Chapter 12
Carcinogenicity of Perfluoroalkyl Compounds

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Abstract This chapter reviews the information available on the carcinogenic potential of perfluoroalkyl acids in both animals and humans. Historically, perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS) have been the most widely used members of this chemical class making these the subject of the largest proportion of the reported studies. Caution needs to be exercised in projecting the biological activities of any of the chemicals in this family based on results from others. For example, considering the three chemicals for which lifetime studies in rats are available, the outcomes were different with no increase in tumors seen with perfluorohexanoic acid (PFHxA), liver adenomas seen with PFOS, and adenomas of the liver, testis, and pancreas seen with PFOA. Mechanistic studies suggest that the liver tumors seen with PFOA reflect the activation of PPARα while the mechanism for tumor formation in the testis and pancreas is less clear. Epidemiologic studies have been reported for several levels of population exposure. Limited evidence of associations with kidney and testicular cancer has been reported in studies among community members exposed to drinking water contaminated by PFOA. Studies in workers exposed to higher levels of both PFOA and PFOS have not shown consistent evidence for an association with any specific cancer type. Studies in populations exposed to low levels of PFOA and PFOS have shown equivocal results for a variety of cancers with no consistent associations. Based on the evidence reported to date, the prospect for developing a carcinogenic outcome following exposure to PFOA and PFOS is remote. For other perfluoroalkyl acids, there is not sufficient evidence regarding their potential carcinogenicity. It should be noted that human exposures to these chemicals is currently quite low and appears to be decreasing.

Keywords PFOA • PFOS • Rodent carcinogenicity • PPAR alpha activation • Worker epidemiology • Community epidemiology

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J.C. DeWitt (ed.), *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances*, Molecular and Integrative Toxicology,
DOI 10.1007/978-3-319-15518-0_12

This chapter will cover the published information relating to the potential carcinogenic activity of perfluoroalkyl acids. The reader will quickly notice that the information cited comes mainly from one member of this family of compounds, perfluorooctanoic acid (PFOA). A natural conclusion might be that this is the key member of the family in terms of potential carcinogenic activity. However, the focus on this chemical comes from the effort to more completely describe potential hazards of this particular chemical because it, like many members of the family, is capable of entering the human body and has attenuated elimination kinetics. Animal studies on PFOA, perfluorooctane sulfonate (PFOS), and perfluorohexanoic acid (PFHxA) evaluating carcinogenic potential have been conducted while other members of this chemical class have not been studied. Industrial use of PFOA and PFOS has resulted in occupationally exposed workers who have been studied for cancer mortality and, less frequently, cancer incidence. Community members living near a chemical plant in West Virginia using PFOA were studied because of exposure through PFOA-contaminated water. This activity resulted in an evaluation of the cancer profile in these surrounding communities. This type of information was designed to look at the potential impact of PFOA on these communities where exposures were greater than seen in other communities but not as great as exposures in workers making and using the chemical.

It might be tempting to look at the structural similarities of these chemicals and use results from one member of the group to predict biological activities of others. Indeed, it has been suggested that, similar to the approach taken for polychlorinated biphenyls, dioxins, and dibenzofurans, the use of Toxic Equivalency factors be employed for risk assessment purposes. Scialli et al. (2007) used data from four different perfluoroalkane acids (PFOS, PFOA, perfluorobutanesulfonate-PFBS, and perfluorodecanoic acid-PFDA) where tests were available on the same species using essentially the same designs, and constructed dose-response curves which could be modeled for concordant endpoints. Scialli and colleagues were unable to identify a scaling system that gave values consistently within an order of magnitude for the same compounds and concluded that combining exposure levels of perfluoroalkane acids for risk assessment was not supportable. A caution to this conclusion was that with additional data being made available, this position could be re-evaluated. Peters and Gonzales (2011) also looked at the appropriateness of using toxic equivalency factors for perfluoroalkyl chemicals and also concluded that the use of such an approach is likely unsuitable. Four facts which do not support predicting the effect of one perfluoroalkyl chemical by using the results from another are that, first, on a mechanistic basis, the effects of these chemicals are modulated by more than one receptor. Second, where comparative data are available, the induced effects are quite discordant. Third, very limited information has been published to evaluate either additivity or synergism with these chemicals. Fourth, the lack of solid data does not allow application of additivity. Importantly, the lack of a strong data base for many of the commonly used commercial perfluoralkyl chemicals seriously limits evaluation. Peters and Gonzales (2011) also presents inherent limitations that would need to be overcome including bioavailability and pharmacokinetics, understanding of the target genes the mediate toxicity, influence of species differences,

identification of potential nonadditive effects, and influence of endogenous chemicals that could modify the effect(s) of these chemicals.

Thus, the reader is cautioned to apply the information presented for a specific chemical to that chemical and not extend the findings (or non-findings) to other perfluoroalkanes. Also, because the reader will note that material covered here is predominantly derived from studies of PFOA, and to a lesser degree studies of PFOS, it must be remembered that this reflects more accurately their use rather than their potential for biologic activity among members of this chemical family. A final introductory note is that when evaluating the human information, those individuals exposed to greater amounts of chemical would be expected to produce a greater, rather than a lesser, chance of response. Therefore, studies in those working directly with the material would have the greatest exposure and would be most likely to respond.

12.1 Animal Studies

12.1.1 Bioassays with APFO

A limited number of long-term studies looking at the carcinogenic potential of PFCs have been published. With APFO (the ammonium salt of PFOA), two long-term feeding studies were conducted in rats (Biegel et al. 2001; Butenhoff et al. 2012). Two-year studies were also conducted in rats fed PFOS (Thomford 2002) and PFHxA (Klaunig et al. 2014). Although the dosing period was only 6 months, studies in monkeys were conducted with PFOA (Butenhoff et al. 2002) and PFOS (Seacat et al. 2002) which included looking at a variety of endpoints associated with long term outcomes which could be linked to cancer (Butenhoff et al. 2002).

For APFO, rats of both sexes were fed either 30 or 300 ppm (approximately 1.5 and 15 mg/kg) for 2 years (Butenhoff et al. 2012). A significant increase in Leydig cell tumors of the testes was seen in the males fed 300 ppm. No increase in tumor incidence of any other tissues or organs was seen in the males fed 30 ppm or in both groups of females (Table 12.1). The conclusion of the original study was that there was no increase in the incidence of proliferative lesions in the mammary gland in the APFO-treated rats above the historical control and normal expected background incidence from the published literature for female Sprague-Dawley rats. However, the incidence of fibroadenomas in the mammary gland was increased in the high-dose group when compared to the concurrent controls; therefore, a Pathology Working Group (PWG) review of this tissue was conducted using current diagnostic criteria. The consensus reached by the PWG was that the incidence of mammary gland neoplasms (lobular hyperplasia, fibroadenoma, and adenoma) was not affected by chronic dietary administration of APFO, and no increase in proliferative lesions in that tissue were produced (Hardisty et al. 2010). The primary difference between the original reported

Table 12.1 Incidence of neoplastic microscopic findings for male and female rats in either control groups fed 30 ppm or 300 ppm APFO in their diet for 2 years

Organ/lesion	Dietary dose group (ppm APFO)					
	Males			Females		
	0	30	300	0	30	300
Adrenal						
Pheochromocytoma, benign	2/49 (4)[a]	4/50 (8)	4/50 (8)	2/50 (4)	0/50 (0)	0/49 (0)
Pheochromocytoma, malignant	0/49 (0)	1/50 (2)	0/50 (0)	0/50 (0)	0/50 (0)	1/49 (2)
Liver						
Hepatocellular adenoma	0/49 (0)	0/50 (0)	0/50 (0)	0/50 (0)	0/50 (0)	0/50 (0)
Hepatocellular carcinoma	3/49 (6)	1/50 (2)	5/50 (10)	0/50 (0)	0/50 (0)	1/50 (2)
Mammary gland	–	–	–	7/46 (15)	14/45 (31)	5/44 (11)
Adenocarcinoma	–	–	–	3/46 (7)	0/45 (0)	0/44 (0)
Adenoma	–	–	–	1/46 (2)	0/45 (0)	0/44 (0)
Carcinoma	–	–	–	10/46 (22)	19/45 (42)	21/44 (48)[*]
Fibroadenoma	–	–	–	0/46 (0)	0/45 (0)	1/44 (2)
Lymphangiosarcoma	–	–	–			
Reevaluation by PWG[b]						
Adenocarcinoma				9/50 (18)	16/50 (32)[c]	5/50 (10)
Adenoma				1/50 (2)	0/50 (0)	0/50 (0)
Fibroadenoma				16/50 (32)	16/50 (32)	20/50 (40)
Fibroadenoma (multiple)				2/50 (4)	6/50 (12)	3/50 (6)
Pituitary						
Adenoma	17/48 (35)	17/47 (36)	13/46 (28)	33/46 (72)	39/47 (83)	36/50 (72)
Testes/epididymis						
Leydig cell adenoma	0/49 (0)	2/50 (4)	7/50 (14)[*]	–	–	–
Thyroid						
C-cell adenoma	0/43 (0)	2/47 (4)	4/47 (9)	1/50 (2)	0/45 (0)	0/41 (0)
C-cell carcinoma	2/43 (5)	0/47 (0)	0/47 (0)	0/50 (90)	0/45 (0)	0/41 (0)

[*]Statistically significant different from controls ($p \leq 0.05$)
[a]Number observed/number examined (%)
[b]Hardisty et al. (2010)
[c]The incidence in the groups sharing this footnote were statistically significantly different from each other ($p < 0.01$, Hardisty et al. 2010)

finding and the PWG involved classifying lesions originally noted as lobular hyperplasia as fibroadenomas and this occurred mainly in the control group.

A dose-related increase in the incidence of ovarian tubular hyperplasia was found in female rats sacrificed at 2 years (Mann and Frame 2004). The significance was unknown and there was no progression to tumors. Using more recently published nomenclature, these lesions were diagnosed as gonadal hyperplasia or tubular adenoma and no statistically significant increase in hyperplasia and adenomas was seen in the PFOA-treated rats. There was some evidence for an increase in stromal size in the 300 ppm group but the total number of rats with either adenoma or hyperplasia was 12, 16, and 17 in the 0, 30, and 300 ppm groups respectively which does not suggest a risk for tumor development.

