trophic level of these organisms. The results for several compounds showed a positive correlation with PFAS concentration and trophic levels. The chemicals that exhibited this positive correlation are shown in the graphs below.

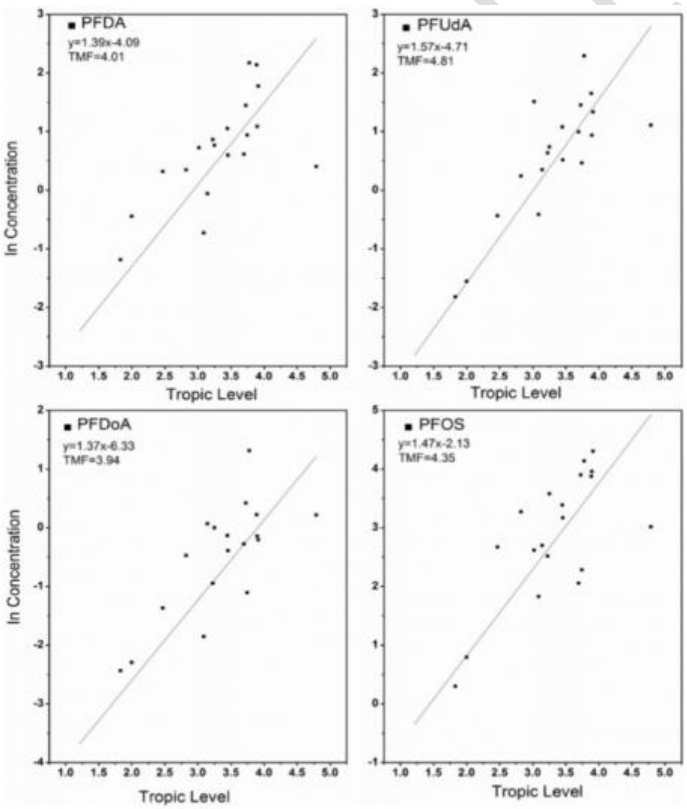

Figure 7: Concentration of various PFAS across tropic levels.

This data is an important factor that shows the large impact these chemicals can have not only on the environment but the significant accumulation/distribution they have in our food chain and subsequently in various tissue systems.

2.3 DIFFERING EFFECTS AMONG MAMMALS

Research that has been done to understand the human health impacts that have been performed on rodents which are poor models for certain toxicological endpoints such as PPAR activation. This was a topic of much debate on the 2005-2008 PFOA SAB Expert Panel. There is a need to further this research to understand key biological differences among species.

Bjork and Wallace, 2009 [19] conducted a study to examine the effects of PFOA on cell proliferation. They examined the impact these compounds had on genes that were a transcriptional response to peroxisome proliferation and how their impact can be compared among humans and rats. Hepatocytes from both rats and humans were obtained and samples were prepared. After incubation individual PFAS were added to the samples while DSMO was added to the control. The same methods were completed with samples containing HepG2/C3A cells. The RNA within these cells were then isolated and a reverse PCR was performed to analyze the gene expression within these cells. The results showed that with exposure to PFOA there was an increase in Didt3 gene expression in rat hepatocytes which is a gene that can contribute to many forms of DNA damage. Another gene analyzed in this experiment was the Acox gene that is transcribed in a response to peroxisome proliferators. The results showed that with the exposure of PFOA primary, rat hepatocytes showed an increase in expression of the Acox gene while there was no differences among human and HepG2/C3A cells compared to the control.

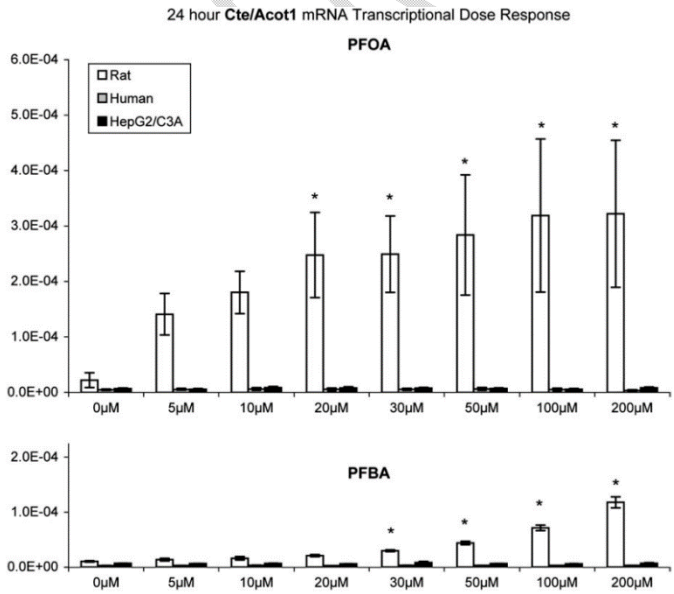

Figure 8: Acox gene expression after exposure.

Another gene that is readily associated with peroxisome proliferator is Cte/Acot1 which exhibits the same trends as seen with the previous genes.

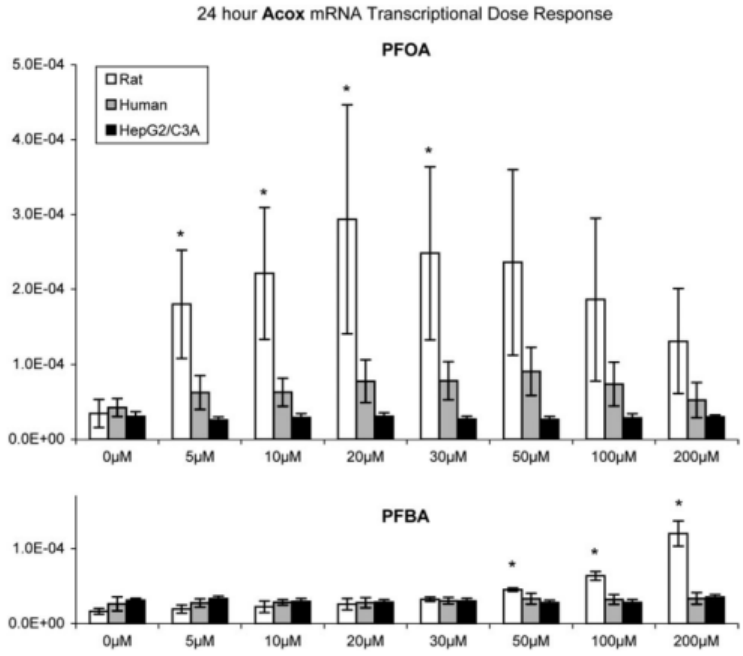

Figure 9: Cte/Acot1 gene expression after exposure.

This study concludes that the length of the carbon chain has a direct impact on how these compounds can interact and cause various levels of potential insult in the body. When comparing the impact of PFOA on human and rat liver cells through the mechanism of activating PPAR, there is significant impact when looking at rat cells however there was no significant impact observed in human cells. Ammerschlaeger et al., 2004 [18] performs a study that further confirmed these results suggesting that differences in the promoter sequences of rats and human cells could play a role in the peroxisomal proliferator differences.

