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The Potential Link between PFAS and Colorectal Cancer: A Narrative Review

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Abstract

Background: Currently, there is information on the detrimental impacts of exposure to pollutants such as per- and polyfluoroalkyl substances (PFAS) but there is still limited information on the impact PFAS has on colorectal cancer.

Aim: To review and examine the evidence available in the existing published literature showing an association between exposure to PFAS and colorectal cancer.

Methods: In this narrative review using searches of PubMed, SCOPUS and Google Scholar, publications between 2003 and 2023 were identified to ensure the inclusion of all recent development in this area of research. Inclusion criteria included a focus on the association between PFAS/PFOS and colorectal cancer. Both epidemiological and toxicology studies conducted globally were examined as well as reviews. In addition, studies that assessed an association between PFAS/PFOS and GI tract/intestinal inflammation were also reviewed, because they were considered relevant for the colorectal cancer outcome.

Results: In the 18 studies included in this review, there is not sufficient evidence to say that PFAS/PFOS causes colorectal cancer. Findings from the animal studies vary, showing negative associations, molecular disruptions and microbiota dynamics, as well as inverse associations between PFAS exposure and reduction of gastrointestinal tumors. Additional studies on colorectal cancer cell lines and metabolic remodeling of cells suggest limited evidence for an association between PFOA exposure and stimulation cell's invasive ability and metabolism. The epidemiological studies also show complex and distinct findings suggesting the need for further research to establish an association between PFAS/PFOS exposure and colorectal cancer.

Conclusion: The current existing body of published literature does not provide sufficient evidence to establish an association between exposure to PFAS/PFOS and colorectal cancer. Additional research is warranted to better understand the mechanisms through which PFAS/PFOS affect colorectal cancer.

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Introduction

Etiology and risk factors of colon/colorectal cancer

Colorectal cancer is the third most commonly diagnosed cancer as well as the third most common cause of cancer-related death in both men and women in the United States. (American Cancer Society, 2023; Siegel et al., 2023). Globally, it accounts for approximately 10% of all cancer cases and is the second leading cause of cancer-related deaths (World Health Organization, 2023). Colorectal cancer is most often diagnosed at advanced stages with the majority of cases occurring in people aged 50 and older as colorectal cancer presents no symptoms in the early stages (World Health Organization, 2023). One of the strongest known risk factors for colorectal cancer is family history of colorectal cancer; individuals with a parent, sibling or child who has been diagnosed with colorectal cancer, have a 2 to 4 times the risk of developing the disease compared to people without a family history (World Health Organization, 2023). In addition to these heredity factors, modifiable risk factors also play a role. More than half (55%) of all colorectal cancers in the United States are attributable to lifestyles including unhealthy diets, insufficient physical activity, high alcohol consumption, smoking and family history as well as environmental factors (American Cancer Society, 2023). Some specific environmental influences that are associated with colorectal cancer have been identified in several epidemiological studies such as diets that are rich in red, processed and grilled meats, smoking and alcohol use, as well as pre-existing diseases such as obesity, type 2 diabetes and inflammatory bowel diseases (Ratray et al., 2017). Inflammatory bowel diseases (IBD) such as either ulcerative colitis or Crohn's disease, significantly increases one's risk of colorectal cancer (American Cancer Society, n.d.). The National Cancer Institute highlights that interrelated factors such as diet, bacteria in the gut and inflammation significantly contribute to the early-onset of colorectal cancer. This review will provide an overview of existing literature investigating the association between PFAS exposure and colorectal cancer.

Background on PFAS/PFOS

Per- and polyfluoroalkyl substances (PFAS) are a group of man-made chemicals often referred to as “forever chemicals” that have been widely used in industry and consumer products since the 1940s and over time, leak into soil, water and air. PFAS are of concern, including many perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) as they do not break down in the environment, bio accumulate in fish and other wildlife, and can use soil and contaminated drinking water sources as reservoirs to move in the environment (Centers for Disease Control and Prevention, 2022a). PFOA and PFOS are widely known as the most commonly detected types of PFAS in various environmental compartments and have been associated with plastic pollution and as being endocrine disruptors (Wee & Aris, 2023). These chemicals are resistant to grease, oil, water and heat and as a result, remain in the environment for a long-time (Centers for Disease Control and Prevention, 2022b). PFAS are widespread in the environment and are commonly found in a variety of products such as clothing, furniture, food packaging, adhesives, heat-resistant non-stick cooking surfaces among others (Centers for Disease Control and Prevention, 2022a).

PFAS have a chain of linked carbon and fluorine atoms and some of these also have a functional group at the end of each chain which are the basis for the different chemical properties and different chemical names. In the structure of perfluoroalkyl substances, all carbons except the last one are attached to fluorine, and the last carbon attaches to the functional group (National Institute of Environmental Health Sciences, n.d.). In the structure of polyfluoroalkyl substances, at least one (but not all) carbons are attached to fluorines. The carbon-fluorine represents one of the strongest bonds, thus, these chemicals do not degrade easily in the environment (National Institute of Environmental Health Sciences, n.d.). PFAS are a group of nearly 15,000 synthetic chemicals (National Institute of Environmental Health Sciences, n.d.). PFAS are often divided into two groups, short and long chains. PFOS is a long-

chain subtype of PFAS. Long-chain PFAS have comparable bioaccumulation potential as other well-known contaminants such as dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCBs) (Durham et al., 2023).

The widespread presence of PFAS has led scientists to study their impact on human health and over the last decade, interest in PFAS has significantly grown with agencies such as ATSDR partnering with state health officials in more than 30 communities across the United States to investigate exposure and the possible human health effects associated with PFAS (Centers for Disease Control and Prevention, 2022b). Humans are most likely exposed to such chemicals through consumption of PFAS-contaminated water or food, using products made with PFAS or through breathing air with PFAS. Since PFAS don't easily degrade, humans and animals continue to repeatedly be exposed and blood levels of some PFAS can build up over time (National Institute of Environmental Health Sciences, n.d.). To measure PFAS exposure, PFAS can be measured in multiple biological matrices including whole blood, serum, plasma, urine, breast milk and hair (Alves et al., 2015). However, most studies predominantly used either serum or plasma to evaluate PFAS exposure and the measured concentrations in serum and plasma of PFAS have been comparable to the measures of PFOA, PFOS and perfluorohexane sulfonate (PFHxS) (Ehresman et al., 2007).

PFAS/PFOS and Cancer

The International Agency for Research on Cancer (IARC) has classified one type of PFAS, PFOA, a human known carcinogen, and PFOS as a possible carcinogen; and various studies have linked exposure to PFOA to cancers such as renal cell carcinoma and testicular cancer (International Agency for Research on Cancer, 2023). This is supported by several cohort studies in the past few decades that have linked PFOA to different kinds of tumorigenesis, including cancers such as pancreatic, liver and testicular among many (Miao et

al., 2015). Despite the findings of the IARC indicating PFOS and PFOA as known or possible carcinogens, there lies gaps in the understanding of the association between PFAS and carcinogenicity. To better address these gaps in understanding of the association between PFAS and carcinogenicity, the Division of Cancer Epidemiology & Genetics (DCEG) has launched several studies aimed at identifying specific cancers associated with PFAS (National Cancer Institute Division of Cancer Epidemiology & Genetics, 2020). Additionally, the National Academies Sciences Engineering Medicine 2022 Guidance on PFAS Exposure, Testing, and Clinical Follow-Up report highlights an association between PFAS link to intestinal inflammation and a rare autoimmune condition of the gastrointestinal (GI) tract. This is supported by the American Cancer Society, which highlights that inflammatory bowel disease (IBD) such as either ulcerative colitis or Crohn's disease, significantly increases one's risk of colorectal cancer (American Cancer Society, n.d.). Furthermore, the National Cancer Institute also highlights that interrelated factors such as diet, bacteria in the gut and inflammation contribute to the early-onset of colorectal cancer (National Cancer Institute, 2020). Despite the findings from the National Academies Sciences Engineering Medicine 2022 Guidance on PFAS Exposure, Testing, and Clinical Follow-Up report, highlighting an association between PFAS and intestinal inflammation and evidence supporting that inflammatory bowel disease (IBD) such as ulcerative colitis or Crohn's disease as well as bacteria in the gut and inflammation contribute to the onset of colorectal cancer, currently there is still little information on the environmental impact of pollutants such as PFAS/PFOS/PFOA on colorectal cancer.