To investigate the time course and mechanism of action of APFO, a 2-year feeding study in rats was conducted with a number of interim sacrifices to measure potential treatment related changes as a function of exposure time (Biegel et al. 2001). To match the exposure conditions in an earlier chronic study in rats (Butenhoff et al. 2012), a single test group exposed to 300 ppm was used along with a group pair-fed to the 300 ppm group to detect any possible influence of changes in feeding amounts. Increase in the incidence of adenomas in the liver, pancreas, and testis were seen in male rats receiving 300 ppm (equivalent to a daily dose of approximately 15 mg/kg) as shown in Table 12.2. Hyperplasia of both the pancreas and the testis was also increased. Cell proliferation was seen in the pancreas but not in either the liver or the testicular Leydig cells (Biegel et al. 2001).

The above tumor triad was produced in rats by clofibrate (Svoboda and Azarnoff 1979), HCFC-123 (Malley et al. 1995), gemfibrozil, and diethyl-hexyl phthalate (DEHP). Trichloroethylene (TCE) produced both liver and Leydig cell tumors (Cook et al. 1999; David et al. 2000; Voss et al. 2005). Nafinopen (Cook et al. 1999) produced both liver and pancreatic tumors. Other chemicals causing Leydig cell tumors in rats include clofibrate, gemfibrozil, methyl clofenazide, perchloroethylene, and TCE (Cook et al. 1999). In mice, estradiol exposure leads to Leydig cell tumors while estrogenic compounds do not induce testicular tumors in rats (Cook

Table 12.2 Incidence of liver, testes, and pancreas tumors in rats fed 300 ppm APFO in the diet for 2 years

		Groups:	Control	Pair fed	PFOA
Tumor	Cancer				
Liver	Adenomas		2/80[a]	1/79	10/76*
	Carcinomas		0/80	2/79	0/76
Testes	Adenomas		0/80	2/78	8/76*
(Leydig cell)	Hyperplasia		11/80	26/78*	35/76*
Pancreas	Adenomas		0/80	1/79	7/76*
	Carcinomas		0/80	0/79	1/76
	Hyperplasia		14/80	8/79	30/76*

*Statistically significant different from controls ($p \leq 0.05$)

[a]Number affected/number of rats tested

et al. 1999). In F344 rats there is an age-related increase in serum estradiol which correlates with Leydig cell hyperplasia and tumor formation (Grunewald et al. 1992).

Pancreas acinar cell tumors are modified by steroid concentrations, growth factors, cholecystokinin (CCK), and dietary fat (Longnecker 1983, 1987; Longnecker and Sumi 1990). CCK is a growth factor found in the gut mucosa which is released by the presence of food in the duodenum, then binds to pancreatic tumor cell receptors to release pancreatic secretions including chymotrypsin. It has been hypothesized that PFOA increases fat content in the gut by enhanced excretion of cholesterol and triglycerides resulting in hyperplasia and adenomas.

To look further at the induction of Leydig cell adenomas by APFO, male rats were treated by oral gavage with either 1, 10, 25, or 50 mg/kg for 14 days along with a group of pair-fed controls to the 50 mg/kg rats (Cook et al. 1992). A decrease in the rate of body weight gain was seen at 10 mg/kg and higher, and, since the body weights of the group of pair-fed and its control group were similar, this was attributed to decreased food intake. At the top two doses, accessory sex organ weight was decreased while testes weights and histopathology were unchanged. Serum estradiol levels were increased at 10 mg/kg and higher being 2.7 times control levels at 50 mg/kg. Serum testosterone concentrations decreased (3.2, 1.6, 1.6, 1.2, 0.8, and 0.7 ng/dl in rats receiving 0, 1, 10, 25, 50, and 50 mg/kg pair-fed respectively). Similarly, interstitial cell testosterone levels were lower in the APFO-treated rats with the greatest effect seen at 50 mg/kg in the pair-fed group. Liver weights at 10 mg/kg and higher were increased and beta oxidation also increased from 8 to 11 times in a dose-related fashion (Cook et al. 1992).

In a series of studies to determine the level of the testosterone lowering lesion, rats were given 50 mg APFO/kg for 14 days followed by treatment with human chorionic gonadotropin, gonadotropin-releasing hormone (GnRH), or naltrexone. Human chorionic gonadoptropin (hCG) affects lesions in the steroidogenic pathway by binding to luteinizing hormone (LH) receptors on Leydig cells to stimulate testosterone synthesis. GnRH affects lesions at the adenohypophysis by stimulating LH release from gonadotropin. Naloxone affects lesions at the hypothalamus by enhancing GnRH release by removal of inhibitory action of opiate neurotransmitters on GnRH controlling neurons. Only hCG led to a 50 % decrease in serum testosterone suggesting the lesion was at the testes modifying the conversion of 17 alpha to androstenedione. No changes seen with GnRH treatment suggests that the lesion was not at the pituitary level, and, for naltrexone, the lack of change suggests that the lesion was not at the hypothalamus level.

In a 6-month study in which cynomolgus monkeys were given daily doses of either 3, 10, or 30/20 (dose reduced to 20 mg/kg after 2 weeks) mg APFO/kg, the effects on biological markers associated with the hepatic, pancreatic, and testicular responses (seen in rats dosed with APFO and other peroxisome proliferating chemicals) were evaluated (Butenhoff et al. 2002). There was no increase in peroxisomal proliferation as measured by palmitoyl CoA oxidase activity. The approximately twofold increase seen at the high dose reflects that this species is not particularly responsive to peroxisome proliferating compounds. No changes in reproductive

hormone levels were seen as estradiol, testosterone, and cholecystokinin concentrations in each monkey were unaltered over the course of the experiment. No evidence of cholestasis as indicated by changes in bile acids, bilirubin, or alkaline phosphatase, was observed. Cell proliferation in the liver, pancreas, or testes, as demonstrated by replicative DNA synthesis, was not affected by APFO treatment. Although the study duration was only 6 months, biological markers associated with responses in the three tissues shown to result in adenomas in the rat were not affected.

12.1.2 Bioassay with PFOS

A 2-year study with PFOS fed to male and female rats at concentrations of 0, 0.5, 2, 5, and 20 ppm (dosing equivalents of 0, 0.02, 0.10, 0.25, and 1.1 mg/mg respectively) was conducted (Thomford 2002). An extra group fed 20 ppm for 1 year followed by a 1 year recovery with no PFOS added to the diet was employed. The incidence of hepatocellular adenomas in male rats showed a positive trend with 7/60 (11.7 %) found in the high-dose group compared to 0/60 (0 %) in the control group. In females, the incidence of hepatocellular adenomas was also increased with 5/60 (8.3 %) observed in the high-dose group compared to 0/60 in the control group. In addition, the only hepatocellular carcinoma in this study occurred in this group (Table 12.3).

Among males fed 20 ppm PFOS for 1 year with 1 year recovery, the incidence of thyroid follicular cell adenomas appeared to be increased. This was not observed in males fed 20 ppm continuously for 2 years or in females.

Another non-dose related observation was the apparent increase in mammary gland tumors in the group fed 0.5 ppm. Combining rats with either a mammary adenoma or a carcinoma, the incidence in all groups including the controls was relatively high. None of the remaining tissues or organs had tumor incidences that could be related to the feeding of PFOS. Further, although some of the incidence values in some of the test groups appear greater than those in the control group, the lack of a dose-response allows only a suggestion of carcinogenicity in the rat.

In a 6-month study in which cynomolgus monkeys were given daily doses of either 0.03, 0.15, or 0.75 mg potassium PFOS/kg, the effects on biological markers associated with the hepatic, pancreatic, and testicular responses seen with APFO in rats were evaluated (Seacat et al. 2002). Hepatic peroxisome proliferation measured by palmitoyl CoA oxidase activity was increased in the females given 0.75 mg/kg but the response was less than the twofold change typically associated with biological significance. No effects on cell proliferation were seen in either the liver, pancreas, or testes using the proliferating cell nuclear antigen immunohistochemistry cell labeling index.

Table 12.3 Incidence of neoplastic microscopic findings for rats fed PFOS for 2 years

	Dietary PFOS (ppm)	0	0.5	2	5	20	20[a]
Males							
Tumors							
Liver-hepatocellular adenoma		0/60	3/50	3/50	1/50	7/60*	0/40
Thyroid							
Follicular cell adenoma		3/60	5/49	4/50	4/49	4/59	9/39*
Follicular cell carcinoma		3/60	1/49	1/50	2/49	1/59	1/39
Females							
Liver							
Hepatocellular adenoma		0/60	1/50	1/49	1/50	5/60*	2/40
Hepatocellular carcinoma		0/60	0/50	0/49	0/50	1/60	0/40
Thyroid							
Follicular cell adenoma		0/60	0/50	0/49	2/50	1/60	1/40
Follicular cell carcinoma		0/60	0/50	0/49	1/50	0/60	0/40
Mammary gland							
Adenoma		23/60	30/50*	22/48	26/50	15/60	16/40
Carcinoma		11/60	12/50	11/50	11/50	14/60	10/40
Combined adenoma and carcinoma		29/60	36/50	31/48	29/50	24/60	17/40

From Thomford (2002)
*$p < 0.05$
[a]Fed PFOS for 1 year, control diet for 1 year

12.1.3 Bioassays with Other Polyfluorinated Compounds

A 2-year rat study was conducted to evaluate both the chronic toxicity and potential carcinogenicity of perfluorohexanoic acid (PFHxA) (Klaunig et al. 2014). Male rats were given daily gavage doses of either 0 (control), 2.5, 15, or 100 mg PFHxA/kg for 104 weeks. Female rats were given daily doses of either 0, 5, 30, or 200 mg PFHxA/kg. No increase in neoplasms related to treatment of PFHxA at any of the three dosage levels examined was seen in either male or female rats (Table 12.4).