Bjork et al., 2011 [20] continued research on the harmful impacts of PFOS and looked to study the impact of PPARa independent effects while looking at both rodent and human cells. The expression of genes was analyzed to understand and recognize the activation of different nuclear receptors. The results indicated variation among these nuclear receptors when exposed to both PFOA and PFOS. The results compared multiple genes such as Nr1i3 and Nr1i2 that are involved in drug metabolism and elimination in cells. Nr1h3, which plays an important role in liver through lipid and cholesterol biosynthesis, was also compared and observed. Lastly, Nr1h4, a gene that is involved in the regulation of carbohydrate metabolism was tested and

compared. The results of this study are included in the table below and a general trend is observed that the rat hepatocytes saw more upregulation among those genes.

Table 1: Comparison of human and rat gene expression

		25uM PFOA		25uM PFOS		Ratio of Ctls
nReceptor (Gene)	Marker transcript (rat/human)	Rat	Human	Rat	Human	Human/Rat
Ppara	*Acox1/ACOX1*	6.5	1.8	3.0	1.4	1.8
	Ehhadh/EHHADH	70.6	1.4	21.8	1.1	23.2
	Cyp4a1/CYP4A11	59.1	3.1	27.3	1.4	15.8
Nr1i3 (Car)	*Cyp2b2/CYP2B6*	11.2	2.1	6.2	3.2	7.7
	Cyp2c6/CYP2C19	1.7	1.2	2.2	1.5	12.7
Nr1i2 (Pxr)	*Cyp3a1/CYP3A4*	1.8	1.6	3.3	2.2	3.0
	Abcb1/ABCB1	1.5	1.6	1.1	1.4	0.9
Nr1h3 (Lxrα)	*Srebf1/SREBF1*	1.9	1.7	1.9	1.5	0.1
	Abcg5/ABCG5	1.9	NA	1.6	NA	NA
	Abca1/ABCA1	1.1	1.5	1.3	1.5	0.3
Nr1h4 (Fxr)	*Abcb11/ABCB11*	1.2	0.7	1.4	1.6	0.02
	Nr0b2/NR0B2	1.1	1.2	1.2	1.6	11.7

Another aspect of this study was to analyze the impact PFOS and PFOA had on metabolic pathways found in both rat and human liver cells. The metabolic pathways that were analyzed was fatty acid metabolism, carbohydrate metabolism, Ammonia metabolism, and pathways involving ketogenesis. The results from the analysis of these pathways are shown in the table below.

Table 2: Comparison of impact on metabolic pathways

Fatty Acid Metabolism		PFOA		PFOS		Ratio of Ctls
		Rat	Hu	Rat	Hu	Human/Rat
Oxidation	*Acox1/ACOX1*	6.5	1.8	3.0	1.4	1.8
	Ehhadh/EHHADH	70.6	1.4	21.8	1.1	23.2
	Cyp4a1/CYP4A11	59.1	3.1	27.3	1.4	15.8
	Aldh1a1/ALDH1A1	1.9	1.0	1.5	1.3	27.7
Transport	*Cd36/CD36*	4.3	1.0	2.6	1.5	0.02
Synthesis	*Fasn/FASN*	0.9	1.8	0.7	0.9	2.4
	Scd1/SCD	4.3	2.3	4.0	1.5	0.2
Glycerolipid synthesis	*Gk/GK*	5.7	1.8	3.6	0.9	1.0
	Gpam/GPAM	1.3	1.4	1.5	1.2	0.2
	Agpat3/AGPAT3	1.5	1.3	1.0	1.3	1.3

Carbohydrate Metabolism		PFOA		PFOS		Ratio of Ctls
		Rat	Hu	Rat	Hu	Human/Rat
Gluconeogenisis	*Pck1/PCK1*	0.8	2.0	0.6	0.9	2.0
	Pck2/PCK2	1.8	1.5	1.5	1.7	2.7
	G6pc/G6PC	1.0	1.3	0.9	1.2	1.2
Glycolysis	*Gaphd/GAPDH*	1.2	1.2	1.1	1.2	0.4
	Pklr/PKLR	0.1	0.9	0.2	0.8	0.3
	Pdk4/PDK4	22.5	3.3	7.3	1.5	0.8
	Slc2a2/SLC2A2	0.4	0.8	0.6	0.9	0.4
Glycogen Synthesis	*Ugp2/UGP2*	0.8	1.2	1.2	1.4	2.6
	Phka2/PHKA2	1.1	1.7	1.1	1.4	2.3
	Ppp2r5c/PPP2R5C	1.0	1.0	1.0	1.0	1.6

Amonia (protein) Metabolism		PFOA		PFOS		Ratio of Ctls
		Rat	Hu	Rat	Hu	Human/Rat
Urea Cycle	*Cps1/CPS1*	0.4	0.6	0.8	0.5	0.6
	OtcOTC	0.4	1.0	0.6	0.8	1.9
	Ass1/ASS1	0.5	1.3	0.6	1.5	0.9
	Asl/ASL	1.1	1.1	1.0	1.1	0.7
	Arg1/ARG1	0.6	0.8	0.9	0.7	0.4

Other Metabolism		PFOA		PFOS		Ratio of Ctls
		Rat	Hu	Rat	Hu	Human/Rat
Ketogenisis	*Hmgcs2/HMGCS2*	3.8	3.3	1.9	1.1	0.3
Bile acid biosynthesis	*Cyp7a1/CYP7A1*	0.9	0.5	2.8	0.7	2.1

Fatty acid metabolism pathways indicate both catabolic and anabolic reactions, therefore researchers believe this could lead to the cycling of lipid intermediates that do not create useful products which wastes energy causing harm. The genetic markers observed in the study, in general**,** were seen to be more impacted in rat cells than human cells. It is important to note in human tissue which have a recognized complex “sink” of bioaccumulated and transformed PFAS compounds that single compound study results must be carefully interpreted when comparing to real world human exposures.

2.4 WATER FLUROIDATION

Fluoride is an inorganic anion that is commonly found in dental products and is also found in water sources such as groundwater. Fluoride is used because it has been shown to help prevent tooth decay. Research began to show that after certain concentrations the fluoride in water began to have harmful effects such as tooth molting. This research is critical because studies have shown that over 200 million people are exposed to fluoride exceeding the concentration that is deemed safe (Nordstrom and Smedley, 2023) [8]. Multiple studies looked to measure at what concentration did signs of teeth molting begin and it was determined to be a concentration of 1mg/L (Dean, 1936) [21].

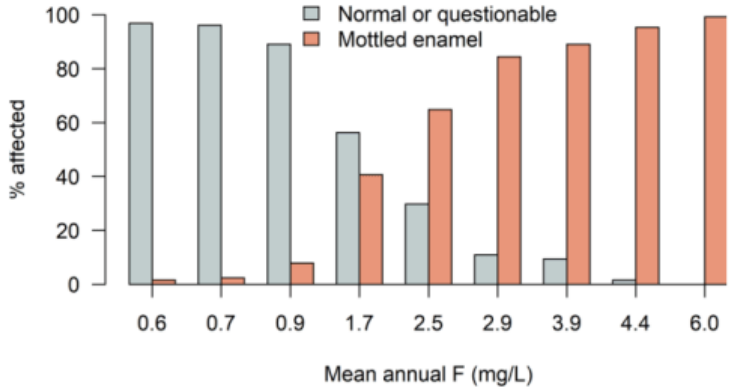

Figure 10: Molted teeth compared to fluoride concentration.