Methods

This is a narrative review where existing literature was examined providing evidence linking PFAS and colorectal cancer. The following databases were searched: PubMed/MEDLINE, Google Scholar and SCOPUS using the following specific search terms:

PFAS, PFOS, PFOA, perfluoroalkyl, colon, colorectal, colon cancer, colorectal cancer, gastrointestinal (GI) tract, perfluorooctane, perfluorooctanesulfonyl fluoride, perfluoro, and polyfluoro. A total of 138 articles were identified as part of the search and the titles and abstracts were manually screened for duplicates, irrelevant articles and articles relating to other types of cancers. After this initial screening, a total of 65 articles were excluded and a second manual screening of the full-text articles was conducted and 55 additional reports were excluded as they did not meet the strict inclusion criteria of assessing PFAS/PFOS and its association with colorectal cancer. As a result, this review includes the 18 articles that met the search criteria. Articles published from 2003 to 2023 were included in this narrative review and the following article types were included: primary research articles, systematic reviews, meta-analyses, various observational studies such as cohort studies and case-control studies, peer-reviewed articles and reports. The following types or studies were excluded: non-peer-reviewed sources, studies with unclear methodology, studies lacking clear evidence of exposure assessment (biological methods such as serum/blood testing, etc.), studies with small sample sizes in human studies such as studies that did not look at large cohorts, and human studies with short follow-up times of less than a year since PFAS bio accumulates over time, it is important to assess populations who have been exposed to PFAS for a longer period of time. The 18 articles included in this review investigate the association between specific PFAS and colorectal cancer including studies on both human subjects or animal studies. From each of the studies, the primary findings were abstracted and assessed if the study looked at a link between PFAS and colorectal cancer. Additional information abstracted from studies include: year of study, study type, study location and population (human/animal model used), sample size, types of PFAS assessed, and samples measured (i.e. environmental, biological).

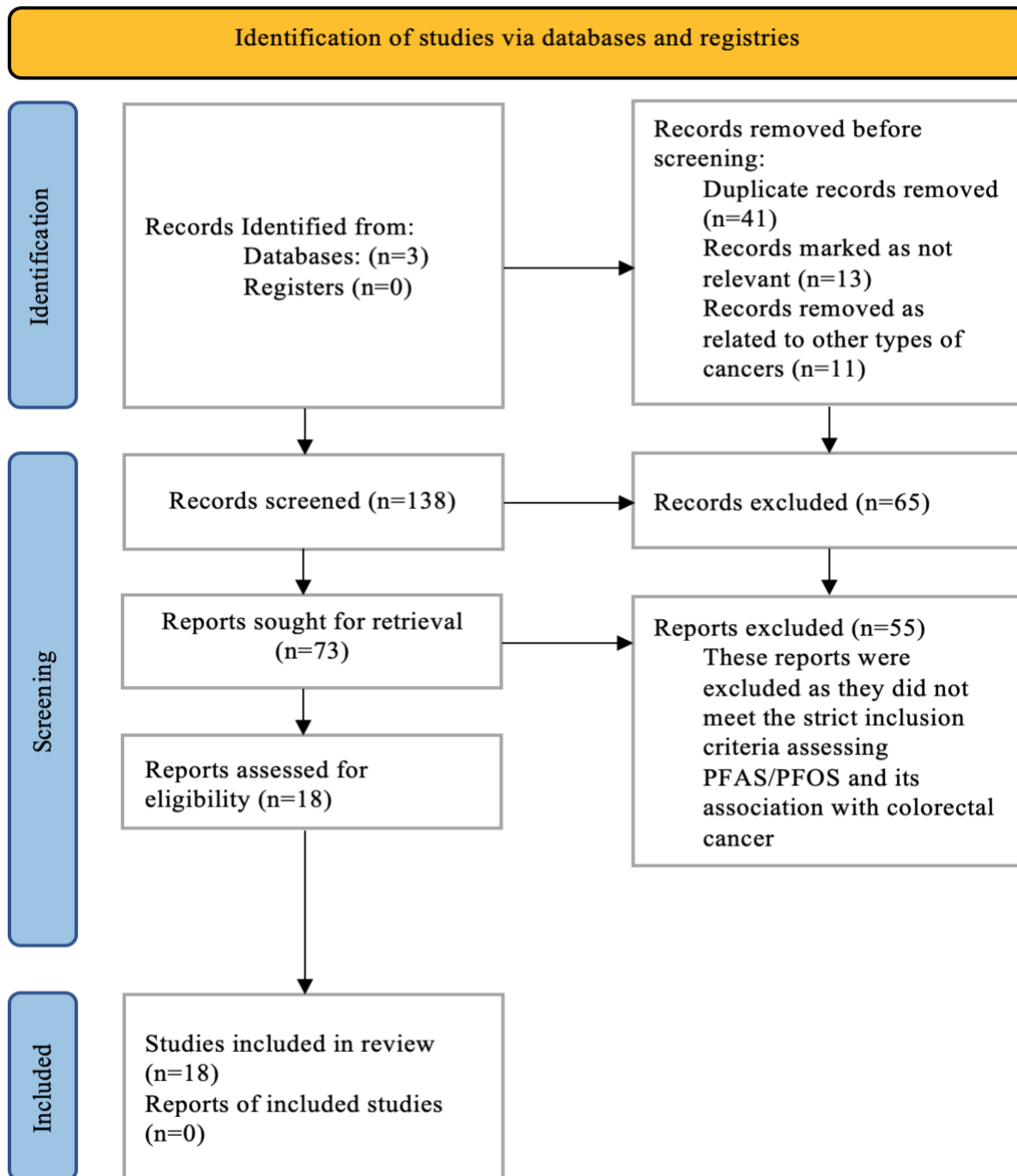


Figure 1: Literature search strategy for studies of PFAS and Colorectal Cancer

This flow chart was created manually after screening the literature. Records are indicated as literature for which only the title and abstract were screened. Reports are indicated as full-text screening of literature.

55 reports were excluded as they did not fit the inclusion criteria: 3 reports looked at effects of polystyrene (PS) particles and PFOS on the human colon adenocarcinoma cell, 8 reports looked at enhanced antitumor efficacy in colon cancer using nanoparticles loaded with perfluorocarbon (these reports did not look at the association between

PFAS and colon cancer), 4 reports were on the analysis in the prostate, lung, colorectal and ovarian cancer screening trial (these reports did not include association on PFAS exposure with colorectal cancer even though the screening trial included those with colorectal cancers), 6 reports looked at the presence of perfluoroalkyls moieties and hyaluronic acid to form nanoparticles and their impact on colon cancer cell lines and only mentioned colon cancer but did not identify a clear association, 2 reports looked at associations between PFAS substances and lymph nodes in colorectal cancer, 18 reports looked at PFAS and the detection of colorectal liver metastases, 14 reports looked at perfluorocarbon or other PFAS such as liposome encapsulated PFAS for ultrasonography, photodynamic therapy, chemotherapy, radiotherapy and other treatment without showing association between PFAS and colorectal cancer.