In a TSCA 8(e) notification, a rat oral gavage study was conducted with 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy) propionic acid, ammonium salt (CAS 62037-80-3) in which female rats were treated with doses of either 1, 50, or 500 mg/kg daily for 23Months (Anand 2013). Doses for male rats were 0.1, 1, and 50 mg/kg and were treated for 24 months. Although actual incidence numbers were not given, an increase in liver adenomas was reported in female rats given 500 mg/kg. This result was not reported for females at the two lower doses. Non-neoplastic liver

Table 12.4 Neoplastic findings in rats dosed with PFHxA for 2 years

Incidence of neoplastic findings[a]										
				Males				Females		
		Dose (mg/kg)	0	2.5	15	100	0	5	30	200
Tissue	Lesion									
Adrenal cortex	Adenoma		0/60	0/60	0/60	1/70	2/60	1/60	0/60	2/70
	Adenocarcinoma		1/60	0/60	1/60	0/70	0/60	0/60	2/60	0/70
Adrenal medulla	Pheochromocytoma		4/60	3/60	2/60	1/70	1/60	1/60	2/60	1/70
Kidney	Tubular adenoma		0/60	2/60	0/60	0/70	0/60	0/60	0/60	0/70
	Tubular carcinoma		1/60	0/60	0/60	0/70	1/60	0/60	0/60	0/70
Liver	Adenoma		1/60	0/60	1/60	1/70	0/60	0/60	1/60	2/70
	Carcinoma		2/60	0/60	1/60	0/70	2/60	0/60	1/60	0/60
Brain	Astrocytoma		0/60	3/60	4/60	0/70	1/60	0/60	1/60	1/70
Pancreas	Ascinar adenoma		0/60	0/60	1/60	0/70	0/60	0/60	0/60	0/70
	Islet cell adenoma		9/60	10/60	5/60	4/70	4/60	2/60	3/60	4/70
	Islet cell carcinoma		0/60	2/60	1/60	0/70	2/60	0/60	0/60	1/70
Pituitary	Pars distalis-adenoma		32/60	38/60	33/60	29/70	50/60	51/60	54/60	55/70
	Pars distalis-carcinoma		0/60	0/60	0/60	0/70	1/60	0/60	0/60	2/70
	Pars intermedia-adenoma		12/60	16/60	16/60	16/70	0/60	1/60	0/60	1/70
	Pars intermedia-carcinoma		0/60	0/60	2/60	0/70	0/60	0/60	0/60	0/70
Prostate	Adenoma		1/60	1/60	0/60	0/70	–	–	–	–
Mammary gland	Fibroadenoma		1/60	0/60	0/60	0/70	14/60	17/60	18/60	20/70
	Adenoma		0/60	0/60	0/60	0/70	2/60	2/60	3/60	6/70
	Adenocarcinoma		0/60	1/60	1/60	1/70	12/60	9/60	14/60	10/60
Skin	Fibroma		0/60	0/60	0/60	1/70	1/60	0/60	0/60	0/70
	Papilloma		1/60	0/60	0/60	2/70	0/60	0/60	0/60	0/70

(continued)

Table 12.4 (continued)

Incidence of neoplastic findings[a]										
				Males				Females		
		Dose (mg/kg)	0	2.5	15	100	0	5	30	200
Tissue	Lesion									
Testes	Interstitial cell		1/60	1/60	1/60	0/70	–	–	–	–
Thyroid	C-cell-adenoma		10/60	5/60	3/60	5/70	9/60	4/60	14/60	7/70
	C-cell-carcinoma		0/60	0/60	0/60	0/70	0/60	1/60	0/60	0/70
	Follicular cell-adenoma		3/60	4/60	2/60	4/70	1/60	3/60	2/60	2/70
Uterus	Leiomyoma		–	–	–	–	1/60	0/60	0/60	0/70
	Endometrial polyp		–	–	–	–	2/60	1/60	3/60	2/70
	Endometrial adenocarcinoma		–	–	–	–	0/60	0/60	1/60	0/70

[a]Number affected/number tested

changes were reported in female rats given either 50 or 500 mg/kg. In males, marginal increases in interstitial cell tumors of the testis and acinar cell tumors of the pancreas were reported. No increase in liver adenomas was reported. Non-neoplastic liver lesions including hypertrophy, degeneration, and necrosis were reported in males given 50 mg/kg but not either 0.1 or 1 mg/kg.

12.1.4 Initiation/Promotion Studies

Both the ammonium and sodium salts of PFOA have been evaluated in a battery of genotoxicity tests (Butenhoff et al. 2014). Although PFOA is a hepatocarcinogen, the weight of evidence from these studies supports the position that PFOA is non-genotoxic and non-mutagenic. Consistent with PFOA being a non-genotoxic hepatocarcinogen, initiation-promotion studies have demonstrated that PFOA is an initiator of liver tumors.

In an initiation promotion study, rats were initiated with diethylnitrosamine (DEN), fed 2-acetylaminofluorene (AAF), and given a single dose of carbon tetrachloride (CCl_4) (Abdellatif et al. 1990). Following this, a group of 12 rats were fed a diet containing 150 ppm APFO for 7 months. Rats were then sacrificed for microscopic examination of the liver. The incidence of hepatocellular carcinomas was 33 % in the APFO fed rats compared to 0 % in the controls. APFO produced an increase in hydrogen peroxide (H_2O_2), catalase, and fatty acid beta-oxidation while having no effect on glycolate oxidase (leads to production of H_2O_2) and urate oxidase (an enzyme not found in humans) and serum triglyceride levels. The cancer effect was attributed in the overproduction of H_2O_2, an effect commonly seen with peroxisome proliferators.

Many peroxisome proliferators have been shown as promoters of liver tumors in rodents including WY-14,643, nafenopin, dichlorophenyl, trichloroethylene, and DEHP (Cook et al. 1999). As mentioned, it has been hypothesized that DNA damage is mediated by a reactive molecular species derived from H_2O_2 generated by peroxisomes during beta-oxidation of fatty acids. Following PFOA exposure, peroxide is observed in rat livers as the result of beta-oxidation. Double bond conjugation and peroxidation of membrane lipids leads to lipofuscin accumulation suggesting oxidative damage.

In a study, a single dose of 200 mg/kg DEN followed 2 weeks later by feeding of 0.03 % AAF for 2 weeks (reference group) or DEN followed 2 weeks later by a single 2 mg/kg CCl_4 dose followed by feeding of 150 ppm PFOA for 2 weeks was conducted (Nilsson et al. 1991). Hepatocellular carcinomas were found in 3 of 12 PFOA-fed rats while 0/12 were seen in the reference group. Focal nodules were seen in both groups, six in the reference group and eight in the PFOA-fed group. Liver weight increases and well as increase acyl Coenzyme A, dicarbonyl Coenzyme A, catalase, and decreased triglyceride levels were seen.

A study of rainbow trout with up to 9 months of chronic exposure to PFOA alone did not produce an increase in liver tumor incidence (Tilton et al. 2008). Trout,

initiated with aflatoxin B1 in water at 0.01 ppm for 30 min, control water for 3Months, then PFOA at either 200 or 1,800 ppm (equivalent to either 5 or 50 mg/kg) for 6 months, showed a very weak promotion effect. Aflatoxin alone resulted in 36 % of the trout developing liver tumors. In aflatoxin B1 initiated trout, the liver tumor incidence in the 200 ppm PFOA group was 34 % while for those given 1,800 ppm PFOA, it was 71 %. The multiplicity of tumors at the higher dose of PFOA was increased with 10 % having six or more tumors. In this experiment, the PPARα agonist DEHP lead to a 100 % liver tumor incidence while no increase was seen with clofibrate.

12.1.5 Studies on the Mechanism of Action

The liver is the main target for perfluoroalkyl compounds in animals. Liver toxicity in rodents results from the ability of these compounds to activate the peroxisome-proliferator-activated-receptor (PPARα), a member of the nuclear receptor superfamily. Studies of PPARα in various species have shown the rat and mouse to be the most sensitive species in response to PPARα agonist, hamsters are moderately responsive, and guinea pigs, primates, and humans are less responsive. Activation of the receptor in rodents initiates a characteristic sequence of biochemical and morphological events mainly in the liver. These events include marked hepatocellular hypertrophy due to an increase in both the number and size of the peroxisomes, a large increase in peroxisomal fatty acid beta-oxidation, an increase in CYP450 mediated gamma-hydroxylation of lauric acid, and alterations in lipid metabolism. PPARα regulates lipid homeostasis through the modulation of expression of genes involved in fatty acid uptake, activation, and oxidation. Both PFOA and PFOS are relatively weak ligands compared to the naturally-occurring long-chain fatty acids such as linoleic and alpha-linoleic acid (Vanden Heuvel et al. 2006).

PFOA appears to induce liver tumors via binding to the PPARα nuclear receptor resulting in peroxisome proliferation and increased liver mitogenesis (Biegel et al. 2001; Maloney and Waxman 1999; Pastoor et al. 1987). The key events following PPARα ligands activating PPARα involve regulation of the transcription of genes involved in peroxisome proliferation, cell cycle/apoptosis, and lipid metabolism. This leads to perturbations in cell proliferation, apoptosis, and peroxisome proliferation. Suppression of apoptosis along with a stimulation of cell proliferation allows DNA-damaged cells to persist and proliferate giving rise to preneoplastic foci. Clonal expansion then leads to tumor formation.

A number of events have an influence on this process. Peroxisome proliferation may lead to oxidative stress which could cause indirect DNA damage or by stimulation of cell proliferation. PPARα ligands also inhibit gap junction intercellular communication and stimulate non-parenchymal hepatic Kupffer cells, both of which could induce cell proliferation. The evidence for these key events from PPARα activation to selective clonal expansion to yield liver tumors is quite convincing (Klaunig et al. 2003).

PFOA has been demonstrated to activate PPARα (Pastoor et al. 1987; Maloney and Waxman 1999). In PPARα knockout mice, PFOA did not increase beta-oxidation unlike that readily produced in wild-type mice (Yang et al. 2002). PFOA induction of hepatomegaly, peroxisomal beta-oxidation, microsomal 1-acylglycerophosphocholine acetyltransferase, and cytosolic long-chain acyl CoA hydrolase can be blocked in castrated male rats showing the effect to be related to the elimination rate (Kawashima et al. 1995). Several key endpoints which could be the initiating effect leading to liver tumors, (and possibly pancreas and testicular tumors) were shown to be modified by PFOA (Liu et al. 1996). These include increasing liver weight, hepatic beta-oxidation, hepatic aromatase (CYP19A1), and hepatic total cytochrome P450. These changes were observed in the 2-year rat study with PFOA (Biegel et al. 2001).