Today researchers are continuing to look and test methods of removing fluoride from water in some areas while, in other areas they are continuing to add this anion into the water. With the continued use of fluoride, it is important to understand the potential harmful effects it has on human health, specifically on the liver, kidneys, and the blood.

A study, completed by Zhao et al., 2014 [22], looked at the impact of fluoride on kidneys and the liver. In this study four groups of rats were tested: control, fluorosis, blocking, and blocking control. The blocking groups were characterized by the rats being injected with either cyclopamine or dimethyl sulfoxide. The purpose of these groups was to analyze the impact cyclopamine had on the Hh pathway and testing it as a potential treatment for fluorosis. The fluorosis group was the focus in this review to understand the impact fluoride has on the Hh pathway and how that can contribute to harm in the body. The control group was given tap water and the other groups were given sodium fluoride. The results of this study showed that after the initial exposure the control group showed no signs of fluorosis while in the fluorosis and blocking group it was expressed in 91.67 percent and in the blocking control 83 percent. Using an ion selective electrode method, urine samples and the bones of the rats were collected to measure the fluoride content, the results are shown in the table below.

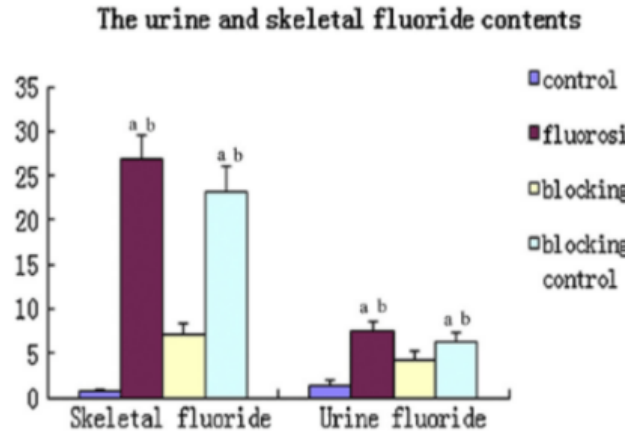

Figure 11: Comparison of Fluoride concentration among groups.

The liver function of these groups were tested by analyzing enzyme and protein concentration found in the blood. The results showed an increase in ALT and AST levels, enzymes associated with liver function, in the fluorosis and blocking control group while there was decrease in albumin and total protein levels. The study contributes the increase in those two enzymes and a decrease in protein due to the rapid uptake of fluoride in the body which causes the generation of free radicals which can ultimately lead to liver damage.

When looking at the liver dissection from the rats a light microscopy was used to analyze the physiological changes. When compared to the control the fluorosis group showed signs of damage such as enlargement, fatty degeneration, partial loss of membrane, and other abnormalities.

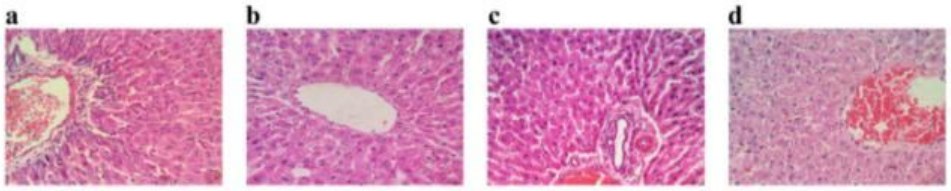

Figure 12: Images of liver dissection comparing the groups.

Proteins that impact the Hh signaling pathway have an important role in tumor suppression, transduction of target genes, and negative feedback helping to maintain self-regulation. A PCR and a Western blot test were both performed to analyze the expression of Shh, Smo and Gli1 proteins involved in this pathway. In both the fluorosis and blocking control group there was an increase in expression of these proteins compared to the control and blocking group. These results indicate that an increase in activation of this pathway due to fluoride exposure could further contribute to liver injury.

Agalakova and Gusev, 2013 [23] performed a study looking at the impact fluoride has on rat erythrocytes and how they can induce death to these cells in vivo. Their previous study showed evidence that in vitro an increase in fluoride exposure can cause premature death in rat erythrocytes (Agalakova & Gusev, 2011) [24]. The control group was given tap water, while the other three received either 2, 10, or 20 mg of fluoride per liter. The results from the blood analysis show that with the higher concentration of Fluoride exposure there was a significant increase in the concentration of F- in plasma. This increase was also accompanied by a decrease in total blood volume percent when looking at the groups exposed to a higher concentration of fluoride. The results are indicated in the table below.

Table 3: Impact of fluoride consumption on rats.

	Control (n = 10)	2 ppm F (n = 10)	10 ppm F (n = 10)	20 ppm F (n = 10)
Body weight (g)	423 ± 9	425 ± 14	424 ± 8	422 ± 10
Water intake (ml animal^{-1} day^{-1})	9.25 ± 0.54	8.64 ± 0.69	10.1 ± 0.68	9.87 ± 0.61
Plasma F concentration (μM)	1.21 ± 0.06	2.31 ± 0.18**	3.90 ± 0.23***	5.94 ± 0.36***
Hematocrit (%)	45.1 ± 0.61	41.3 ± 1.17*	37.1 ± 1.71***	33.7 ± 1.59***
Spleen (g)	1.07 ± 0.03	1.09 ± 0.03	1.07 ± 0.02	0.91 ± 0.04*

The values are means ± SE
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$—in comparison to the values obtained for control rats

The study suggests these results contributed to the premature death of red blood cells. This conclusion was determined by using annexin V binding analysis to indicate any Phosphatidylserine present on the outer membrane of these cells. PS is an indicator of cell death, and the results showed an increase of PS presenting cells in the group exposed to 20 mg/L. The researchers also tested this in vitro due to how quickly PS cells leave the blood stream and the results showed a significant increase in all three groups.

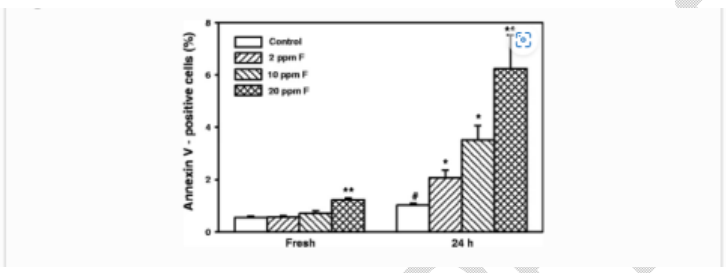

Figure 13: percent of Annexin V among the groups

The results indicated that formation of reticulocytes in all three groups increased to compensate for the loss of red blood cells, however the formation was not to the extent that the researchers had expected. When looking at the morphology of the cells there were deformations in the cells that were exposed to higher concentrations of fluoride. The image below shows the changes in cell morphology and the study believes these changes further contributed to the idea that excess fluoride could lead to anemia.

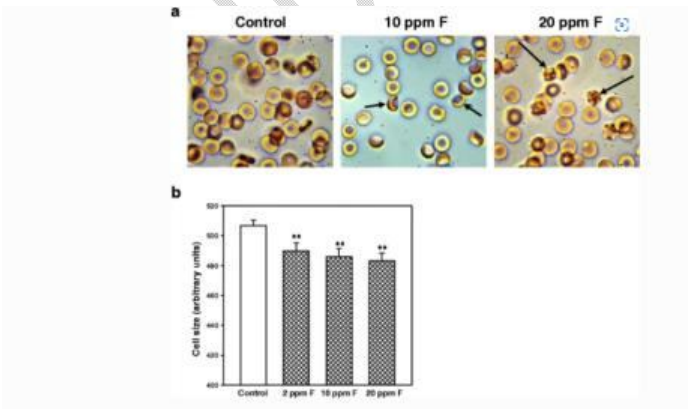

Figure 14: Morphology of the cells.