Results

Table 1: Summary of studies assessing association of PFAS/PFOS exposure and Colorectal Cancer development

Study	Year of Study	Study Type	Study Location and Population (human/animal model used)	Sample Size	Types of PFAS assessed	Samples Measured (i.e. environmental, biological)	Primary Findings
Wimsat et al. (2016)	2016	Animal study	United States, APC ^{min} mice	N = 24 APC ^{min} female mice N = 24 APC ^{min} male mice	PFOS (dosed in drinking water)	Gastrointestinal tumors counted and scored, blood PFOS levels measured	APC ^{min} male and female mice were randomized at 5-6 weeks of age to receive different doses of PFOS through their drinking water. Female APC ^{min} mice were given 0, 20, 250 mg PFOS/kg and male APC ^{min} mice were given 0, 10, 50 and 200 mg PFOS/kg. At 15 weeks of age, gastrointestinal tumors were counted and scored and blood PFOS levels were also measured. Chronic exposure to PFOS in drinking water resulted in an inverse dose-response effect, resulting in reduced formation of gastrointestinal tumors. The highest dose groups show the largest effects and suggest that PFOS could potentially be a new form of treatment for familial colorectal cancer.
Wimsat et al. (2018)	2018	Animal study	United States, NSG mice	N = 45 NSG mice	PFOS (human colorectal cancer cell xenografts implanted)	Tumors from mice, human colorectal tissues from patients who had not previously received any therapy	Primary patient colorectal cancer cell xenografts were implanted into 45 NSG mice aged 34-103 days. PFOS reduced tumor size in one group of tumors, particularly those from the right ascending colon. In two mice, by 5 weeks of treatment, PFOS eliminated their 52.4 mm ³ and 124.6 mm ³ masses completely, and treatment was sustained for 10 weeks, however their corresponding matched vehicle control mice had tumors that grew significantly to 472.7 mm ³ and 340.1 mm ³ in size during the same 10-week treatment period. In a third xenograft mouse, PFOS resulted in tumor growth to be significantly blunted although not eliminated and this was also the case in the matched vehicle control for the same time-period.

Ngo et al. (2014)	2014	Animal study	Male and female Min/+ and wild-type mice	Wild type female mice with n=104 in experimental block 1 and n=100 in experimental block 2	PFAAs (dosed in drinking water), PFOA (dosed in drinking water), PFOS (dosed in drinking water)	Blood glucose measured	Investigated whether PFOA or PFOS resulted in obesogenic effects and increased spontaneous intestinal tumorigenesis after in utero exposure. Dams were exposed to PFOA or PFOS (0.01, 0.1 or 2.0 mg/kg bw/day) by po gavage on GD1-17 and Min/+ and wild-type offspring terminated at 11 weeks old for intestinal tumorigenesis examination or at week 20 to assess obesogenic effect. Food intake assessed at weeks 6 and 10, blood glucose measured at weeks 6 and 11. Study found indications of PFOA toxicity only, with lower survival of pups and lower body weight in pups after 3.0mg/kg PFOA, and increased relative liver weight after exposure to 0.01 and 0.1mg/kg PFOA. PFOA and PFOS did not increase the incidence/number of tumors in the small intestine/colon of Min/+ mice and it did not affect their location along the intestines.
Rashid et al. (2020)	2020	Animal study	United States, CD1 female mice	15 female mice	PFOA (dosed in drinking water)	Blood serum	CD1 mice were dosed with PFOA in drinking water with concentrations of 1, 5, 10 or 20 mg/kg/day for 10 days to evaluate the accumulation of PFOA and the induced alterations in the expression of epigenetic and tight junction genes in the small intestine and colon. In the CD1 mice, after 10 days of PFOA exposure, the small intestine and colon tissues of the female CD1 mice had a significant amount of PFOA accumulation compared to the control group with no PFOA detected. Expression of epigenetic and tight junction proteins Claudins (Cldn)m Occludin (Ocln), and Tight Junction Protein (Tjp) were heavily altered in the small intestine. PFOA triggered DNA methylation changes and altered the expression of genes that are essential to maintain the physical barrier of the intestine, with more profound effects in the small intestine compared to the colon.
Rashid et al. (2023)	2023	Animal study	United States Adult CD-1 male mice	N = 44 adult male mice	PFOS (dosed in deionized water), GenX (dosed in deionized water)	DNA from 200 mg gut microbiota samples of mice small intestine and colon extracted from each mouse, liver tissue, and liver organs harvested	44 adult male mice in this study were grouped based on a diet containing either PFOS or GenX in three different concentrations as well as a vehicle control (control, 5 mg/kg, 10 mg/kg, and 20 mg/kg for PFOS and control, 10 mg/kg, 20 mg/kg, and 100 mg/kg for GenX) for a two-week period. PFOS exhibited a stronger impact on colon microbiota and liver metabolome compared to GenX, possibly due to the high potency of PFOS. PFOS and GenX exposure altered the gut microbiota and can potentially disrupt the microbial functional pathways as well as the liver metabolome.

Innes et al. (2014)	2014	Cross-sectional study	mid-Ohio Valley Large Appalachian population	N = 47,151 cancer-free adults age ≥ 21 N = 208 cases of primary colorectal cancer adults age ≥ 21	PFOA (measured in serum), PFOS (measured in serum)	Serum levels of PFOA, PFOS, and a range of other blood markers	Investigated the association between PFAS exposure and colorectal cancer in adults ages 21 and above with 47,151 cancer-free and 208 primary colorectal cancer cases. There is a strong, inverse association between PFOS and likelihood of colorectal cancer diagnosis and a more modest inverse association between PFOA and colorectal cancer. Individuals with serum PFOS levels in the highest quartile were about 80% less likely to have received a colorectal cancer diagnosis and individuals in the highest quartile of PFOA were approximately 40% less likely to have received a colorectal cancer diagnosis.
Messmer et al. (2022)	2022	Ecological study	Residents of Merrimack, New Hampshire with contaminated drinking water by PFAS compared to demographically similar communities in New England	n/a	PFHxS (measured in drinking water), PFOA (measured in drinking water), PFOS (measured in drinking water)	n/a	Investigated the risk of Merrimack, New Hampshire residents from PFAS contaminated water exposure for 24 types of cancers between 2005 and 2014 as well as their risk of all-cause cancers experienced, compared to the U.S. national cancer rates and cancer rates in 4 demographically similar towns in New England. Merrimack residents were exposed to 140 parts per trillion (ppt). Merrimack residents had a significantly higher risk of thyroid cancer and prostate cancer and when compared to pooled data from demographically similar New England towns with documented exposure to PFAS in drinking water supplies and had significantly higher risks of colon cancer.
Li et al. (2022)	2022	Cohort study	Ronneby, Sweden,	N = 60,507 individuals from the Ronneby Register Cohort	PFAS (measured in drinking water), PFHxS (measured in drinking water), PFOS (measured in drinking water), PFPeA (measured in drinking water), PFHxA (measured in drinking water), PFHpA (measured in drinking water), PFOA (measured in drinking water), PFBS (measured in drinking water), PFHpS (measured in drinking water)	Water samples, serum PFAS levels	Investigated PFAS exposure in Ronneby households exposed to PFAS in drinking water between 1985 and 2013. PFAS exposure was extremely high, particularly by PFOS and PFHxS and to a lesser degree by PFOA. In a subset of approximately 3,300 Ronneby residents, serum PFHxS level in the Ronneby population was on average more than 100 times higher than the general population of Sweden and approximately 30 times higher than the C8 study findings. PFOS, on average the residents of Ronneby were approximately 35 times higher than the general population of Sweden and 7 times higher than the C8 study findings. There was a moderately increased risk of kidney cancer and a moderately increased standardized cancer incidence ratios for rectum cancer among those in Ronneby.

Miao et al. (2015)	2015	Laboratory-based experimental study	n/a	n/a	PFOA (dosed in cells)	Human colorectal cancer cells DLD-1 purchased from the American Type Culture Collection	Collected human colorectal cancer cells DLD-1 that were purchased from the American Type Culture Collection and seeded the cells into a 96-well plate with serum-free medium, 2,000 cells per well, and 24 hours later, the cells were treated with different doses of PFOA. After the cells were exposed to PFOA, trans-well filter assays were used and the assays indicated that PFOA treatment can stimulate DLD-1 colorectal cancer cell invasion significantly. PFOA could induce colorectal cancer cell DLD-1 invasive ability and MMP-2/-9 over-expression particularly through NF-κB activation.
Zhang et al. (2023)	2023	Experimental animal model study	n/a	n/a	PFOA (dosed in human colorectal cancer (DLD-1) cells)	n/a	A total of 20 targeted metabolites related to metabolism of glucose, glutamine and fatty acids in PFOA-treated human colorectal cancer (DLD-1) cells were analyzed using C metabolic flux analysis (MFA). PFOA-treated DLD-1 cells showed significant changes in glucose consumption and decreased glucose flux into the tricarboxylic acid (TCA) cycle and biosynthesis of fatty acids. Metabolic remodeling could occur in intestinal cells that are exposed to PFOA and this was most likely because of PFOA toxicity relevant with the loss of glucose in biomass synthesis and energy metabolism.