The induction of Leydig cell tumors by PFOA is postulated to be due to a hormonal mechanism whereby PFOA inhibits testosterone biosynthesis and increases serum estradiol levels via induction of hepatic aromatase activity (Biegel et al. 1995; Cook et al. 1992; Liu et al. 1996). This mechanism appears to be influenced and perhaps mediated by PPARα. The induction of pancreatic acinar cell tumors is postulated to be secondary to the liver effects, specifically a sustained increase in plasma cholecystokinin (CCK) secondary to reduced bile flow or altered bile acid composition resulting in an indirect inhibition of trypsin. An indirect inhibition of trypsin by WY-14,643 (a strong PPARα activator) results in an increase in CCK levels (Obourn et al. 1997).

Like some other PPARα agonists, PFOA induces hepatocellular adenoma, Leydig cell adenomas, and pancreatic acinar cell adenomas in rats. Although humans possess PPARα at sufficient levels to mediate the hypolipidemic response to therapeutic fibrate drugs, there are enough qualitative and quantitative differences between the response of the human liver to PPARα agonists relative to the response of the rat liver. These differences include gene promoters, receptor activities, and receptor levels that make the mode of action for liver tumors unlikely to be operative in humans. There is inadequate evidence to link PPARα and the induction of either Leydig cell adenomas or pancreatic acinar cell adenomas. Additionally, there is insufficient evidence to link other mode-of-actions with PFOA-induced testicular or pancreatic adenomas.

12.1.6 Ancillary Information

The occurrence of liver tumor with two other peroxisome proliferators, WY14643 and DEHP, was studied in both in wild-type and PPARα null mice (Ito et al. 2007). Mice fed DEHP at 12,000 ppm for 6 months developed liver enlargement, an increased number of peroxisomes, and eosinophilia, a series of findings not seen in the PPARα null mice. In this report, groups of Sv/129 mice, either null or wild type, were fed either 100 or 500 ppm DEHP from 3 weeks of age to 23Months. The incidence of hepatocellular adenomas (and possibly carcinomas) was slightly

increased in the null mice. Inflammatory cell infiltration and 8-OHdG levels (oxidative stress) were higher in null mice than wild type- both elevated from controls suggesting that oxidative stress may lead to induction of inflammation, expression of proto-oncogenes, and an increase in tumors in null mice. Thus, different mechanisms were shown to induce hepatocellular tumors in wild-type and PPARα null mice (Ito et al. 2007; Takashima et al. 2008). The mechanistic hypothesis included that either oxidative stress from increased beta-oxidation induced by peroxisome proliferators produces excess ROS leading to DNA damage and cancer or an imbalance in hepatocyte growth control reflected by increased cell proliferation and suppression of apoptosis disrupting hepatocyte growth control. The authors concluded that most likely both contributed.

To look at the activity of aromatase as a mechanism for the increased estradiol observed, rats were given oral gavage doses of 0, 0.1, 2, 20, or 40 mg APFO/kg for 14 days with a pair-fed group matching the top dose (Liu et al. 1996). Both testicular and hepatic aromatase activity along with body weights, liver weights, microsomal protein, and estradiol were measured. Aromatase activity in the liver was increased by up to 16 times but no significant effect on this testicular enzyme was seen. Body weight decreases were seen as well as increased liver weight along with increased hepatic beta-oxidation, cytochrome P450 activity, and protein content of microsomes. A doubling of serum estradiol was seen along with a linear correlation between serum estradiol and hepatic aromatase.

12.1.7 PFOA as an Anti-tumorigenic Agent

Some PPARα ligands have been shown to possess anti-tumorigenic properties, such as suppression of growth of several types of human cancer cells *in vitro* and inhibition of carcinogenesis *in vivo* making PPARα a potential candidate for cancer therapy (Pozzi and Capdevila 2008). PPARα ligands such as fibrates, which cause tumors in rodents, are commonly used therapeutically in humans with no evidence of carcinogenicity (Peters et al. 2005).

A Phase 1 clinical trial was conducted to assess the tolerability, safety, and pharmacokinetics of APFO administered orally once a week to human patients (Macpherson et al. 2010). A total of 42 patients, who had both advanced refractory solid tumors and clinically normal liver and kidney function were enrolled. Dose escalation, starting with a dose of 50 mg once a week followed a standard 3 + 3 design until dose-limiting toxicity was observed in two or more patients. The largest group of patients presented with colorectal cancer (N = 16) with pancreas, esophageal, and kidney cancers, each represented by two of more patients. Doses of up to 1,200 mg were tested without producing clinical changes in either the liver or kidney; thus, the goal of finding a dose-limiting toxicity was not attained. As a practical matter, APFO was given in 50 mg capsules so those given the highest dose tested needed to take 24 capsules orally. Although the study was not designed to evaluate efficacy, stable disease at 12 weeks or greater was observed in eight of the

first 37 patients enrolled and included a case with anaplastic thyroid at 40 weeks, one with pancreatic cancer at 35 weeks, and one with cervical cancer at 34 weeks. Further studies have not been conducted at this time.

The proposed mechanism for the anti-tumorigenic response is through inhibition of PIM-1 kinase. PIM proteins belong to a family of serine and threonine kinases which play a role in cell cycle regulation and have a potent anti-apoptotic activity. Increased expression of PIM kinase is associated with malignant subtypes of leukemia and lymphoma (Adam et al. 2006; Cohen et al. 2004; Brault 2010) and a number of solid tumors including pancreatic (Li et al. 2006; Chen et al. 2009; Reiser-Erkan et al. 2008), colorectal (Popivanova 2007), esophageal (Beier et al. 2007), and prostate (Chen et al. 2005; Mumenthaler et al. 2009; Roh et al. 2008) cancers.

APFO has been tested in four human tumor xenograft models, HT-29 (colon), PC3 (prostate), PANC-1 (pancreatic), and HepG2 (liver) (Elcombe et al. 2011). Anti-tumor effects were detected in all models. No significant toxicity was observed in the treated mice although there was liver weight enlargement and some evidence of changes in liver enzyme function. The effect of PFOA on HT-29 (colon adenocarcinoma) tumors was assessed in nude mouse xenografts. Mice were inoculated with a tumor cell suspension on each flank and tumors were allowed to grow for 16 days. APFO was given by intraperitoneal injection of 25 mg/kg 3 times a week for 4 weeks. The HT-29 tumor volumes at 30 days were 280 mm^3 in the saline injected controls compared to 175 mm^3 in the APFO treated mice. Relatively few animals were used in each group but a suggestion of anti-tumor effect was noted. In a parallel experiment using a prostate tumor cell line PC3, APFO intraperitoneal doses of either 5, 15, or 25 mg/kg were used. All of the APFO mice showed decreased tumor volume with a volume of 10 mm^3 in the highest APFO group compared to 50 mm^3 in the saline-treated controls.

Two other xenograft models were tested with similar results. Using the human pancreatic cell line PANC-1 (a slow growing tumor in vivo), a fourfold increase in size over a 90-day test period was seen in the controls compared to a 2.5-fold increase in mice receiving 25 mg/kg APFO. Tumor weights were 0.5 g in the APFO-treated mice compared to 1.2 g in the controls. A lesser response was seen in a test using a xenograft model of liver carcinoma in cell line HepG2. After 24 days of test, APFO-treated mice had a tumor volume of 1,000 mm^3 and a weight of 1.5 g while the controls had a volume of 1,200 mm^3 and a weight of 1.8 g.

12.2 Studies Involving Exposed Humans

12.2.1 General Epidemiologic Concepts

For the purpose of this review, risk estimates reported by epidemiologic studies are described as measures of potential associations between cancer, either as all cancers or for specific diagnostic types, and PFOA and other perfluorinated alkyl acids

(PFAAs) including PFOS. For mortality studies, typically reported for occupational cohorts, the Standardized Mortality Ratio (SMR) is estimated as the ratio of observed number of cancer deaths among a study group relative to an expected count of cancer deaths estimated from a defined reference population rate (Checkoway et al. 2004). In addition, relative risk (RR) estimates evaluate the probability of a cancer death or diagnosis among those assigned to a higher exposure category relative to those persons classified as less exposed. A related measure of RR is the odds ratio (OR) which is a measure of association based on the same relative comparison of exposure groups and describes the odds of having cancer among exposed cases relative to the odds of not having cancer among exposed controls (Gordis 2009). Finally, several studies report the hazard ratio (HR) which is estimated using a proportional hazards (PH) model, usually the Cox PH model. The HR is the ratio of the rate of cancer events between different levels of exposure using time to the event (*i.e.*, cancer diagnosis or death) as the time-scale variable. An increased HR indicates an earlier occurrence of the event among the exposed group relative to the reference group assuming that the underlying hazard rates are proportional for the two groups (Kleinbaum 1996).

For all measures of risk, estimates are presented with the reported 95 % confidence interval (CI) as a standard convention. In addition, statistical significance of risk estimates is interpreted based on the lower and upper values of the 95 % CI and the corresponding p-value for the association. Risk estimates measuring association between exposure and cancer are considered to be significant when the 95 % CI does not include 1.0 in its range, consistent with $p<0.05$. Associations that have a reported 95 % CI that does include the value 1.0 (*i.e.*, $p>0.05$), cannot exclude random chance as an explanation for the measured association. Many published studies emphasize observed increased risk estimates that are not statistically significant when describing and interpreting results; however, these estimates are not indicative of a valid, non-random increase in risk any more than non-significant risk estimates less than 1.0 point to a possible lowering of risk associated with exposure.

Studies may categorize exposure for study participants by defining a classification approach based on subjective levels of exposure potential or by applying a quantitative distribution such as quartiles. These studies may also present a test for trend, usually indicated by a p-value for an analysis of the ordered categorical risk estimates. In many cases, the trend test is based on the assumption of a monotonic relationship between exposure category and outcome as evaluated by a linear regression model. The p-value from such tests conflates the test of significance for the slope coefficient from a regression model with an assumed monotonic dose-response without estimation of the actual exposure-response relationship at biologically plausible exposure values (Maclure and Greenland 1992). In particular, this approach is problematic when applied to a naive method such as percentile classification (*i.e.*, quartiled exposure groups) when exposure is within a very narrow range of values (Greenland 1995). Caution should be taken into account when interpreting studies that report non-significant categorical associations but rely on a significant trend test p-value for inference of an association.