2.5 FLUORIDE INTERACTION WITH PFAS

With the continued addition of fluoride in the water this adds another ion that can influence and interact with various PFAS. The full effect of this interaction is still unknown, but there are several studies looking at the impact inorganic molecules such as fluoride can have on organic compounds. A study conducted by Calza and Pelizzetti, 2001 [25], looked at the photocatalytic reaction of organic compounds and how this process can be influenced by the presence of inorganic anions. The photocatalytic process occurs when the photocatalyst, in this study TiO2, is exposed to UV light. This process takes place on the surface of a semiconductor and results in the creation of electron hole pairs that facilitate multiple reactions such as surface trapping or recombination. The study indicates that presence of various halides can impact these reactions that take place. When looking specifically at fluoride, the results indicated a strong interaction with the photocatalyst TiO2. This interaction is seen by the fluoride ion replacing the hydroxyl group of TiO2 which can modify its catalytic properties. To observe the impact fluoride has on organic compounds such as phenol, both fluoridated TiO2 and pure TiO2 were added to the phenol suspension. The results of this test indicated that when phenol was exposed to a higher concentration of fluoride the rate of disappearance of phenol increased which is illustrated in the table below.

Table 4: The rate constant of disappearance of phenol in response to pH and fluoride

The researchers contribute these results to various mechanisms such as initiating changes in absorption and surface interactions for organic compounds. Looking at the impact of fluoride, it can have a significant impact on organic compounds and has been shown to increase the rate

Rate, min^{-1}	0 F^-	1×10^{-3} M F^-	3×10^{-3} M F^-	1×10^{-2} M F^-
pH 2.5	–		–	0.09
pH 3.6	0.056	0.098	0.12	0.15
pH 4	–		–	0.17
pH 5	–	–	–	0.17
pH 6	–	–	–	**0.11**

of degradation of these compounds.

Sansotera et al., 2014 [9] also conducted a study that analyzed the photocatalytic process and the effect titanium dioxide had on PFOA. In addition to looking at the impact of fluoride on the photocatalytic process, this study looked at how this process broke down PFOA and generated fluoride ions. To test this reaction a sample of PFOA was created that was exposed to UV light with the addition of the photocatalyst TiO2. When looking specifically at the generation of fluoride ions, ion chromatography was used. The results showed that during the photocatalyst process breaking down PFOA there was an increase in the concentration of fluoride ions measured in the solution which correlated with decrease in PFOA. The study suggest that 15 moles of fluoride ions were released per mole of PFOA that was degraded. The study went on to analyze the impact these fluoride ions may have on the catalyst involved in the photocatalytic reactions. An x-ray photoelectron spectroscopy and Fourier-transform infrared spectroscopy was used to analyze the samples before the degradation reaction and at various times during the reaction. The researchers analyzed peaks at various wavelengths during the reaction time of 2 and 4 hours but after 9 hours to understand multiple different characteristics during the reaction. The results from this analysis indicate that the fluoride ions interacted with the TiO2 catalyst and contrary to previous research it was suggested that this interaction lowers the photocatalytic activity of the catalyst. The study contributed these differing results to the

influence of other factors such as pH that may influence the catalytic activity in response to fluoride ions.

2.6 PRECURSOR MOLECULES

When looking at the source of these polyfluorinated compounds research has begun to surface that a major contributor to PFAA compounds comes from the transformation of other PFAS such as perfluroocctane sulfonamide and fluorotelomer alcohols. These precursor molecules have been shown to have their own harmful impact and research has begun to show they act as an indirect source for PFAA compounds such as PFOA and PFOS (Zhang et al., 2021) [26]. This process can occur through numerous transformation pathways such as oxidation and defluorination. This concept is important because there are many sources that can contribute to these precursor molecules and one example is said to be Aqueous firefighting foam substances. AFFF is an example of a substance that consist of chemical compounds which can contribute to PFAS contamination found in the environment either directly or through the transformation of precursor molecules.

A study conducted by Yuan et al., 2022 [27], looked to research and understand PFOA and PFOS removal through absorption using granular activated carbon filters. The study evaluated 6 GAC filter absorbers in three different water treatment plants to analyze the efficiency of removing PFOA and PFOS. One aspect of the study looked at the possibility of PFOS and PFOA degradation and found that this process did not occur, instead in one sample site PFOS and PFOA was formed likely due to the biotransformation of precursor molecules. Researchers tested the possible biodegradation of these molecules by taking the three water plant sample sites and sterilized the GAC to measure the amount of PFOA and PFOS absorbed. One of the plant sites (plant B) showed a significant increase in absorption with the sterilized GAC compared to the unsterilized, while the other two sites showed no significant difference. Multiple hypotheses were constructed to explain the improvement of PFOA and PFOS absorption but the one that was determined most probable was a result of the formation of PFOA and PFOS due to the transformation of precursor molecules. Plant B contained perfluoroalkyl surfactants and other related chemicals that could have acted as precursor molecules and formed PFOS and PFOA that were found absorbed in the GAC filter. This study begins to introduce the idea that precursor molecules play a significant role in the formation of PFAS such as PFOS and PFOA.

One hypothesis mentioned in the above study for the degradation of precursor molecules leading to PFOA and PFOS formation was through microbial activity. A review conducted by Berhanu et al., 2023 [29], examine studies that focused on the impact microbial communities have on PFAS and their precursors. The review suggests that microbial communities play a major role in transforming and degrading fluorinated compounds found throughout contaminated environments. This ability could also be contributed to unique properties found in microbial communities that make them tolerate high levels of fluoride. A study mentioned in this review conducted by Wang et al., 2009 [28], looked at the biodegradation of 8-2 Fluorotelomer alcohol in soil that contained a mixture of microbial communities. Three soil samples were collected from three different locations that did not contain 8-2 Fluorotelomer alcohol, and then a solution of this alcohol was added to each. Two experiments were performed, a closed bottle study that limited oxygen exposure and a flow through study that allowed for the exchange of constant air. The techniques used to analyze the soil over time was LC/MS/MS which is a technique that combines both liquid and mass chromatography and liquid chromatography accurate radioisotope counting (LC/ARC) both can

be used to identify metabolites. The results showed various metabolites produced over time and those metabolites and their peaks are listed in the table below.

Table 5: LC/ARC results that show the various metabolite products.