This table includes 10 studies identified through the very specific and stringent inclusion and exclusion criteria. The studies in this table were organized by study type.

Types of PFAS measured in Table 1 abbreviated and listed out here: Per- and polyfluoroalkyl substances (PFAS), perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluoroalkyl acids (PFAAs), Hexafluoropropylene Oxide (HFPO) Dimer Acid and its Ammonium Salt (GenX), Perfluorohexane sulfonate (PFHxS), Perfluoropentanoic Acid (PFPeA), Perfluorohexanoic Acid (PFHxA), Perfluoroheptanoic Acid (PFHpA), Perfluorobutane sulfonic acid (PFBS), Perfluoroheptane sulfonic acid (PFHpS)

Table 2: Other relevant literature assessing association of PFAS/PFOS exposure and Colorectal Cancer development

Study	Year of Study	Study Type	Study Location and Population (human/animal model used)	Sample Size	Types of PFAS assessed	Samples Measured (i.e. environmental, biological)	Primary Findings
Olén et al. (2020)	2020	Cohort study	Denmark and Sweden, Patients with ulcerative colitis (UC in Denmark and Sweden (total cohort N = 96,447)	N = 32,919 UC in Denmark N = 63,528 UC in Sweden	n/a	n/a	Investigated the risk of individuals with ulcerative colitis (UC) developing colorectal cancer. There were 1336 incidences of colorectal cancers observed in the UC cohort and 9,544 incidences of colorectal cancers in the reference individuals. 639 patients died from colorectal cancer in the UC cohort, compared to 4,451 in the reference individuals. Individuals with UC are at an increased risk of developing colorectal cancer, often diagnosed with less advanced colorectal cancer and are at an increased risk of dying from colorectal cancer compared to individuals without UC.
Kunovszki et al. (2020)	2020	Population-based study	Hungary, All adult patients who experienced at least two events in outpatient care or at least two medication prescriptions, or at least one inpatient event with ulcerative colitis (UC) diagnosis	N = 36,315 patients suffered from UC between 2010 and 2016	n/a	n/a	Investigated the epidemiology and mortality of the Hungarian ulcerative colitis (UC) population between 2010 and 2016. 8.5% individuals diagnosed with colorectal cancer in the incident patient subpopulation. 33% of the patients with colorectal cancer died during the study of which 25% are attributed to colorectal cancer. Survival of UC patients who suffer from colorectal cancer than that of the general population is worse.
Xu et al. (2020)	2020	Registry study	Ronneby and Karlshamn municipalities of Sweden,	N = 189	PFOS (measured in fecal biomarkers), PFHxS (measured in fecal biomarkers), PFAS (measured in fecal biomarkers), PFOA (measured in fecal biomarkers)	Serum PFAS, fecal biomarkers (fecal calprotectin and zonulin)	Investigated the association between PFAS and inflammatory bowel disease (IBD) in the Ronneby cohort which has a high PFAS exposure from Aqueous Film-Forming Foam (fire-fighting foam) through drinking water. Association of PFAS exposure with fecal calprotectin and zonulin which are biomarkers of gut inflammation and permeability were explored in a subset of n=189 participants from Ronneby and a nearby control municipality, Karlshamn. Sufficient evidence was not found to support linkage between PFAS exposure from drinking water as a risk factor for the development of IBD.

Fart et al. (2021)	2021	Case-control study	Sweden, Inflammatory bowel disease (IBD) patients from previously described cohort and patients with Crohn's disease (CD) or Ulcerative colitis (UC) recruited at the outpatient IBD clinic of Örebro University Hospital	N = 20 patients diagnosed with ulcerative colitis age ≥55, N = 20 patients diagnosed with Crohn's disease age ≥55, N = 20 age and sex-matched blood donors as healthy controls	PFAS (measured in serum), PFOS (measured in serum), PFOA (dosed in ileal and colonic murine tissue)	Serum concentrations of PFAS and bile acids (BAs), blood samples	Investigated the association between exposure to high levels of PFAS with 1) late-onset IBD and 2) disturbances of the bile acid pool with a study sample size of 60 individuals with ulcerative colitis, Crohn's disease, and age and sex matched blood donor healthy controls. Also conducted ex vivo Ussing chamber experiments assessing the effect of PFOA on ileal and colonic murine tissue n=9 which found that exposure to PFOA resulted in increased ileal and colonic permeability as well as an enhanced colonic secretory response carbachol. The total amount of PFAS substances was significantly increased among patients with ulcerative colitis when compared to those patients with Crohn's disease and healthy controls. PFAS substances levels were higher among patients with late-onset ulcerative colitis
Steenl and Winquist (2021)	2020	Scoping Review	n/a	n/a	n/a (this is a review that examines studies assessing different PFAS)	n/a	Analyzed 28 studies as part of this scoping review. Included one cross-sectional study that evaluated the prevalence of colorectal cancer in a high exposure setting of PFOA serum concentrations. The particular study showed little additional information due to its cross-sectional design and potential reverse-causation for PFOA exposure and development of colorectal cancer. Overall findings of the review were that the several epidemiologic studies that investigated the association between PFAS and cancer, were informative but not conclusive in finding an association.
Rosenfeld et al. (2023)	2023	Review of studies about firefighters and their exposure to PFASs	Firefighters	n/a	n/a (this is a review that examines studies assessing different PFAS)	n/a	Summarized the history of firefighters' occupational exposure particularly to aqueous film-forming foam (AFFF) as a source of PFAS exposure. Determined that firefighters are exposed to PFAS from AFFF and contaminated turnout gear through dermal exposure, ingestion and inhalation of AFFF and turnout gear textiles, ingestion of contaminated food and water, smoke inhalation and dust ingestion. International, national and state agencies concluded that PFOA is a long-chain PFAS that is potentially carcinogenic and firefighters also have elevated levels of long-chain PFAS in their serum. Firefighters have an increased cancer risk for thyroid, kidney, bladder, testicular, prostate, and colon cancer.

Li et al., (2022)	2022	Review of current relationships between PFAS exposure and damage to the intestinal barrier	n/a	n/a	n/a (this is a review that examines studies assessing different PFAS)	n/a	Investigated current relationships between PFAS exposure and damage to the intestinal barrier. PFAS entering the gastrointestinal tract (GI tract) can disrupt the intestinal barrier system by causing inflammation in the gut, destruction of the gut epithelium and tight junction structure, reduction of the mucus layer, and induction of the toxicity of immune cells. PFAS accumulation induces microbial disorders and metabolic products changes. Intestinal barrier defects are associated with a broad range of diseases, such as gastrointestinal diseases (like celiac disease), inflammatory bowel disease, irritable bowel syndrome, and colon carcinoma among, and other extra-intestinal disorders. There are some PFAS alternatives with specific intestinal toxicity in animal studies.
Durham et al. (2023)	2023	Review	n/a	n/a	PFAS, PFOS	n/a	Investigated current population and preclinical studies on PFOS exposure and GI inflammation, metabolism, immune responses, and carcinogenesis. There is a link between long-term PFOS exposure, lipid metabolism dysregulation, inflammation, microbiome dysfunction and the etiology of colorectal cancer. Evidence for an association between PFOS and GI cancer remains sparse. Healthy dietary interventions such as diets high in fiber can help reduce or prevent PFOS-mediated disease risks.

This table includes 8 studies identified through the search and includes relevant literature including reviews and articles assessing PFAS/PFOS exposure with other cancers, as well as the association between PFAS and risk factors for colorectal cancer.

As part of this review, 18 studies were included in Table 1 and Table 2 that were identified using the search strategy in Figure 1. A total of 10 of the studies met the study search criteria assessing the association between PFAS/PFOS and colorectal cancer and as a result, were included in Table 1. Table 1 includes, 5 animal studies, 2 studies assessing colorectal cancer cell lines and metabolism, and 3 epidemiological studies consisting of: 1 cohort study, 1 cross-

sectional study and 1 ecological study. A total of 8 of the studies were included in Table 2 as they included relevant information assessing PFAS/PFOS exposure and the association with other cancers, as well as with the association of risk factors for colorectal cancer such as ulcerative colitis, Crohn's disease and inflammatory bowel diseases (IBD).