12.2.2 Occupational Studies – PFOA

A number of studies have looked at the potential carcinogenic effects of PFOA in exposed persons, particularly exposed chemical workers. These studies include workers based in manufacturing plants using the chemical for industrial purposes with occupational exposure to PFOA estimated by a job exposure matrix (JEM) approach. The occupational cohorts studied have involved industrial facilities of the 3M Corporation (manufacturing plants at Cottage Grove, Minnesota and Decatur, Alabama), and DuPont (a polymer production facility, the Washington Works plant, located in Washington, West Virginia). The DuPont plant primarily used APFO in polyethylene production processes. A separate cohort study for tetrafluoroethylene (TFE) synthesis and polymerization workers comprised workers at six facilities operated by four companies including employees from the DuPont Washington Works facility. In addition, there are a series of studies among a community population who were residents of 6 water districts in the Mid-Ohio River Valley in Ohio and West Virginia exposed to drinking water contaminated with PFOA. Exposure assessment for the Mid-Ohio River Valley community studies included both measurement of concentrations in blood serum samples as well as cumulative estimates of drinking water exposure determined by environmental fate and transport modeling. A third group of studies include individual population-based studies of various human cancers among persons with general background levels of exposure to PFOA as measured by serum concentrations taken from biologic samples.

A proportional mortality analysis among 3M plant workers exposed to industrial fluorochemicals including primarily PFOA and PFOS at the Cottage Grove plant was reported (Ubel et al. 1980). A total of 3,688 employees employed during the years 1948 to 1978 were included in the cohort and 180 deaths were recorded through the end of follow-up (159 males and 21 females) of which 177 were matched with death certificates providing information as to underlying cause. The number of female deaths was considered to be too few for meaningful statistical analysis. Among male workers, observed mortality counts agreed with expected numbers for specific causes of death due to cancer. This study provides limited evidence to evaluate the potential association between PFOA and PFOS exposures and cancer mortality with no notable increases observed among fluorochemical workers at the Cottage Grove plant.

A subsequent retrospective cohort mortality involving 2,788 males and 749 females employed at the Cottage Grove plant from 1947 to 1983 was reported (Gilliland and Mandel 1993). Inclusion in the PFOA-exposed category was based on any job history in the Chemical Division for 1 month or more while the unexposed category comprised workers who either never worked in Chemical Division or did so for less than 1 month. Vital status was ascertained through 1989 for the cohort and expected mortality numbers were estimated from United States (U.S.) and Minnesota population rates. For all female employees, the overall cancer SMR was 0.71 (95 % CI: 0.42, 1.14) with no significant increase for any specific cancer

type. The overall cancer SMR for all male employees was 1.05 (95 % CI: 0.86, 1.27) with no significant increase for any single cancer type.

Among the 1,339 male workers who worked at least 1 month or more in the Chemical Division, no significantly increased SMRs were reported for cancers of the gastrointestinal tract including specific results for the colon and pancreas, respiratory tract including the lung, testis, bladder, or lymphopoietic system including leukemia. The authors note that for prostate cancer deaths, workers in the Chemical Division had an SMR of 2.0 (95 % CI: 0.6, 4.6) for four observed deaths compared to approximately two deaths that were expected based on Minnesota White male mortality rates. Among these four cases, only one of the employees appears to have worked directly in the PFOA production building (Olsen et al. 1998).

Gilliland and Mandel (1993) included the use of an internal cohort of non-Chemical Division workers considered to be non-exposed as a comparison group to minimize the potential for the healthy worker effect, a bias widely noted when observed mortality is lower for occupational cohorts relative to expected mortality based on general population rates (Monson 1986). The authors applied a proportional hazards regression model to estimate the HR for all cancer deaths and for prostate cancer deaths among male employees for four occupational metrics: year and age of first employment, duration of employment, and months spent in the chemical division. Although, all cancer deaths were not significantly increased with increasing number of months in the Chemical Division, the rate of prostate cancer death was significantly increased for each month spent in the Chemical Division. The estimate of the HR for each year in the chemical division associated with prostate cancer mortality was 1.13 (95 % CI: 1.01, 1.27); however, the authors note that this finding is based on a small number of cases and could be biased by unmeasured confounders as occupational exposure to PFOA or PFOS was not estimated for any worker.

An updated mortality study in a cohort of 3,993 employees at the Cottage Grove plant was reported (Lundin et al. 2009). Three general categories of PFOA exposure were identified: ever definite exposure (primarily jobs in electrochemical fluorination), probable occupational exposure (jobs in other Chemical Division areas where exposure was possible but assumed to be lower and transient), and no or minimal exposure (jobs in the Non-Chemical Division of the plant). No increase in the SMR for deaths from all cancers was seen in any of the three groups. The all cancer SMRs were 0.9 (95 % CI: 0.5, 1.4), 0.9 (95 % CI: 0.8, 1.1), and 0.8 (95 % CI: 0.6, 1.0) in the ever definite exposure group, the probable exposure group, and the minimal exposure group, respectively. SMRs for cancers of the biliary passages and liver; pancreas; respiratory cancers of the trachea, bronchus, and lung; and bladder and other urinary organs showed no evidence of exposure-related associations. The prostate cancer SMRs were 2.1 (95 % CI: 0.4, 6.1), 0.9 (95 % CI: 0.4, 1.8), and 0.4 (95 % CI: 0.1, 0.9) in the ever definite exposure group, the probable exposure group, and the minimal exposure group, respectively.

Lundin and co-authors (2009) created additional exposure categories: high exposure (included workers with definite exposure for 6 months or more), moderate exposure (included workers with probable exposure or those with definite exposure for less than 6 months), and low exposure (included workers primarily in the

nonchemical division of the plant). Prostate cancer mortality was significantly increased among workers in the high exposure group (HR = 6.6, 95 % CI: 1.1, 37.7, two deaths) with a non-significant increase estimated for the moderate exposure group (HR = 3.0, 95 % CI: 0.9, 9.7, ten deaths) when compared to the low exposure group (four deaths). For the combined high and moderate exposure groups, the HR was 3.2 (95 % CI: 1.0, 10.3, 12 deaths) when compared to the low exposure group. Interpretation of the relative risk estimates for prostate cancer mortality is complicated by a deficit of prostate cancer mortality in the low exposure group which was assigned as the referent group. Workers in this exposure category had an abnormally low occurrence of prostate cancer death as indicated by a significantly reduced SMR when compared to expected prostate cancer deaths based on the Minnesota male population (SMR = 0.4, 95 % CI: 0.1, 0.9). The authors cautioned that the prostate cancer risk should be elucidated using incident cases, rather than deaths from the disease.

In the most recent report from this cohort, both cancer mortality and incidence were assessed for two groups of 3M workers comprising 9,027 total employees (Raleigh et al. 2014). The cohort included 4,668 workers with potential occupational exposure to PFOA at the Cottage Grove plant and 4,359 workers with no occupational exposure to PFOA at a non-related production facility in St. Paul, Minnesota. Mortality and cancer incidence for this combined cohort were determined from linkage of workers with the National Death Index and with cancer registries for the states of Minnesota and Wisconsin. Industrial hygiene data and expert evaluation were used to create a task-based JEM to estimate cumulative PFOA exposure. SMRs were estimated using expected mortality numbers based on Minnesota population mortality rates. HRs for time-dependent cumulative PFOA exposure were estimated from an extended Cox PH model. Outcomes of *a priori* interest included mortality and incidence for cancers of the liver, pancreas, testes, kidney, prostate, and breast.

Observed mortality counts in the PFOA-exposed cohort were less than the numbers expected for deaths based on Minnesota residents resulting in SMRs less than 1.0 for all listed cancers (Table 12.5). When assessing selected causes of deaths based on cumulative PFOA exposure categorized by quartiles, the HRs for mortality from cancer outcomes of interest did not show an association with increasing exposure. Similarly, there was little evidence that incident cancers were associated with PFOA exposure (Table 12.6). Compared to the non-exposed population of workers from the St. Paul facility, there were no significant HRs observed for incident cancers in the combined two highest exposure quartiles of PFOA among workers at the Cottage Grove plant. No association was observed between PFOA exposure and incident cases of kidney, prostate, or breast cancer when analyzed by quartile of cumulative exposure. The authors conclude that this analysis did not support an association between occupational exposure and cancer mortality or incidence but caution that for some of the cancers of interest, the study had limited ability to detect a precise association due to small numbers of cases.

Cancer mortality among workers at the DuPont Washington Works plant has been reported by Leonard et al. (2008) who conducted a study with the primary

Table 12.5 Standardized mortality ratios (SMR) for selected causes of death for the Cottage Grove and Saint Paul cohorts

	Cottage Grove plant		Saint Paul plant	
Cause	Obs	SMR (95 % CI)	Obs	SMR (95 % CI)
All causes	1,125	0.85 (0.80, 0.90)*	1,829	0.98 (0.94, 1.03)
All cancers	332	0.87 (0.78, 0.97)*	514	1.04 (0.95, 1.13)
Liver cancer	8	0.81 (0.35, 1.59)	7	0.55 (0.22, 1.14)
Pancreatic cancer	18	0.85 (0.50, 1.34)	30	1.09 (0.74, 1.56)
Prostate cancer	24	0.83 (0.53, 1.23)	48	1.03 (0.76, 1.37)
Kidney cancer	6	0.53 (0.20, 1.16)	18	1.23 (0.73, 1.95)
Breast cancer	11	0.82 (0.41, 1.47)	15	1.39 (0.78, 2.29)
Bladder cancer	8	0.89 (0.38, 1.76)	8	0.62 (0.27, 1.22)
Diabetes mellitus	27	0.76 (0.50, 1.11)	64	1.42 (1.09, 1.81)*
Ischaemic heart disease	248	0.84 (0.74, 0.95)*	444	0.95 (0.87, 1.05)
Cerebrovascular disease	57	0.81 (0.61, 1.05)	112	1.02 (0.84, 1.23)
Chronic renal disease	14	1.09 (0.60, 1.84)	13	0.72 (0.38, 1.24)