Metabolite	LC/ARC Peak number[a]	Acronym	Molecular weight
$F(CF_2)_8CFH^{14}COOH$	2	2*H*-PFOA	398
$F(CF_2)_7{}^{14}COOH$	3	PFOA[c]	416
$F(CF_2)_7{}^{14}CHOHCH_2COOH$	4	3-*OH*-7-3 Acid	460
$F(CF_2)_7{}^{14}CF{=}CHCOOH$	5,6	8-2 FTUA[c]	460
$F(CF_2)_7{}^{14}CH{=}CHCOOH$	5,6	7-3 U Acid[c]	442
$F(CF_2)_7{}^{14}CH_2CH_2COOH$	7	7-3 Acid[c]	444
$F(CF_2)_7{}^{14}COCH_3$	8	7-2 FT Ketone	414
$F(CF_2)_7{}^{14}CHOHCH_3$	9	7-2 sFTOH[c]	416
$F(CF_2)_7{}^{14}CF_2CH_2CHO$	10	8-2 FTAL[c]	464
$F(CF_2)_7{}^{14}CF_2CH_2CH_2OH$	11	8-2 FTOH	466
$F(CF_2)_5COOH$	N/A[b]	PFHxA[c]	314

[a] Corresponds to LC/ARC peak number in Fig. 2.
[b] No ^{14}C.
[c] Previously observed metabolite.

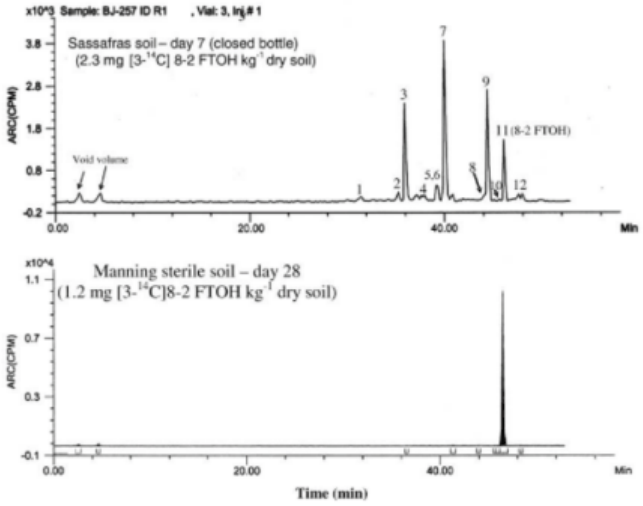

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Figure 15: metabolites and their formation over time compared to the control which only exhibited at peak for 8-2 FTOH.

This study showed that in various soil samples when 8-2 FTOH was added it was degraded into various PFA metabolites, the largest being PFOA which had an average yield of 25 percent among all three soil samples. Further research is needed to understand the exact pathways of this degradation, but it is believed that the mixed microbial communities found in soil can play a role in the biotransformation of PFAS and their subsequent toxicity to humans.

Fluorotelomer alcohols are compounds that are thought to be significant precursor molecules for various PFAS including PFOA. This is shown through a study conducted by Martin et al., 2005 [30], who looked at the presence of these compounds in the atmosphere and how they are transformed in the blood. This study was conducted by first analyzing two groups of rats. One group was exposed to 400 mg/kg of 8:2 FTOH that had been dissolved in corn oil while the control group was dosed with just corn oil. After 6 hours blood samples were taken from the rats and samples of both their liver and kidneys were taken. Multiple techniques were used to identify the metabolites that were released during the reaction such as a reversed

phase chromatography and mass spectral detection. The results showed three metabolites that was released from 8:2 FTOH, PFOA, 8:2 FTCA, and 8:2 FTUCA.

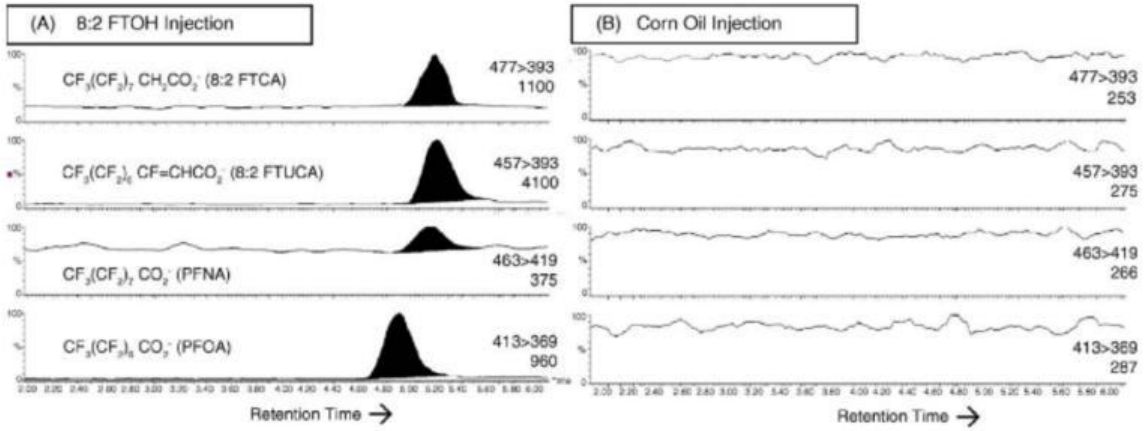

Figure 16: Metabolites released from 8:2 FTOH injection and corn oil injection.

This study continued to look at these metabolites through an in vitro study that was conducted by taking hepatocytes from rats and suspending them with various forms of FTOH and enzymes. 4:2, 6:2, 8:2, and 10:2 FTOH was added to different samples of rat hepatocytes. After 4 hours of incubation the results indicate that 78 percent of the material had been transformed but the known metabolites only made-up 8.5 percent. It was discovered that there was other polyfluorinated substances detected as a metabolite of FTOH but in this study they lack the ability to identify and quantify these compounds.

A study conducted by Houtz et al., 2016 [31], illustrates the impact AFFF can have through PFAS and precursor molecules. The researchers looked at various water treatment plants to analyze the presence of PFAS compounds. To conduct this experiment, effluent from 8 different water treatment plants was collected and for each sample site there was an unoxidized sample analyzed and an oxidized sample. The oxidized sample was used to measure the presence of precursor molecules and their reaction to being oxidized. The top four PFAS compounds found in these sample sites were PFHxA at (24 ng/L), PFOA (23 ng/L), PFBA (19 ng/L), and PFOS (15 ng/L). The study found that 2 of the sample sites contained a significantly higher concentration of PFAS compounds. 6 of the sample sites had a concentration between 80- 160 ng/L while 2 sample sites had total PFAS concentrations of 390 and 2900 ng/L. The study hypothesized the result of a higher concentration was due to the location of these two sample sites being near an airport and a military base that frequently uses AFFFs and a history of previous PFOA contamination still present. The oxidized samples analyzed showed the presence of precursor molecules and the molar fraction of PFAA precursors ranged from 33-63 percent in all sites and accounted for a 3-18 percent increase in PFCAs observed in the samples. 6:2 FtS is a polyfluorinated precursor molecule that was measured and contributed to the greatest fraction of products after oxidation. This study begins to introduce the idea that PFAA precursor molecules are contributing to the transformation of PFAS compound in the environment. The results also show that AFFF compounds can be associated with releasing these precursor molecules and can contribute to higher concentrations of PFAS contaminate in water sources.

EtFOSE is one example of a precursor molecule that can be in sources such as sewage sludge and other sources. A study conducted by Nguyen et al., 2013 [32], examined this

compound and how it can be transformed in water. The purpose of this study was to observe the degradation of N-EtFOSE and monitor its kinetic data and metabolites that are produced. Multiple analysis techniques were used during this study such as irradiation studies. This was conducted by a PFC stock solution mixed with methanol then was placed under irradiation and then injected into a liquid chromatography coupled with a mass spectrometer. 4-tert-octyl phenol (OP) was used as a reference compound throughout the study due to the bimolecular rate already being determined and researchers could analyze this compound simultaneously. Short term kinetic experiments (3 hours) were done to measure the initial first order characteristic such as product formation rate constants. Competitive kinetics were used to measure second order characteristics and long-term kinetic experiments (2 days) were used to compare the concentration profiles. After testing and analyzing the data, multiple equations and models were used to compare and evaluate the data.