Animal studies assessing the association between PFAS/PFOS and colorectal cancer

The 5 animal studies included are all mouse studies that assessed the relationship between PFAS/PFOS exposure and colorectal cancer. Of the 5 studies, 3 studies assessed the role of PFOS/PFOA on the small intestine and colon and 2 studies found that there is an inverse relationship between PFOS exposure and colorectal cancer. The study Rashid et al. (2020), evaluated the accumulation of PFOA and the induced alterations in the expression of epigenetic and tight junction genes in the small intestine and colon in CD1 mice. Although there was strong evidence of PFOA induced epigenetic effects in colon tissue, the study found that the expression of epigenetic and tight junction proteins Claudins (Cldn), Occludin (Ocln), and Tight Junction Protein (Tjp) were heavily altered in the small intestine. PFOA triggers DNA methylation changes and alters the expression of genes that are essential to maintain the physical barrier of the intestine, with more profound effects in the small intestine compared to the colon. More importantly, the study assessment after 10 days of PFOA exposure, showed that the small intestine and colon tissues of the female CD1 mice were shown to have a significant amount of PFOA accumulation compared to the control group with no PFOA detected. This is further supported in the study Rashid et al. (2023) which assessed the impact of PFOS and GenX exposure in gut microbiota. A total of 44 adult male mice were included in this study and assessed in three different concentrations with their vehicle controls. The study found that both PFOS and GenX exposure resulted in alterations of the gut microbiota and potentially disrupted the microbial functional pathways as well as the liver metabolome. The

study concluded that PFOS exhibited a stronger impact on colon microbiota and liver metabolome compared to GenX, possibly due to the high potency of PFOS. While these two animal studies assessed alterations caused by exposure to either PFOA or PFOS including GenX, the study Rashid et al. (2020) highlights more profound effects of PFOA in the small intestine compared to the colon and the study Rashid et al. (2023), highlights stronger alterations caused in the gut microbiota and liver metabolome by PFOS than GenX exposure. In contrast to these two studies, the study Ngo et al. (2014) investigated whether PFOA or PFOS resulted in obesogenic effects as well as if they resulted in the increase in spontaneous intestinal tumorigenesis in the mouse model after in utero exposure. The study found that PFOA or PFOS did not increase the incidence or number of tumors in the small intestine or colon of the Min/+ mice and it did not affect their location along the intestines.

The remaining 2 animal studies assessed the relationship between PFOS exposure and colorectal cancer. The study Wimsatt et al. (2016) randomized APC^{min} male and female mice at 5-6 weeks of age to receive different doses of PFOS through their drinking water. Gastrointestinal tumors were counted and scored, and blood PFOS levels were measured to investigate the effect of PFOS exposure. The study found that chronic exposure to PFOS in drinking water has an inverse dose-response effect, resulting in reduced formation of gastrointestinal tumors. These reductions were found to be both significant and dose-dependent with the highest dose groups showing the largest effects. The study concluded that this inverse dose-response effect could suggest that PFOS could potentially be a new form of treatment for familial colorectal cancer. The Wimsatt et al. (2018) study was conducted using primary patient colorectal cancer cell xenografts into NSG mice with goals to 1) assess potential factors supporting the successful use of colorectal cancer cells from heterogeneous tumors for PDX studies and 2) evaluate PFOS as a therapy in tumor matched pairs of mice randomized to receive PFOS or vehicle. In two mice, by 5 weeks of treatment, PFOS eliminated their 52.4 mm³

and 124.6 mm³ masses completely. These observations led to the findings that PFOS reduces tumor size in one group of tumors, particularly tumors located in the right ascending colon, suggesting that PFOS may be utilized in the treatment of colorectal cancer and may potentially offer a new treatment modality.

The 5 mouse studies indicate inconsistent relationships between PFOS/PFOA exposure and colorectal cancer. The 3 studies, Rashid et al. (2020), Rashid et al. (2023) and Ngo et al. (2014) show distinct results of the impact of PFOS/PFOA exposure on the small intestine and colon such as more profound effects of PFOA in the small intestine compared to the colon, stronger alterations in the gut microbiota and liver metabolome by PFOS than GenX, and results that indicate no increase in the number of tumors in the small intestine or colon, suggesting a negative association. On the contrary, the 2 studies, Wimsatt et al. (2016) and Wimsatt et al. (2018) suggest that PFOS can potentially be utilized as treatment for colorectal cancer and may offer a new treatment modality. In conclusion, these 5 mouse studies show inconsistent association between PFOS/PFOA and colorectal cancer, with 2 studies showing a moderate association, 1 study showing a negative association and 2 studies suggesting an inverse association.

Epidemiological studies assessing the association between PFAS/PFOS and colorectal cancer

The three epidemiological studies that assess the association between PFAS/PFOS and colorectal cancer are Innes et al. (2014), Messmer et al. (2022), and Li et al. (2022). The study by Innes et al. (2014) is cross-sectional study and conducted on a large Appalachian population of 47,151 cancer-free adults ages 21 and older and 208 cases of primary colorectal cancer in adults ages 21 and older in the mid-Ohio valley. The study assessed serum levels of PFOA, PFOS, and a range of other blood markers and after adjusting for multiple potential confounders, found that individuals who had serum PFOS levels in the highest quartile were

approximately 80% less likely to have received a colorectal cancer diagnosis and individuals in the highest quartile of PFOA were approximately 40% less likely to have received a colorectal cancer diagnosis. The study concluded that there was a strong inverse association between PFOS and individuals with a colorectal cancer diagnosis and a more modest inverse association between exposure to PFOA and colorectal cancer.

Another epidemiological study, Messmer et al. (2022), is an ecological study that assessed residents of Merrimack, New Hampshire who had at least 65 square miles of drinking water contaminated by PFAS from a plastic coating plant in the area. This study compared the risks of the residents of Merrimack, New Hampshire with demographically similar towns in New England between 2005 and 2014, assessing 24 types of cancers identified from a previous study, as well as the risk of all-cause cancers experienced and compared it to both the U.S. national cancer rates and cancer rates in 4 demographically similar towns in New England. The exposure of PFOA in drinking water in Merrimack, New Hampshire was 140 parts per trillion (ppt). Comparator drinking water PFOA exposure for South Portland, ME Water District was 2 ppt, Bennington, VT was between 40 to 2880 ppt and the districts Auburn, ME Water district, Sanford, ME Water District and Colchester, VT did not detect any levels of PFOA in their drinking water. Merrimack residents were found to have a 14% risk of all-cause cancers, a significant 49% higher risk of colon cancer and 45% higher risk of prostate cancer when compared to the demographically similar community, Colchester. Additionally, another demographically similar town, Bennington was also found to have a 14% increased risk for all-cancers, 100% increased risk for bladder cancer, a 31% increased risk for colon cancer and a 37% reduced risk for prostate cancer when compared to the U.S. average. In comparison to similarly exposed New England communities, Merrimack residents were found to experience significantly higher risk of thyroid cancer and prostate cancer, and to experience significantly higher risks of colon cancer when compared to pooled data from demographically similar New

England towns that have documented exposure to PFAS in drinking water supplies. While, the residents of Merrimack were found to be at significantly higher risks of colon cancer when compared to pooled data of similar towns, overall the residents experienced a significantly higher risk of at least 4 types of cancer over ten years (thyroid, bladder, esophageal and mesothelioma) when compared to national averages.