From Raleigh et al. (2014)
*Statistically significant ($p \leq 0.05$)

Table 12.6 Hazard Ratios for selected cancers comparing APFO exposure quartiles to the referent population

	Exposure quartile									
	Referent		Q1		Q2		Q3		Q4	
Cancer	Obs	HR(95 % CI)	Obs	HR(95 % CI)	Obs	HR(95 % CI)	Obs	HR(95 % CI)	Obs	HR (95 % CI)
Prostate cancer	253	1 (referent)	42	0.80 (0.57, 1.11)	42	0.85 (0.61, 1.19)	49	0.89 (0.66, 1.21)	55	1.11 (0.82, 1.49)
Kidney cancer	19	1 (referent)	4	1.07 (0.36, 3.16)	4	1.07 (0.36, 3.17)	4	0.98 (0.33, 2.92)	4	0.73 (0.21, 2.48)
Pancreatic cancer	15	1 (referent)	Combined with Q2		1	0.13 (0.02, 1.03)	Combined with Q4		9	1.36 (0.59, 3.11)
Bladder cancer	43	1 (referent)	7	0.81 (0.36, 1.81)	6	0.78 (0.33, 1.85)	15	1.50 (0.80, 2.81)	12	1.66 (0.86, 3.18)
Breast cancer	28	1 (referent)	8	0.36 (0.16, 0.79)	8	0.65 (0.29, 1.42)	14	1.47 (0.77, 2.80)	4	0.85 (0.29, 2.46)

From Raleigh et al. (2014)
Referent population = Saint Paul, MN plant

Table 12.7 Cancer mortality for DuPont Washington works employees compared to three external reference populations

	WW cohort	US population			WV population			DuPont region I workers		
Cause of death	O	E	SMR	CI	E	SMR	CI	E	SMR	CI
All malignant neoplasms	234	315	0.74	0.65, 0.84*	340	0.69	0.60, 0.78*	229	1.02	0.89, 1.16
Liver	8	8.1	0.99	0.43, 1.96	6.9	1.15	0.50, 2.27	5.5	1.45	0.63, 2.86
Pancreas	11	15.4	0.71	0.36, 1.28	13.7	0.80	0.40, 1.43	11.2	0.98	0.49, 1.76
Breast	2	3.7	0.55	0.07, 1.97	3.5	0.57	0.07, 2.05	2.8	0.70	0.09, 2.54
Prostate	12	23.2	0.52	0.27, 0.91*	20.9	0.58	0.30, 1.00	18.4	0.65	0.34, 1.14
Testes	1	1.2	0.87	0.02, 4.84	1.3	0.76	0.02, 4.22	0.6	1.70	0.04, 9.46
Kidney	12	7.9	1.52	0.78, 2.65	7.9	1.51	0.78, 2.64	6.6	1.81	0.94, 3.16
Thyroid/other endocrine glands	3	1.0	3.12	0.64, 9.12	1.1	2.86	0.59, 8.35	0.5	6.29	1.30, 18.37*

From Leonard et al. (2008)
O observed, *E* expected, *SMR* standardized mortality ratio, *CI* = 95 % confidence interval
*Statistically significant ($p \leq 0.05$)

objective to determine if mortality from ischemic heart disease was increased in a cohort based on a previous association between PFOA exposure and increased lipids (Sakr et al. 2009). The secondary objective of the study was to examine a broad range of other causes of mortality including cancer outcomes. The cohort included 6,027 individuals working at the plant from January 1, 1948 through December 31, 2002, the end date for mortality ascertainment. SMRs were estimated based on three reference population rates: the U.S. population, the West Virginia population, and an eight-state regional population of over 74,000 DuPont employees with no work history at the Washington Works facility. Similar to retrospective cohort study of the Cottage Grove plant, all Washington Works employees were considered to have PFOA exposure even though only 23 % of 1,025 workers who participated in a previous health survey had work assignments in PFOA areas of the plant (Sakr et al. 2007).

All cancer mortality was significantly lower among the workers compared to the U.S. and the West Virginia population rates and was no different from the DuPont employee reference rates (Table 12.7). For specific cancer mortality outcomes, no significant increases were reported for observed deaths due to liver, pancreas, testicular, prostate, or breast cancers. For kidney cancer (12 deaths observed through 2002), SMRs for workers were 1.52 (95 % CI: 0.78, 2.65) when compared to the US reference rate; 1.51 (95 % CI: 0.78, 2.64) compared to the West Virginia reference rate; and 1.81, (95 % CI: 0.94, 3.61) compared to the DuPont regional worker

reference rate. Bladder cancer mortality (seven deaths observed through 2002) was similar to expected numbers based on the U.S. and West Virginia population rates and resulted in an SMR of 1.30 (95 % CI: 0.52, 2.69) when compared to the DuPont regional worker reference rate. Of interest is that the SMR for prostate cancer was significantly decreased compared to the U.S. population (SMR = 0.52, 95 % CI: 0.27, 0.91), and prostate cancer SMRs were reduced compared to West Virginia residents (SMR = 0.58, 95 % CI: 0.30, 1.00) and the DuPont regional employees (SMR = 0.65, 95 % CI: 0.34, 1.14).

An update of mortality ascertainment for the Washington Works cohort extended through 2008 was reported by Steenland and Woskie (2012). The updated study observed an increase in the total number of deaths in the cohort from 806 to 1,084 during the six additional years of follow-up through December 31, 2008. This update also analyzed cancer mortality based on occupational exposure to PFOA. Using a job exposure matrix developed by Kreckmann et al. (2009), workers were assigned to one of eight job category and job group combinations for estimation of cumulative PFOA exposure (Woskie et al. 2012). Modeled serum PFOA levels among workers in each job category and group combination were correlated with measured levels by job category overall and across time to derive cumulative exposure estimates for 5,791 workers with sufficient work history records. Cumulative exposure was categorized by a quartile distribution with the lowest quartile assigned as the referent category for analyses.

The SMR for total cancer mortality did not differ significantly for plant workers in any quartile of estimated cumulative serum PFOA when compared to the DuPont regional employee reference rates (SMR = 0.93, 95 % CI: 0.83, 1.04) and was significantly lower than expected based on the U.S. reference rate (SMR = 0.74, 95 % CI: 0.66, 0.83). Although six additional years of mortality ascertainment were added, the number of kidney cancer deaths (12) was equal to the number reported by Leonard et al. (2008) as no kidney cancer deaths occurred among cohort members from 2003 to 2008. The SMR for kidney cancer among all workers combined was 1.28 (95 % CI: 0.66, 2.24) while the SMR for the highest quartile (quartile 4) of cumulative PFOA exposure was significantly increased (SMR = 2.66, 95 % CI: 1.15, 5.24) with no significant increase observed for the other exposure quartiles (quartiles 1, 2, and 3). For mesothelioma, a significant positive exposure-response trend was observed when compared to other DuPont regional workers based on six deaths (SMR = 2.85, 95 % CI: 1.05, 6.20) with five deaths observed in the highest quartile of PFOA exposure (SMR = 6.27, 95 % CI: 2.04, 14.63). The authors state that the increased SMR for mesothelioma did not appear to be specific to PFOA exposure and suggested that it was the result of co-exposure to asbestos among workers that was highly correlated with estimates of cumulative PFOA exposure.

In addition, Steenland et al. (2015) presented additional analyses based on medical record review among 3,713 workers at the Washington Works facility. Eighteen disease outcomes with incident cases greater than or equal to 20 were analyzed. Among the four incident cancer outcomes reported, prostate cancer showed a positive non-significant trend (p-value for categorical trend test = 0.11, 129 cases). Bladder cancer had a significant negative trend such that higher PFOA exposure

Table 12.8 Mortality by cumulative exposure to APFO (unit–years) among 4,773 male workers ever exposed to TFE, 1950–2008

Cumulative exposure to APFO (unit-years)				
Cause of death				
	Never exposed	Low (<16)	Medium (16–138)	High (139+)
	Obs/Exp	Obs/Exp	Obs/Exp	Obs/Exp
	SMR (95 % CI)	SMR (95 % CI)	SMR (95 % CI)	SMR (95 % CI)
All causes	101/132.3	178/243.3	178/220.9	178/225.2
	0.76 (0.62, 0.93)*	0.73 (0.63, 0.85)*	0.81 (0.69, 0.93)*	0.79 (0.68, 0.92)*
All cancer	28/40.1	51/65.8	53/65.4	55/70.3
	0.70 (0.46, 1.01)	0.78 (0.58, 1.02)	0.81 (0.61, 1.06)	0.78 (0.59, 1.02)
Esophageal cancer	0/1.3	4/2.5	4/2.6	3/2.6
	–	1.62 (0.44, 4.14)	1.54 (0.42, 3.93)	1.16 (0.24, 3.39)
Liver cancer	1/1.4	1/1.4	2/1.6	4/1.9
	0.72 (0.02, 4.02)	0.70 (0.02, 3.87)	1.25 (0.15, 4.52)	2.14 (0.58, 5.49)
Pancreatic cancer	3/1.8	0/3.2	4/3.1	6/3.3
	1.66 (0.34, 4.84)	–	1.30 (0.35, 3.33)	1.84 (0.67, 4.00)
Lung cancer	10/13.3	20/21.9	16/21.3	13/23.9
	0.75 (0.36, 1.39)	0.91 (0.56, 1.41)	0.75 (0.43, 1.22)	0.54 (0.29, 0.93)*
Kidney and other urinary organs cancer	0/1.0	3/1.9	3/2.0	4/2.0
	–	1.57 (0.32, 4.59)	1.50 (0.31, 4.39)	2.00 (0.54, 5.12)
Leukemia	1/1.3	4/2.4	3/2.2	4/2.2
	0.79 (0.02, 4.40)	1.64 (0.45, 4.20)	1.35 (0.28, 3.94)	1.85 (0.50, 4.74)

Reference: National Rates
From Consonni et al. (2013) supplement
*Statistically significant ($p \leq 0.05$)

quartiles had a lower relative risk for this incident disease (p-value for log cumulative exposure trend = 0.04, 29 cases). No significant trend tests were reported for either colorectal cancer (41 cases) or melanoma (41 cases) (Steenland et al. 2015).