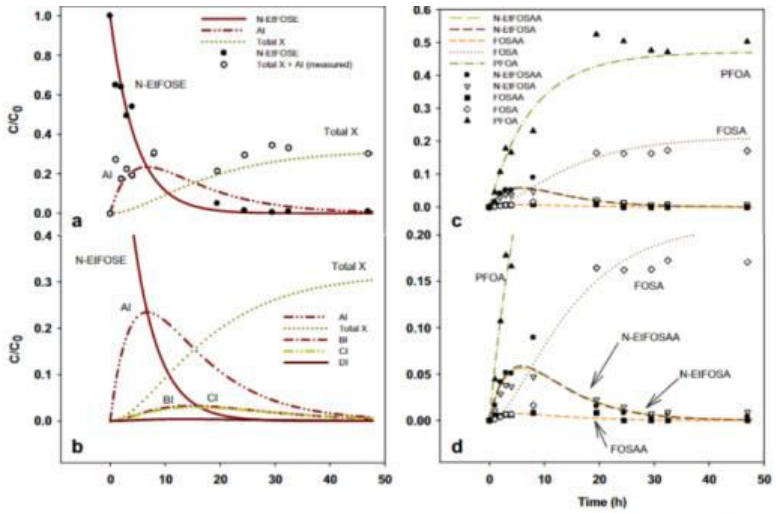

Figure 17: the concentration of various compounds compared to the time of exposure during the irradiation experiments.

The data from this study suggest that N-EtFOSE is degraded by hydroxyl radical that forms various intermediates and other products such as PFOA. This study helps introduce another possible source and precursor that can lead to the formation of PFOA in the environment. Similar data is found in a study conducted by Wen et al., 2018 [33], that looked at the degradation of N-EtFOSAA in plant species. The results of this study showed that over time the concentration of PFOA increased continuously while precursor molecules such as N-EtFOSA was shown to degrade. An example of one species tested is shown in the figure below.

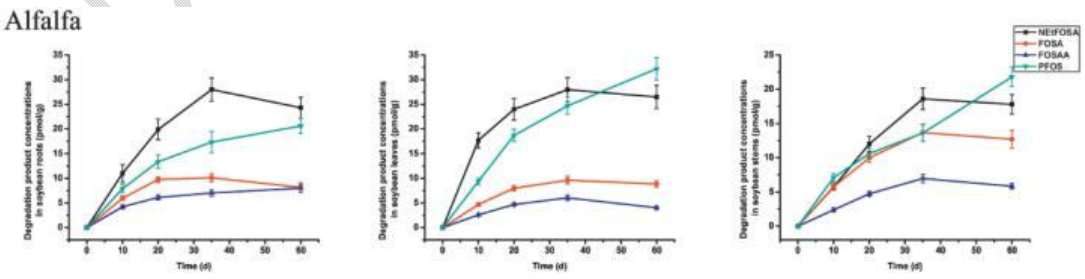

Figure 18: degradation of EtFOSAA in Alfalfa

Many of these precursor molecules consist of other polyfluorinated compounds that go through multiple transformation pathways such as oxidation and are found in various sources such as water treatment plants, sewage sludge, and AFFF compounds.

2.7 IMPACT ON KIDNEYS AND OTHER HEALTH IMPACTS

Kidneys are one organ in the body that has been shown to be impacted by the presence of PFOA. A review written by Liu et al., 2023 [34], states that kidneys are recognized as a target organ for PFOA due to their ability to bind to proteins and accumulates in organs. Glomerular filtration is an important function performed in the kidneys that help filter blood and play a role in the excretion of certain compounds in the body. Studies have indicated that PFOA can impact on the glomerular filtration rate and that with an increase in PFOA exposure the glomerular filtration rate decreases which could be an indication of kidney complications. Two studies came to this same conclusion while another study showed the opposite effect, but this is believed to be due to differences in exposure level or population studied. Uric acid, when found in serum at high levels is an indicator for multiple disorders such as gout and hyperuricemia which is an indicator for kidney disease. Studies have been conducted to analyze this impact and found a correlation between high levels of PFOA exposure [35], to higher levels of uric acid.

One study conducted by Li et al., 2022examined the association of renal cancer and high PFAS exposure by conducting a population study. The setting took place in a location in Sweden that had contaminated drinking water due to the use of AFFFs in a nearby military base. The water was contaminated with PFOS, PFHxS, and PFOA. The study was organized with subjects that were put into groups by gender and based on if their residence was supplied with the highly contaminated water. The group that was supplied with the contaminated water was known as the ever-high group. The population characteristics were determined, and the population was analyzed for the number of cancer incidences. When looking at the incidence of kidney cancer the SIR, the cancer incidence ratio, increases in both females and males when comparing the never-high and ever-high groups shown in the table below.

Table 6: Cancer incidence ratio found among various cancers and exposure groups.