The Li et al. (2022) study is a cohort study that was conducted in Ronneby, Sweden on a large cohort population of 60,507 individuals from the Ronneby Register Cohort who lived there between 1985 and 2013. PFAS exposure in the Ronneby municipality was found to be extremely high, particularly dominated by PFOS and PFHxS and to a lesser degree by PFOA. It was found that in a subset of approximately 3,300 Ronneby residents who participated in a biomonitoring study, serum PFHxS level in the Ronneby population was on average more than 100 times higher than the general population of Sweden and approximately 30 times higher than the findings in the C8 study. For PFOS, on average the residents of Ronneby were approximately 35 times higher than the general population of Sweden and 7 times higher than the findings in the C8 study. Of the Ronneby Register Cohort of 60,507, 5,702 individuals with cancer were identified to assess their risks associated with contaminated water. The study found a moderate increased risk of kidney cancer, and showed moderately increased standardized cancer incidence ratios for rectum cancer among those who resided in Ronneby at an address that was supplied with highly contaminated waterworks at any time between 1985 and 2013 for both men and women. However, internal estimates for colon cancer were below or at unity in those who resided in Ronneby at an address that was supplied with highly contaminated waterworks at any time between 1985 and 2013. Overall analysis of this large cohort exposed to high levels of PFAS showed no evidence for an overall increased risk for cancer resulting from PFAS exposure.

The three epidemiological studies were all conducted on large populations but show inconsistent results when the association between PFAS exposure and colorectal cancer are assessed. The study Innes et al. (2014) shows an inverse association between PFOS/PFOA and colorectal cancer, while the Messmer et al. (2022) study shows Merrimack residents to be at significantly higher risks of colon cancer when compared to pooled data of similar towns in, and the Li et al. (2022) indicates estimates of colon cancer for Ronneby residents to be below or at unity, suggesting no association.

Studies assessing the association between PFAS/PFOS and colorectal cancer cell lines and metabolism

The 2 studies by Miao et al. (2015) and Zhang et al. (2023) both assess the association between PFOA and human colorectal cancer (DLD-1) cells. Miao et al. (2015) is a laboratory-based experimental study that used human immortalized colorectal cancer cells DLD-1 that were purchased from the American Type Culture Collection and seeded into a 96-well plate with serum-free medium, 2,000 cells per well. After 24 hours, the cells were treated with different doses of PFOA and after the cells were exposed, trans-well filter assays were used to assess cell migration. The results of the assays indicated that PFOA treatment can stimulate DLD-1 colorectal cancer cell invasion significantly. The Zhang et al. (2023) study is an experimental animal model study that investigates, a total of 20 targeted metabolites related to metabolism of glucose, glutamine and fatty acids in PFOA-treated human colorectal cancer (DLD-1) cells and analyzes them using C metabolic flux analysis (MFA). The results of the 20 metabolites indicated that PFOA-treated DLD-1 cells showed significant changes in glucose consumption and decreased glucose flux into the tricarboxylic acid (TCA) cycle and biosynthesis of fatty acids. This suggests that metabolic remodeling could occur in intestinal cells that are exposed to PFOA and this was most likely because of PFOA toxicity relevant with the loss of glucose in

biomass synthesis and energy metabolism. These two studies show a pattern in the findings associated with PFOA exposure and cell lines, particularly assessing the impacts on human colorectal cancer (DLD-1) cells and intestinal cells. The Miao et al. (2015) study suggests that PFOA exposure can potentially stimulate DLD-1 cell invasion, and the Zhang et al. (2023) study indicates that PFOA exposure can result in metabolic remodeling in intestinal cells.

Other relevant literature

The 4 studies Olén et al. (2020), Kunovszki et al. (2020), Xu et al. (2020), and Fart et al. (2021) discuss common causes of colorectal cancer and assess the role of different factors such as exposure to PFAS/PFOS as well as other diseases such as inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis and inflammation. The study Fart et al. (2021) is a case-control study that investigated the association between exposure to high levels of PFAS with 1) late-onset IBD and 2) disturbances of the bile acid pool. The study found that the total amount of PFAS substances was significantly increased among patients with ulcerative colitis when compared to those patients with Crohn's disease and healthy controls, and found that PFAS substance levels were higher among patients with late-onset ulcerative colitis and may contribute further to the disease by inducing a dysfunctional intestinal barrier. The Olén et al. (2020) cohort study during the follow-up period, observed that 639 of the 1336 incidences died from colorectal cancer in the ulcerative colitis (UC) cohort and that 4,451 of the 9,544 incidences died of colorectal cancers in the reference individuals. The study found that compared to individuals without UC, individuals with UC are at an increased risk of developing colorectal cancer, are often diagnosed with less advanced colorectal cancer and are at an increased risk of dying from colorectal cancer. The relationship between ulcerative colitis and colorectal cancer is also supported in the Kunovszki et al. (2020) study which is a population-based study that assessed the epidemiology and mortality of the Hungarian UC population

between 2010 and 2016. The study found that colorectal cancer was the most common cancer and 8.5% of the ulcerative colitis incident subpopulation was diagnosed with colorectal cancer. The study found that there is significantly worse survival of UC patients who suffer from colorectal cancer than that of the general population. The study, Xu et al. (2020) on the other hand, investigated the association between PFAS and inflammatory bowel disease (IBD) in the Ronneby cohort which has a high PFAS exposure from Aqueous Film-Forming Foam (fire-fighting foam) through drinking water. The study explored association of PFAS exposure with fecal calprotectin and zonulin which are biomarkers of gut inflammation and permeability in a subset of 189 participants from Ronneby and a nearby control municipality, Karlshamn. Using serum PFAS measures and fecal biomarkers, the study did not find sufficient evidence to support PFAS exposure from drinking water as a risk factor for the development of IBD.

Findings from the 4 studies show distinct study findings on the relationship between PFAS and ulcerative colitis and the relationship between ulcerative colitis and colorectal cancer. The 2 studies Olén et al. (2020) and Kunovszki et al. (2020) assess the relationship between ulcerative colitis and colorectal cancer and have found that patients with ulcerative colitis are at a greater risk of being diagnosed with and dying from colorectal cancer. The Fart et al. (2021) study showed that PFAS substance levels were higher among patients with late-onset ulcerative colitis and may contribute further to the disease by inducing a dysfunctional intestinal barrier. The study Xu et al. (2020) on the other hand, found that there isn't sufficient evidence to support that PFAS exposure from drinking water is associated with the development of IBD.

As part of the search strategy, Table 2 consists of 8 relevant literature that, while did not fit the strict inclusion criteria, were relevant to assess the association between PFAS/PFOS exposure and colorectal cancer. Table 2 includes 4 reviews assessing existing literature on the effects of PFAS/PFOS exposure and 4 epidemiological studies consisting of 1 registry study, 1 case-control study, 1 cohort study, and 1 population-based study. Of the 4 reviews, the scoping

review by Steenland and Winquist (2020) assessed 28 studies looking at PFAS/PFOS exposure and its association with different types of cancer and included one particular study on the prevalence of colorectal cancer which showed a reverse-causation for PFOA exposure and colorectal cancer development. This review concluded that the findings of studies included, provide informative but not entirely conclusive findings on the association between PFAS and cancer.

Some groups of people are particularly more at risk to being exposed to PFAS/PFOS due to occupational exposures, such as firefighters. Rosenfeld et al. (2023) is a review that summarizes the history of firefighters' occupational exposure particularly to aqueous film-forming foam (AFFF) as a source of PFAS exposure. Exposure of PFAS from AFFF can be from contaminated turnout gear through dermal exposure, ingestion and inhalation of AFFF and turnout gear textiles, ingestion of contaminated food and water, smoke inhalation and dust ingestion. This review concludes from the findings of international, national and state agencies that PFOA, a long-chain PFAS, is potentially carcinogenic and firefighters have elevated levels of long-chain PFAS in their serum. Studies included in this review provide a robust analysis, indicating that firefighters have an increased cancer risk for thyroid, kidney, bladder, testicular, prostate, and colon cancer.