A retrospective cohort mortality study including 5,879 male workers from six tetrafluoroethylene (TFE) production sites in Europe and the U.S. was reported (Consonni et al. 2013). Occupational TFE exposure was the main focus with cumulative exposure to PFOA estimated using an exposure matrix that was highly correlated with TFE exposure estimates (Sleeuwenhoek and Cherrie 2012). The TFE study sites differed for duration of ascertainment period with an average of 25 years of follow-up overall. Among 4,205 workers classified as ever having occupational exposure to PFOA among those workers with TFE exposure, the SMR for all cancer deaths was significantly reduced (SMR = 0.79, 95 % CI: 0.67, 0.92) compared to an expected number estimated from national rates (Table 12.8 – Consonni

et al. 2013 supplement). For workers categorized in the highest tertile of cumulative PFOA exposure, there was no significant increased SMR for cancers of the esophagus, liver, pancreas, lung, or kidney, or for leukemia. The authors conclude that no exposure-response trend was observed for any of these outcomes and the study was limited by the inability to separate the potential effects from either PFOA or TFE.

12.2.3 Occupational Studies – PFOS

All available epidemiologic studies of cancer risk and occupational exposure to PFOS have been conducted among the employee cohort at a 3M facility in Decatur, Alabama that manufactured PFOS-based fluorochemicals in its chemical division from 1961 to 2002. Because the Decatur plant primarily manufactured PFOS-based chemicals, it has been studied only with respect to PFOS exposure; however, PFOA is a residual by-product of PFOS production. Therefore, chemical workers were potentially exposed to PFOA as well as other chemicals (Sigurdson et al. 2003).

A retrospective cohort mortality study of individuals who worked at least 1 year at the 3M facility in Decatur, Alabama was reported (Alexander et al. 2003). The site contained two plants, one producing specialty chemicals and the other making a specialty film. Perfluorooctanesulfonyl fluoride (POSF) is the major fluorochemical produced at this plant. POSF-based products can be metabolized to PFOS in humans. A cohort of 2,083 employees with 1-year or more of employment was classified as either non-exposed, low exposed, or high exposed based on biological monitoring data for PFOS and work site. A previous study reported that the mean concentration of PFOS in chemical plant workers was approximately 900 ppb while the mean PFOS concentration in film plant workers was approximately 100 ppb. The authors assigned all workers in the film plant to the non-exposed group while the low and high exposure groups included workers at the chemical plant categorized by their potential for exposure to POSF based on job role.

A total of 39 cancer deaths occurred in the cohort through 1997. For all three groups, observed cancer mortality was lower than that expected based on general population rates. SMRs for all cancer deaths were 0.84 (95 % CI: 0.50, 1.32, 18 deaths), 0.52 (95 % CI: 0.19, 1.44, 6 deaths), and 0.73 (95 % CI: 0.41, 1.21, 15 deaths) for the high, low, and non-exposed groups, respectively. For bladder cancer, three deaths occurred in the cohort (SMR = 4.81, 95 % CI: 0.99, 14.06) with all three cases having at least 1 year in a high exposed job. The authors conclude that bladder cancer mortality in this study could not be attributed to fluorochemical exposures due to the small number of cases and the possibility for unknown exposures to other substances that are potential bladder carcinogens either at work or due to lifestyle factors such as smoking (Alexander et al. 2003).

A follow-up study was conducted to determine whether bladder cancer mortality among workers with high potential workplace exposure to POFS-based fluorochemicals was representative of the overall bladder cancer experience of the cohort

(Alexander and Olsen 2007). Exposures to PFOS were estimated from work history and weighted using biological monitoring data. Categories of exposure included: no direct workplace exposure (serum PFOS concentrations between 100 and 290 ppb), job assignments with low potential for exposure (serum concentrations between 390 and 890 ppb), and job assignments with high potential for exposure (serum concentrations between 1,300 and 1,970 ppb). Mortality ascertainment was extended through 2002 with two additional deaths due to bladder cancer observed. In addition 1,400 of 1,845 cohort members responded to a questionnaire administered in 2002 with six bladder cancer cases reported. Of these, two were validated by medical records and four were not confirmed due to lack of consent for medical record review. Combining the 11 bladder cancers for an incidence rate analysis, the authors estimated a standardized incidence ratio (SIR) for all workers of 1.41 (95 % CI: 0.79, 2.33). No SIR based on stratification by exposure potential and duration of employment in a high exposure group was significantly increased. The authors conclude that the incidence of bladder cancer in workers is similar to that of the US population.

Health claims data for 652 chemical division employees (PFOS exposed) were analyzed against claims for 659 film division workers (non-PFOS exposed) at the Decatur plant (Olsen et al. 2004). Health claims were grouped into episodes of care defined as sets of one or more claims records which could be categorized into a discrete disease diagnosis. Two analyses were conducted: one comparing the all chemical workers to all film workers, and a second analysis of 211 workers with high exposure jobs in the chemical division compared to 345 workers who had similar jobs in the film division without POSF exposure for at least 10 years. Episodes of care were compared to similar health claims for approximately 20,000 manufacturing workers of the 3M Company in the U.S. No difference in the number of episodes of care per year was seen for those in the chemical division (average 2.7 per year) compared to those in the film division (average 3.0 per year). Relative risk (RR) was estimated for the ratio of episodes of care for specific diagnoses. For prostate cancer, five episodes were seen in the chemical division compared to 3.1 expected based on company-wide rates. The film division had one prostate cancer episode compared to 4.7 expected. Overall, the results of this study appear to show that the risk of cancer in the chemical division workers exposed primarily to PFOS was no different than that of the film plant workers. For bladder cancer, no episodes of care were recorded for chemical division workers during the period of the study.

A separate study of self-reported health conditions including cancer diagnoses among 1,400 workers at the Decatur facility was conducted for responses from the 2002 questionnaire (Grice et al. 2007). PFOS-exposure groupings were based assignments made previously (Alexander and Olsen 2007). Cancer diagnoses were validated by medical record review and included 12 cases of colon cancer, 8 cases of melanoma, and 22 cases of prostate cancer. No significant association between these cancers and any of the PFOS-exposure categories was observed.

12.2.4 Studies in a Community with PFOA-Contaminated Drinking Water

The C8 Health Project, a cross-sectional survey and biomarker study in 2005 and 2006 among 69,030 residents of the mid-Ohio Valley, was conducted in response to a legal settlement from a class-action lawsuit against DuPont (Frisbee et al. 2009). The aim was to investigate the potential human health effects of PFOA exposure from contaminated drinking water. Among the series of studies conducted to address this aim, a cancer-registry based case-control study was reported assessing the relationship between PFOA exposure via drinking water and cancer in residents living in the 6 water districts with contaminated drinking water and 13 adjacent counties surrounding the DuPont Washington Works plant (Vieira et al. 2013). Data on incident cases of 18 types of cancer diagnosed from 1996 through 2005 in five Ohio and eight West Virginia counties reported to the state cancer registries for Ohio and West Virginia were used. The study included 7,869 cancer cases in Ohio and 17,238 cancer cases in West Virginia. Serum PFOA levels were estimated using combined environmental, exposure, and pharmacokinetic models and were based on residential water district at the time of diagnosis (Shin et al. 2011). For comparative analyses, the authors fit logistic regression models to estimate the adjusted odds ratio (OR) for specific types of cancer cases using incident cancers from all other cancer categories as controls after excluding cases of kidney, pancreatic, testicular, and liver cancers. These cancer types were excluded from control groups due to the previous reports of associations with PFOA.

A positive association was found between kidney cancer and either the high or very high exposure categories with ORs of 2.0 (95 % CI: 1.3, 3.2) and 2.0 (95 % CI: 1.0, 3.9) for the high and very high categories, respectively. Among the nine cases in the very high exposure group stratified by sex, the association was observed for women (OR = 3.5, 95 % CI: 1.4, 8.3, six cases), but not for men (OR = 1.0, 95 % CI: 0.3, 3.4, three cases). For testicular cancer, there was a small number of cases overall (n = 18) with ORs above 1.0 reported for the very high exposure category (OR = 2.8, 95 % CI: 0.8, 9.2, six cases) and the Little Hocking water district which had the highest estimated exposure to PFOA (OR = 5.1, 95 % CI: 1.6, 15.6, eight cases). However, no exposure –response pattern was observed as the ORs for the low to high exposure categories and the other water districts were all non-significant and less than 1.0. Associations in the very high exposure group were also noted for prostate, and ovarian cancers, and for non-Hodgkin's lymphoma. The authors note that the primary limitation to their study was the use of other cancer cases as control subjects for comparative analyses. In addition, although the study included an area with a population estimate of over 500,000 persons, precision of OR estimates was limited due to small numbers of cases for specific cancer types following categorization to exposure groups or assignment to specific water districts with varying levels of PFOA exposure.

A second C8 Science Panel study involved a retrospective cohort design that included 32,254 participants living in the mid-Ohio River Valley in one of the six

water districts near the DuPont Washington Works plant (Barry et al. 2013). Of this cohort, 3,713 had ever worked at the DuPont Washington Works facility. Among these community residents and plant workers, 2,507 validated cancer cases comprising 21 different diagnostic types were observed. Cancer risk was analyzed based on cumulative PFOA exposure estimated from residential history as described by Shin et al. (2011) combined with additional occupational exposure estimates for workers (Woskie et al. 2012). The authors fit a proportional hazards regression model for each cancer type as the outcome and age at either diagnosis or last follow-up as the time scale. HRs were estimated for time-varying cumulative exposure to PFOA calculated as the sum of yearly drinking water concentrations. PH models were adjusted for sex, 5-year birth period, educational attainment, and time-dependent measures of smoking and alcohol consumption.