Cancer type	Male				Female			
	Never-high [a]		Ever-high [b]		Never-high [a]		Ever-high [b]	
	N	SIR (95% CI)	*N*	SIR (95% CI)	*N*	SIR (95% CI)	*N*	SIR (95% CI)
Overall	2368	1.00 (0.96, 1.05)	725	1.04 (0.96, 1.12)	1949	0.89 (0.85, 0.93)	600	0.89 (0.82, 0.96)
Lip	10	0.74 (0.36, 1.36)	2	0.57 (0.07, 2.05)	2	0.38 (0.05, 1.38)	3	1.96 (0.40, 5.72)
Oral	18	1.05 (0.62, 1.67)	6	1.14 (0.42, 2.49)	10	0.64 (0.31, 1.18)	3	0.59 (0.12, 1.73)
Salivary gland	2	0.47 (0.06, 1.70)	2	1.68 (0.20, 6.06)	3	1.14 (0.23, 3.32)	0	0.00 (0.00, 5.29)
Pharynx	16	0.88 (0.50, 1.43)	8	1.45 (0.63, 2.85)	6	0.92 (0.34, 2.00)	2	0.98 (0.12, 3.53)
Oesophagus	33	1.02 (0.70, 1.44)	7	0.71 (0.29, 1.47)	11	1.03 (0.51, 1.83)	2	0.64 (0.08, 2.31)
Stomach	82	1.00 (0.80, 1.24)	24	1.10 (0.70, 1.64)	37	0.85 (0.60, 1.17)	13	1.03 (0.55, 1.76)
Colon	172	1.01 (0.87, 1.18)	50	0.99 (0.73, 1.30)	156	0.88 (0.75, 1.03)	45	0.84 (0.62, 1.13)
Rectum	109	0.96 (0.79, 1.16)	41	1.25 (0.89, 1.69)	80	1.00 (0.79, 1.24)	32	1.33 (0.91, 1.88)
Liver	24	1.12 (0.72, 1.66)	9	1.52 (0.70, 2.89)	9	0.98 (0.45, 1.86)	4	1.52 (0.41, 3.88)
Gallbladder, bile duct	11	0.56 (0.28, 1.00)	6	1.10 (0.40, 2.40)	32	1.21 (0.83, 1.70)	7	0.99 (0.40, 2.05)
Pancreas	38	0.84 (0.60, 1.16)	6	0.46 (0.17, 1.01)	39	0.93 (0.66, 1.27)	10	0.81 (0.39, 1.50)
Nose, sinuses	6	1.43 (0.52, 3.11)	0	0.00 (0.00, 3.08)	0	0.00 (0.00, 1.80)	0	0.00 (0.00, 5.15)
Larynx	10	0.48 (0.23, 0.89)	4	0.67 (0.18, 1.71)	1	0.43 (0.01, 2.37)	3	4.54 (0.94, 13.3)
Trachea, lung	177	1.11 (0.96, 1.29)	64	1.42 (1.09, 1.81)	100	0.94 (0.76, 1.14)	29	0.88 (0.59, 1.27)
Pleura	12	0.61 (0.32, 1.07)	2	0.35 (0.04, 1.25)	1	0.57 (0.01, 3.19)	1	1.97 (0.05, 11.0)
Breast	2	0.44 (0.05, 1.57)	1	0.74 (0.02, 4.14)	525	0.80 (0.73, 0.87)	156	0.75 (0.64, 0.88)
Cervix	–	–	–	–	55	0.97 (0.73, 1.26)	15	0.81 (0.45, 1.33)
Uterus	–	–	–	–	113	0.94 (0.77, 1.13)	30	0.82 (0.55, 1.17)
Ovarian	–	–	–	–	68	0.87 (0.68, 1.11)	25	1.12 (0.72, 1.65)
Vulva, vagina	–	–	–	–	17	0.80 (0.47, 1.29)	4	0.61 (0.17, 1.57)
Prostate	712	1.14 (1.05, 1.22)	181	0.96 (0.82, 1.11)	–	–	–	–
Testicle	30	0.85 (0.57, 1.21)	14	1.28 (0.70, 2.15)	–	–	–	–
Kidney	46	0.67 (0.49, 0.90)	17	0.86 (0.50, 1.38)	43	1.17 (0.84, 1.57)	16	1.47 (0.84, 2.39)

Shearer et al., 2020 [36], conducted a study that also examined the association of PFOA exposure and kidney cancer. In this study, a random sample of 150,000 individuals were recruited and tested for Renal Cell carcinoma (RCC). 324 individuals exhibited RCC and were participants in the study. In the study individuals with RCC were compared to a control (an individual without RCC) based on individual characteristics such as age and gender. Research then analyzed the blood serum of these individuals and tested for the presence of various PFAS. The data was then analyzed and used to determine odd ratios and other statistical measurements. The results indicated a positive correlation between RCC and PFAS concentration for compounds such as PFOA, PFOS, and PFHxS. The table below illustrates that with a greater PFAS concentration found in serum the greater the odds ratio calculated is. While there are still many limitations in the study and further research is needed; the data suggests a correlation between PFAS concentration and increased risk for renal carcinomas.

Table 7: Odds ration compared to PFAS concentration found in serum.

PFAS	Controls, No.	Cases, No.	μg/L[a]	OR (95% CI)[b]	P_{trend}[c]	OR (95% CI)[d]	P_{trend}[c]
PFOA	81	47	<4.0	1.00 (Reference)	.007	1.00 (Reference)	.13
	79	83	≥4.0-5.5	1.47 (0.77 to 2.80)		1.41 (0.69 to 2.90)	
	83	69	>5.5-7.3	1.24 (0.64 to 2.41)		1.12 (0.52 to 2.42)	
	81	125	>7.3-27.2	2.63 (1.33 to 5.20)		2.19 (0.86 to 5.61)	
			Continuous[e]	1.71 (1.23 to 2.37)		1.68 (1.07 to 2.63)	
PFOS	81	60	≤26.3	1.00 (Reference)	.009	1.00 (Reference)	.64
	81	82	>26.3-38.4	1.67 (0.84 to 3.30)		1.24 (0.59 to 2.57)	
	81	61	>38.4-49.9	0.92 (0.45 to 1.88)		0.53 (0.22 to 1.24)	
	81	121	>49.9-154.2	2.51 (1.28 to 4.92)		1.14 (0.45 to 2.88)	
			Continuous[e]	1.39 (1.04 to 1.86)		0.92 (0.60 to 1.42)	
PFHxS	88	75	≤2.2	1.00 (Reference)	.04	1.00 (Reference)	.40
	83	74	>2.2-3.4	1.41 (0.75 to 2.64)		1.28 (0.66 to 2.51)	
	76	88	>3.4-5.5	1.14 (0.59 to 2.20)		0.89 (0.43 to 1.85)	
	77	87	>5.5-37.4	2.07 (1.06 to 4.04)		1.46 (0.67 to 3.18)	
			Continuous[e]	1.27 (1.03 to 1.56)		1.12 (0.88 to 1.43)	

Chronic kidney disease was another concept that was mentioned to be impacted by PFOA exposure. A study conducted by Zhao et al., 2020 [37], looked to study PFOA and PFOS due to their ability to bioaccumulate in the kidneys. To conduct this study data was taken from the NHANES and used to measure the association between decreased kidney function and chemicals such as PFOA. The concentration of the chemical was determined by measuring the concentration of metabolites found in the urine. Kidney function was measured by the glomerular filtration rate which in this experiment was estimated based on a creatinine equation, which is based on serum creatinine and adjusted based on characteristics such as age and gender. The concentration of PFA in the urine was analyzed and broken into quartiles, which were compared to the various factors such as demographics and clinical measurements and analyzed from each case. Patients in the fourth quartile of PFA concentration was shown to have a significant decrease in eGFR indicating a decrease in kidney function. The mean measured eGFR found in fourth quartile patient was 84.88 compared to the first quartile patients who had a mean of 101.79. Another trend found was that patients in the fourth quartile had a higher prevalence of CKD (14.96 percent) than those in the first quartile (5.21 percent). The study contributed these results to the idea that the fourth quartile patients were exposed to higher levels of PFA. This quartile analysis was used to further explore the association between PFA exposure and eGFR by using GPS and regression models. These two models also supported the conclusion that an increase in PFA exposure led to a decrease in eGFR, which is a sign of kidney disease. This study helps support the need for further research when studying the association and possibly risk between PFA and kidney function.

Testicular cancer is another health concern that researchers are beginning to study and look for an association with PFOA exposure. The C8 panel in 2012 concluded that certain cancers such as kidney and testicular decided there was a probable link associated with PFOA exposure (Bao et al., 2017) [38]. One study used to make this decision was conducted by Barry et al., 2013 [39], who surveyed 69,030 people who lived within 6 districts that were known to have contaminated water. Demographic and health data was collected from each participant as well as serum was collected to test for PFOA concentrations. After collecting all the data, such as the number of cancer incidences and PFOA concentrations, the cancer risk for various types of cancer was estimated and it was determined that testicular cancer had a high cancer risk at 1.34.