Two reviews particularly assessed the impact of PFAS/PFOS exposure on colorectal cancer risk factors. Li et al. (2022) found that PFAS entering the gastrointestinal tract (GI tract) can result in disruption of the intestinal barrier system by causing inflammation in the gut, destruction of the gut epithelium and tight junction structure, reduction of the mucus layer, and induction of the toxicity of immune cells. Additionally, this review identified that there are some PFAS alternatives which showed specific intestinal toxicity in animal studies. PFAS accumulation was also found to induce microbial disorders and metabolic product changes and intestinal barrier defects were found to be associated with a broad range of diseases, such as

gastrointestinal diseases (like celiac disease), inflammatory bowel disease, irritable bowel syndrome, and colon carcinoma among other extra-intestinal disorders. The Durham et al. (2023) review analyzed existing literature examining the association of PFOS exposure and GI inflammation, metabolism, immune responses, and carcinogenesis. The findings from this review suggested that while there is a link between long-term PFOS exposure, lipid metabolism dysregulation, inflammation, microbiome dysfunction and the etiology of colorectal cancer, healthy dietary interventions such as diets high in fiber can help reduce or prevent PFOS-mediated disease risks. The findings of this review however, show that evidence for an association between PFOS and GI cancer remains sparse.

Discussion

In the current research landscape on PFAS, particularly PFOA and PFOS the International Agency for Research on Cancer (IARC), the Agency for Toxic Substances and Disease Registry (ATSDR), and the EPA show exposure linked with multiple cancers and other adverse health effects (National Academies of Sciences, Engineering, and Medicine, 2022). In this review, the following question was investigated: based on the current existing literature, is there an association between PFAS/PFOS exposure and colorectal cancer? Of the 18 published literature examined as part of this review, there isn't sufficient evidence to establish a relationship between PFAS/PFOS exposure and colorectal cancer.

PFAS/PFOS and Colorectal Cancer

Of the 18 included studies, 10 studies are included in Table 1, looking at the association of exposure to PFAS/PFOS and colorectal cancer. There are 5 animal studies, 2 studies assessing cell lines and metabolism and 3 epidemiological studies with distinct findings on the

association between PFAS/PFOS exposure and colorectal cancer. The study Ngo et al. (2014) suggests that exposure to PFOA or PFOS does not increase the incidence or number of tumors in the small intestine or colon whereas the study Rashid et al. (2020) shows contrasting observations that PFOA accumulation occurs in the small intestine and colon and is associated with alterations in the expression of epigenetic and tight junction genes. This observation suggests that there could be potential molecular disruptions that could contribute to the onset of colorectal cancer development. Additionally, the study Rashid et al. (2023) assesses the impact of PFOS and GenX on gut microbiota and liver metabolome, finding that PFOS indicates a significantly stronger influence. This suggests that there is a complex relationship between exposure to PFAS and microbiota dynamics with the risk of colorectal cancer development. On the contrary, the 2 studies Wimsatt et al. (2016) and Wimsatt et al. (2018) introduce a new dimension of PFAS/PFOS exposure effects, suggesting that PFOS exposure may result in reduced formation of gastrointestinal tumors, reduction of tumor sizes and may also offer a new avenue to potentially be utilized as treatment for colorectal cancer. The overall evidence presented in the 5 animal studies underscores the multifaceted and intricate effects of PFOS/PFOA exposure on overall colorectal health and shows inconsistent findings with 1 study pointing towards a negative association with PFOS/PFOA exposure not increasing the incidence of tumors, 2 studies suggesting the possibility of PFAS resulting in molecular disruptions and microbiota dynamics, and 2 studies that look at an inverse association between PFAS exposure and reduction of gastrointestinal tumors, suggesting exploration of PFOS as a new therapeutic agent to treat colorectal cancer.

Taking a look at the 3 epidemiological studies assessing PFAS/PFOS exposure and colorectal cancer in different populations, the studies show distinct findings. The Messmer et al. (2022) study conducted in Merrimack, New Hampshire where the public water supply was contaminated by PFAS, found significantly elevated risks for colon cancer among residents

compared to demographically similar towns in New England. However, this study also highlighted the complexity of PFAS exposure as it showed both an increased risk and a decreased risk for various other cancers in residents of Merrimack, New Hampshire compared to national averages as well as when compared to demographically similar exposed communities in New England. The findings of this study warrant further research to assess PFAS exposure and different cancer types, particularly colorectal cancer. Another study assessing the impacts of drinking water contaminated by PFAS exposure Li et al. (2022) revealed a moderate increase in the risk of kidney cancer but found estimates for colon cancer to be below or at unity, indicating no clear association for colon cancer and PFAS exposure. Analysis of the overall cohort, found that there isn't sufficient evidence showing that PFAS exposure results in an increased risk of overall cancer, suggesting that further research is needed for a comprehensive understanding of the various impacts of PFAS and different cancers. In contrast to the findings of the two epidemiological studies, the study Innes et al. (2014), a cross-sectional study conducted on a large Appalachian population revealed a strong inverse association between PFOS and colorectal cancer. This study contradicts some of the associations observed in previous studies, thus highlighting and suggesting a multifaceted and distinct relationship between PFAS exposure and colorectal cancer dependent on different contexts.

These 10 studies reveal contradicting associations between PFAS/PFOS exposure and colorectal cancer. Even within the animal studies, study findings vary with 1 study showing a negative association with PFOS/PFOA exposure and incidence of tumors, 2 studies suggesting the possibility of PFAS resulting in molecular disruptions and microbiota dynamics, and 2 studies that look at an inverse association between PFAS exposure and reduction of gastrointestinal tumors, suggesting that PFOS is being explored as a new therapeutic agent for colorectal cancer. The two studies assessing colorectal cancer cell lines and metabolism show

a pattern in evidence for an association between PFOA exposure and stimulation of colorectal cancer cell invasive ability and metabolism but this association is not enough to establish an association. Lastly, taking a look at the 3 epidemiological studies, there are complex findings, with one study showing significantly elevated risks for colon cancer among residents compared to demographically similar towns in New England, another study revealing estimates for colon cancer to be below or at unity, indicating no clear association for colon cancer and PFAS exposure and the third study resulting in a strong inverse association between PFOS and colorectal cancer. While there are some studies of the 10 included as part of the strict search criteria, that suggest an association between PFAS/PFOS exposure and colorectal cancer, the findings do not show sufficient evidence to establish an association between PFAS/PFOS exposure and the development of colorectal cancer, suggesting that further research is warranted to explore the association between exposure of PFAS/PFOS and colorectal cancer.

Assessing further, the two studies that looked at colorectal cancer cell lines and metabolism, by Zhang et al. (2023) and Miao et al. (2015), analyze the multifaceted and intricate relationship between PFAS and colorectal cancer. The study Zhang et al. (2023), a metabolic analysis investigating PFOA-treated human colorectal cancer (DLD-1) cells found significant alterations in glucose metabolism as well as decreased cellular processes such as tricarboxylic acid (TCA) cycle and fatty acid biosynthesis, highlighting the potential link between PFOA exposure toxicity and the processes in colorectal cancer cells. These findings are further supported by the Miao et al. (2015) study that explored the invasive ability of DLD-1 colorectal cancer cells. The study found that PFOA treatment can significantly stimulate DLD-1 colorectal cancer cell invasion, indicating the impact that PFOA exposure has on the behavior of colorectal cancer cells particularly its potential role in enhancing the invasive ability of DLD-1 colorectal cancer cells. These two studies show evidence for an association between PFOA exposure and significant alterations in colorectal cancer cells and metabolism such as stimulation of colorectal

cancer cell invasive ability and metabolic remodeling of intestinal cells, but additional research is needed to better understand the mechanisms of PFOA exposure on colorectal cancer (DLD-1) cells and metabolic remodeling of intestinal cells.

Other risk factors for Colorectal Cancer

As part of this review, while Table 1 includes 10 publications that met the strict search criteria, Table 2 includes the remaining 8 publications identified through the search as critical to assess the risk of colorectal cancer. Of the 8 publications, 4 are reviews and 4 are epidemiological studies. The findings from the 4 epidemiological studies provide a comprehensive assessment of a potential indirect association between exposure of PFAS, particularly PFOA and PFOS, and colorectal cancer through pathways such as inflammatory bowel disease (IBD).