Table 8: Hazards regression model results for various cancer in an area of PFOA exposure

Cancer[a]	No. of cases[b]	No lag		10-year lag	
		HR (95% CI)[c]	*p*-Value	HR (95% CI)[c]	*p*-Value
Bladder	105	1.00 (0.89, 1.12)	0.98	0.98 (0.88, 1.10)	0.77
Brain	17	1.13 (0.84, 1.51)	0.43	1.06 (0.79, 1.41)	0.70
Breast	559	0.94 (0.89, 1.00)	0.05	0.93 (0.88, 0.99)	0.03
Cervical	22	0.89 (0.63, 1.24)	0.48	0.98 (0.69, 1.38)	0.90
Colorectal	264	0.99 (0.92, 1.07)	0.84	0.99 (0.92, 1.07)	0.77
Esophagus	15	0.96 (0.70, 1.32)	0.82	0.97 (0.72, 1.31)	0.84
Kidney	105	1.10 (0.98, 1.24)	0.10	1.09 (0.97, 1.21)	0.15
Leukemia	66	1.01 (0.87, 1.18)	0.88	1.02 (0.88, 1.18)	0.80
Liver	9	0.73 (0.43, 1.23)	0.23	0.74 (0.43, 1.26)	0.26
Lung	108	0.88 (0.78, 1.00)	0.05	0.92 (0.81, 1.04)	0.17
Lymphoma	136	1.01 (0.91, 1.12)	0.88	0.98 (0.88, 1.10)	0.78
Melanoma	241	1.00 (0.92, 1.09)	0.97	1.04 (0.96, 1.13)	0.30
Oral	18	0.89 (0.65, 1.22)	0.46	0.66 (0.43, 1.02)	0.06
Ovarian	43	0.95 (0.76, 1.19)	0.64	0.90 (0.69, 1.16)	0.42
Pancreatic	24	1.00 (0.78, 1.29)	0.99	0.96 (0.75, 1.22)	0.72
Prostate	446	0.99 (0.93, 1.04)	0.63	0.99 (0.94, 1.05)	0.80
Soft tissue	15	0.75 (0.51, 1.10)	0.14	0.72 (0.48, 1.09)	0.12
Stomach	12	0.72 (0.45, 1.14)	0.16	0.77 (0.49, 1.22)	0.27
Testicular	17	1.34 (1.00, 1.79)	0.05	1.28 (0.95, 1.73)	0.10
Thyroid	86	1.10 (0.95, 1.26)	0.20	1.04 (0.89, 1.20)	0.65
Uterine	103	1.05 (0.91, 1.20)	0.53	0.99 (0.86, 1.15)	0.94

[a]A proportional hazards regression model was run for each cancer; each model was adjusted for time-varying smoking, time-varying alcohol consumption, sex, education, and stratified by 5-year period of birth year; time began at age 20 years if the person's 20th birthday was in 1952 or later, otherwise time began at the age the person was in 1952; time ended at the age of cancer diagnosis, age at the last follow-up survey, or age on 31 December 2011, whichever came first. [b]Number of cancer cases used in the regression model (i.e., no missing data for any of the model's covariates). [c]Per unit of log estimated cumulative PFOA serum concentration (ng/mL).

2. DISCUSSION

When looking at PFAS and their possible impact on human health it is important to understand the various compounds and their physiochemical properties. These properties impact a compound's ability to bind, which can impact health and the environment. Studies have indicated the importance of carbon chain length and how that plays a significant role in the ability for these compounds to bind. The compounds that consist of longer chain lengths were shown to have a higher affinity to bind and an increase in cytotoxicity (Sheng et al., 2018) [12]. Another factor that was shown to influence binding affinity was a compound's hydrophobic nature. When these compounds bind to proteins, they are binding to a hydrophobic pocket which has a stronger interaction with hydrophobic compounds (Sheng et al., 2018) [12]. The structure of these compounds also impacts their binding affinity, this is shown by the low binding affinity of GenX, which is a nonlinear structure (Alesio et al., 2022) [13]. The exposure of these chemicals occurs not just through exposure to a single compound but through various compounds in a mixture found in the environment. (Kah et al., 2021) [7]. The research shows that because certain compounds have stronger binding affinity, there is competition between these compounds when it comes to binding and absorption.

In this review the impacts of PFAS on health were analyzed and the differences among species were studied. These chemicals can act on various pathways found in the body and there are many differences expressed among species specifically between rats and humans.

When looking at various studies, there is a general trend of hepatocyte cells in the liver to be more sensitive to PFAS exposure when looking at specific pathways. The research showed up-regulation of various genes indicating the possibility of tumor formation and other heath disorders such as cell damage (Bjork & Wallace, 2009) [20].

The fluoridation of water is another rising concern when looking at the impact it may have on human health and the environment. These inorganic anions found in the water have also been studied to understand their interaction with PFAS. The studies indicate that the main form of interaction is through the photocatalytic pathway. This pathway shows the ability to break down PFOA and the reaction contributes to more fluoride anions being present in the water (Sansotera et al., 2014). Another study indicates that the presence of more fluoride anions can impact the rate of reaction through the interaction of the catalyst involved with the photocatalytic process (Calza & Pelizzetti, 2001) [25]. This research indicates a potential positive feedback loop type interaction, that with the breakdown of PFOA there is an increase in fluoride which can in turn increase the breakdown of PFOA. While the breakdown of these contaminants may seem beneficial, the addition of more fluoride anions into the water may create more health concerns that are seen with high fluoride concentration exposure.

Looking at the impact precursor molecules have on PFAS formation is a significant area in which research needs to continue. PFAS precursor molecules are found in many different sources and can be transformed into compounds such as PFOA, which can be damaging to both the environment and human health. There are a vast number of compounds that can act as precursors but the most common are FTOH and 6:2 FtS which are compounds found in many different substances including AFFF (Houtz et al., 2016) [31]. N-EtFOSA and N-EtFOSE are also common precursors that can be found in sewage sludge and biosolids found in the soil (Wen et al., 2018) [33]. When looking at the possible damage these compounds can have, the kidneys are one organ studied because research has indicated that PFOA can bioaccumulate in the kidneys due to their high protein structure. A vast number of complications have begun to be associated with increased PFOA exposure such as CKD, increased uric acid concentration, and renal carcinomas (Liu et al., 2023) [34]. This research begins to introduce the importance of studying the indirect exposure of PFAS compounds because by releasing PFAS precursor molecules it can continue to contribute to the health consequences. Further research is needed in many aspects to understand these compounds, especially when looking at the association with cancer. A public health statement released by the CDC warns to interpret some studies cautiously because there are inconsistencies and other factors that could influence the results (*Public Health Statement: Perfluoroalkyls* 2015) [2].

3. CONCLUSION

Overall, this review provides important information to gain a better understanding of PFAS and their impact throughout the body and the environment. These results suggest that the various compounds considered PFAS have differing physiochemical properties that play a role in how they are able to bind and act in the body. The interaction of these compounds between each other play a key role in the impact they have on the environment and human health because of their ability to compete and displace. Differences among species show the need for further research to understand the vast number of pathways that can be impacted by the exposure of these various chemicals. The interaction of PFAS and fluoride also contribute to the idea that the breakdown of these chemicals can release fluoride ions raising even more of a concern. PFAS precursor molecules is an important concept to research when trying to understand these compounds. The ability for PFAS molecules to bio transform into various PFAS molecules such as PFOA is a concept that can have a huge impact on how to monitor these compounds and their human implications. PFAS are a broad category of compounds that

interact in many ways making it difficult to fully understand the impact of these chemicals and require future analysis to continue to learn and understand their impact.

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