The study Xu et al. (2020) investigated the Ronneby cohort which was exposed to high PFAS from Aqueous Film-Forming Foam (fire-fighting foam) in their drinking water and measuring fecal biomarkers, the study found that there isn't sufficient evidence to support that PFAS exposure is a risk factor for development of inflammatory bowel disease (IBD) in this cohort, suggesting that there is limited evidence for an association between PFAS and risk of IBD. On the other hand, Fart et al. (2021) is a case-control study that investigated the association between PFAS exposure with late onset IBD and disturbances of the bile acid pool. The study found that the total amount of PFAS substances was significantly increased among patients with ulcerative colitis, when compared to patients with Crohn's disease and healthy controls, suggesting its contribution to the disease by inducing a dysfunctional intestinal barrier. Additionally, the ex vivo experiment resulted in PFOA exposure increasing ileal and colonic permeability, suggesting that PFAS could play a role in intestinal barrier dysfunction.

The studies Olen et al. (2020) and Kunovszki et al. (2020) also provide insights

investigating the association between IBD and colorectal cancer. Olen et al. (2020) found that individuals who were diagnosed with ulcerative colitis (UC) are at an increased risk of developing colorectal cancer and at an elevated risk of mortality from colorectal cancer than individuals who do not have ulcerative colitis. The findings from Kunovszki et al. (2020) also support this as the study investigated and found that survival rates of ulcerative colitis patients with colorectal cancer were significantly worse than the general population. The evidence from the 4 epidemiological studies suggest that there is a complex and indirect relationship between PFAS exposure, IBD development and the subsequent risk of colorectal cancer. While current studies show a limited understanding on the link between PFAS and IBD, the association between IBD and colorectal cancer is emphasized in these studies, suggesting that there could be an indirect link between PFAS exposure and colorectal cancer and thus further research is warranted to establish links in this multifaceted relationship between PFAS and colorectal cancer through pathways such as IBD.

The Steenland and Winquist (2020) review is a scoping review that assesses current literature on the association of different cancers and exposure to PFAS. This review included only one study that assessed the association between PFOA and colorectal cancer. The overall findings of this review suggest that existing evidence is limited and that there isn't sufficient evidence to establish an association between PFAS exposure and colorectal cancer. The review Rosenfeld et al. (2023) on the other hand, particularly assesses the risk of firefighters and conducts a comprehensive review on the occupational exposures to PFAS, particularly aqueous film-forming foam (AFFF) resulting in an increased risk of cancer. The review assesses exposure pathways as well as elevated levels of long-chain PFAS in firefighters resulting in an increased risk for various cancers including colon cancer. While this review does not show sufficient evidence to establish an association between PFAS and colon cancer in firefighters, it suggests that further research is needed as firefighters are at imminent risk of various cancers

resulting from PFAS exposure.

The review Li et al. (2022) on the other hand, delved into the multifaceted relationship between PFAS exposure and gastrointestinal tract health, assessing the disruption of the intestinal barrier system by causing inflammation in the gut, destruction of the gut epithelium and tight junction structure, reduction of the mucus layer, and induction of the toxicity of immune cells. The review assesses PFAS accumulation in the gastrointestinal tract which can result in a number of diseases such as inflammatory bowel disease, irritable bowel syndrome, and colon carcinoma and the findings suggest that PFAS may result in an indirect association with the development of colorectal cancer. The Durham et al. (2023) review also looks at the potential link between long-term PFOS exposure, lipid metabolism dysregulation, inflammation, microbiome dysfunction and the etiology of colorectal cancer. This suggests that there isn't sufficient evidence for an association between PFOS and gastrointestinal cancer as data is still sparse and rather assesses a potential avenue of diets high in fiber to reduce or prevent PFOS-mediated disease. The 4 reviews underscore the complex nature of the relationship between PFAS exposure and colorectal cancer as there are variations in exposure, mechanisms of toxicity, as well as gaps in evidence, indicating the need for further research to explore the impact of PFAS on colorectal health and carcinoma.

The review of current literature assessing the link between PFAS and colorectal cancer, did not find sufficient evidence to establish an association. Taking into account the findings from the National Academies of Sciences, Engineering, and Medicine 2022 report, the findings from reviews by the EPA, OECD and C-8 Science Panel, that there is an association between PFAS exposure and ulcerative colitis, a rare autoimmune condition of the gastrointestinal (GI) tract, several other risk factors for colorectal cancer were identified, including inflammatory bowel disease (IBD) such as either ulcerative colitis or Crohn's disease (American Cancer Society, n.d.). PFAS/PFOS may contribute to the development of inflammatory bowel diseases (IBD)

and this is supported in several studies that have shown evidence to support an association between PFAS/PFOS exposure and increased inflammatory responses in the GI tract. These findings underscore the need for further investigation to assess not just the potential direct link between PFAS/PFOS exposure and colorectal cancer but the multifaceted and potential indirect link between PFAS/PFOS and risk of developing colorectal cancer through the pathways of PFAS exposure resulting in inflammation of the gut, intestinal tissues, and other inflammatory bowel diseases.

Environmental Factors and Colorectal Cancer

While further research is to be undertaken on assessing the relationship between PFAS exposure and colorectal cancer, it is critical to assess other environmental factors that contribute to the development of colorectal cancer. Environmental factors involve a broad range of influences such as lifestyle, diets, and exposures to contaminants/pollutants. Several epidemiological studies have implicated dietary choices as a key factor in colorectal cancer risk. Diets high in processed meats, red meats and alcoholic drinks have been associated with the development of colorectal cancer (Murphy et al., 2019). Sedentary behaviors such as physical inactivity and excess body weight and obesity have also been found in several epidemiologic studies to be factors of developing colorectal cancer. Additionally, with dietary risk factors, environmental exposures resulting from consuming contaminated drinking water or breathing in polluted air over time, can indirectly influence the development of colorectal cancer. The various environmental factors need to be investigated further to assess the impact on development of colorectal cancer.

Limitations and Recommendations

PFAS are widely spread in the environment today and while their adverse health effects

are well known, their impact on the development of colorectal cancer is not clear. Firstly, exposure to PFAS can be from several diverse sources and the multifaceted pathways of exposure can make it difficult to establish a direct relationship between PFAS and the development of colorectal cancer. Studies identified in this review illustrate different populations, different chemicals as well as half of the 10 studies that are part of Table 1, are animal studies with distinct results. A potential limitation is that several studies included in this review are retrospective study designs looking at large cohorts which may introduce recall bias as participants' work to recall exposures.

To further assess the association between PFAS/PFOS exposure and colorectal cancer, more studies need to be conducted on large human cohorts and studies must include rigorous study designs when working with large sample sizes. Longitudinal studies as well as more mechanistic studies assessing the indirect biological mechanisms such as inflammation, oxidative stress, and hormonal disruptions through which PFAS may influence colorectal cancer need to be conducted. The inclusion of diverse populations could enhance the findings of the relationship between PFAS/PFOS and colorectal cancer as it could account for genetic and environmental influences for colorectal cancer. Additionally, mouse studies need to have strict criteria so the study results can be applied to better understand human risks. Lastly, when assessing PFAS/PFOS exposure with the risk of colorectal cancer, it is critical to take into account confounding factors such as environmental, genetics and lifestyle to be able to distinguish the contribution PFAS exposure has on the risk of colorectal cancer.

Conclusion

The current existing body of published literature does not provide sufficient evidence to establish an association between exposure to PFAS/PFOS and risk of colorectal cancer development. Findings from the literature included, show complex and distinct results such as

negative associations, molecular disruptions and microbiota dynamics, cell lines invasive ability, metabolic remodeling of cells, as well as inverse associations between PFAS exposure and colorectal cancer. While some studies suggest an indirect relationship between PFAS/PFOS exposure and colorectal cancer through the pathway of PFAS exposure and development of inflammatory bowel diseases, additional research is warranted to further explore the mechanisms of PFAS exposure, intestinal inflammation, inflammatory bowel diseases and development of colorectal cancer. It is also important to assess the environmental factors that play a role in the development of colorectal cancer. In conclusion, while current existing literature does not provide sufficient evidence to establish an association between PFAS/PFOS exposure and development of colorectal cancer, there is some evidence indicating an indirect relationship between PFAS/PFOS exposure and colorectal cancer through the pathway of PFAS exposure resulting in intestinal inflammation and inflammatory bowel disease which are risk factors for colorectal cancer. The findings in this review suggest that further research is needed to better understand the mechanisms through which PFAS/PFOS affect colorectal cancer and establish an association between PFAS/PFOS exposure and colorectal cancer.

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