In the combined cohort, positive associations were noted for testicular cancer (HR = 1.34, 95 % CI: 1.00, 1.79), kidney cancer (HR = 1.10, 95 % CI: 0.98, 1.24), and thyroid cancer (HR = 1.10, 95 % CI: 0.95, 1.26). When analyzed by cumulative exposure quartile, the HRs for 17 testicular cancer cases distributed by increasing exposure quartiles were 1.0 (referent), 1.04 (95 % CI: 0.26, 4.22), 1.91 (95 % CI: 0.47, 7.75), and 3.17 (95 % CI: 0.75, 13.45), with significant trend tests reported ($p < 0.05$ for both trend tests). For the 105 kidney cancer cases, the HRs were 1.0 (referent), 1.23 (95 % CI: 0.70, 2.17), 1.48 (95 % CI: 0.84, 2.60), and 1.58 (95 % CI: 0.88, 2.84). For 86 thyroid cancer cases, the HRs were 1.0 (referent), 1.54 (95 % CI: 0.77, 3.12), 1.48 (95 % CI: 0.74, 2.93), and 1.73 (95 % CI: 0.85, 3.54). However, trend tests across quartiles based on increasing serum PFOA concentrations were not significant for either kidney or thyroid cancers ($p > 0.10$ for all tests).

Further, HRs for the 21 cancer types were stratified for community and occupational exposure groups (Barry et al. 2013 supplement). The numbers of cases and HRs among 28,541 community members with no occupational exposure are shown in Table 12.9 with the number of cases and HRs for the occupationally exposed group listed in Table 12.10. Among community residents only, the HR for testicular cancer was significantly increased (HR = 1.73, 95 % CI: 1.24, 2.40, 15 cases) while the HR for lung cancer was significantly decreased (HR = 0.85, 95 % CI: 0.73, 1.00, 95 cases) for increasing cumulative PFOA exposure. Among those with occupational exposure to PFOA, thyroid cancer was significantly increased (HR = 1.93, 95 % CI: 1.00, 3.71, 8 cases) and bladder cancer was significantly decreased (HR = 0.65, 95 % CI: 0.44, 0.95, 29 cases) for increasing cumulative exposure.

In a separate study of persons residing in six water districts in the mid-Ohio River Valley with PFOA contamination, a health survey of 47,359 adults with one-time serum PFOA and PFOS measures taken from blood samples collected in 2005 and 2006 was conducted (Innes et al. 2014). There were 292 colorectal cancer cases reported for this group, and the authors were able to confirm 208 cases by medical record validation. The median serum PFOA concentration among all adults was 27.9 ppb considered to be elevated compared to general population levels due primarily to exposure to contaminated drinking water. Meanwhile, the median serum concentration of PFOS was 20.2 ppb which was considered similar to the general U.S. population level at the time of serum sampling. The distribution of cases across

Table 12.9 Effect of estimated cumulative PFOA serum concentration on cancer risk in the community group (n = 28,541)

Cancer	# Cases	HR (95 % CI)
Bladder	76	0.96 (0.81, 1.14)
Brain	13	1.14 (0.78, 1.65)
Breast	546	0.96 (0.90, 1.02)
Cervical	21	0.94 (0.67, 1.32)
Colorectal	223	0.98 (0.89, 1.08)
Esophagus	12	1.00 (0.66, 1.51)
Kidney	87	1.14 (0.99, 1.32)
Leukemia	53	0.92 (0.76, 1.13)
Liver	8	0.62 (0.29, 1.29)
Lung	95	0.85 (0.73, 1.00)*
Lymphoma	121	1.05 (0.92, 1.19)
Melanoma	200	0.99 (0.89, 1.10)
Oral	17	0.96 (0.65, 1.40)
Ovarian	43	1.00 (0.79,1.25)
Pancreatic	21	1.06 (0.79, 1.43)
Prostate	317	0.97 (0.90, 1.05)
Soft tissue	13	0.68 (0.40, 1.14)
Stomach	11	0.70 (0.40, 1.23)
Testicular	15	1.73 (1.24, 2.40)*
Thyroid	78	1.04 (0.89, 1.23)
Uterine	96	1.02 (0.88, 1.18)

From Barry et al. (2013) supplement
*Statistically significant ($p \leq 0.05$)

quartiles of serum PFOA and PFOS concentration was evaluated. An inverse relationship was observed such that there were fewer cases of colorectal cancer reported among those persons categorized to higher quartiles of serum PFOA and PFOS concentrations. The fully adjusted ORs for PFOA serum concentration by quartile were 1.0 (referent), 0.48 (95 % CI: 0.31, 0.75), 0.51 (95 % CI: 0.34, 0.77), and 0.64 (95 % CI: 0.44, 0.94) with a significant trend test ($p = 0.002$), although the trend was not significant when PFOA serum concentration was evaluated as a linear, continuous variable ($p = 0.46$). Moreover, a similar inverse relationship was observed for higher serum PFOS concentrations. The fully adjusted ORs by quartile were 1.0 (referent), 0.38 (95 % CI: 0.25, 0.59), 0.27 (95 % CI: 0.17, 0.42), and 0.24 (95 % CI: 0.16, 0.37) with significant trend test values for both categorical and linear tests ($p < 0.00001$). Among several limitations to this study, the authors note that the study analyzed cross-sectional data that comprised measured PFOA concentrations collected for prevalent colorectal cancer cases. This limits the ability to assess causality due to the absence of a temporal relationship between PFOA exposure and colorectal cancer as both are determined simultaneously.

Table 12.10 Effect of estimated cumulative PFOA serum concentration on cancer risk in the occupational group (n = 3,713)

Cancer	# Cases	HR (95 % CI)
Bladder	29	0.65 (0.44, 0.95)*
Brain	4	0.82 (0.26, 2.59)
Breast	13	1.01 (0.59, 1.74)
Cervical	1	–
Colorectal	41	1.12 (0.81, 1.54)
Esophagus	3	1.42 (0.21, 9.74)
Kidney	18	0.95 (0.59, 1.52)
Leukemia	13	1.30 (0.73, 2.33)
Liver	1	–
Lung	13	0.87 (0.51, 1.47)
Lymphoma	15	1.24 (0.72, 2.14)
Melanoma	41	0.80 (0.59, 1.08)
Oral	1	–
Ovarian	0	–
Pancreatic	3	0.98 (0.21, 4.65)
Prostate	129	0.94 (0.77, 1.17)
Soft tissue	2	1.20 (0.30, 4.76)
Stomach	1	–
Testicular	2	0.85 (0.04, 19.7)
Thyroid	8	1.93 (1.00, 3.71)*
Uterine	7	1.05 (0.56, 1.97)

From Barry et al. (2013) supplement
*Statistically significant ($p \leq 0.05$)

12.2.5 General Population Studies

A number of studies have reported on cancer outcomes in general populations and related the finding to either PFOA or PFOS. In the blood of a representative sampling of individuals in the general population of the United States, four polyfluoroalkyl substances (PFOA, PFOS, perfluorohexane sulfonate-PFHxS, and perfluorononanoate-PFNA) have been found in more than 95 % of those sampled in the NHANES survey (Kato et al. 2011). The geometric mean serum concentrations of each of these four chemicals are presented in Table 12.11. Attributing an association between a cancer outcome and any one of these chemicals (or any of the other chemicals contained in these blood samples) must be done carefully as it is obvious from this data that multi-chemical exposures are occurring.

The association between plasma (serum) concentrations of PFOA and PFOS with cancer risk was determined for a prospective Danish cohort of 57,053 participants with no previous cancer diagnosis at enrollment (Eriksen et al. 2009). From 1997 through 2006, 1,240 incident cancer cases were ascertained through the Danish Cancer Registry. The study included 713 prostate cancer cases, 332 bladder cancer cases, 128 pancreatic cancer cases, and 67 liver cancer cases. The PFOA and

Table 12.11 Serum perfluorochemical concentrations in the general population (participants ≥12 years old)

	Geometric mean in ppb				
Sampling wave	1999–2000	2003–2004	2005–2006	2007–2008	2009–2010
Chemical					
PFOA	5.21	3.59	3.56	3.99	2.84
PFOS	30.40	19.43	15.61	13.19	8.76
PFNA	0.56	0.88	1.01	1.46	1.49
PFHxS	2.30	1.90	1.55	1.93	1.51

Individual serum measurements not available in 2001–2002
Estimated from NHANES data based on Kato et al. (2011)

PFOS concentrations for these cases were compared to concentrations for a representatively selected referent sub-cohort of 772 persons (668 men and 92 women) without a cancer diagnosis during the ascertainment period. The median PFOA concentrations ranged from 5.4 to 6.9 ppb for the cancer groups with a median concentration of 6.6 ppb for the comparison sub-cohort. For PFOS, median concentrations for the cancer groups ranged from 31.0 to 36.8 ppb and the comparison sub-cohort had a median concentration of 34.3 ppb.

For prostate cancer, the adjusted incident rate ratios (RR) for quartiles 1 through 4 for PFOA were 1.00 (referent), 1.09 (95 % CI: 0.78, 1.53), 0.94 (95 % CI: 0.67, 1.32), and 1.18 (95 % CI: 0.84, 1.65), respectively. The same analyses for PFOS estimated incident RRs of 1.00 (referent), 1.35 (95 % CI: 0.97, 1.87), 1.31 (95 % CI: 0.94, 1.82), and 1.38 (95 % CI: 0.99, 1.93), respectively. The authors note that the lack of an increasing exposure-response trend suggests that the similar risk estimates at higher PFOS concentration levels are likely due to a chance finding of a lower incidence in the referent quartile rather than an increased risk with increasing PFOS concentrations. The authors conclude that plasma concentrations of PFOA and PFOS in the general Danish population do not appear to be associated with increased risk of prostate, bladder, pancreatic, or liver cancer (Eriksen et al. 2009).

In a study of persistent organic pollutants (POPs) and breast cancer in an Inuit population, 31 breast cancer cases were selected from a hospital registry in Greenland and 115 control subjects without a cancer diagnosis were sampled from an ongoing POPs monitoring study (Bonefeld-Jorgensen et al. 2011). Serum levels of PFOA as well other perfluorinated carboxylates and sulfonates were reported at higher concentrations for those with breast cancer relative to control subjects. The median concentrations of PFOA were 2.5 ppb for breast cancer cases and 1.6 ppb for control subjects. For PFOS, the median concentrations were 45.6 ppb among breast cancer cases and 21.9 ppb for control subjects.

No significant association with breast cancer case status was observed for increasing PFOA exposure while a significant association was reported for increasing PFOS exposure. The raw (crude) OR for a 1 ppb increase in PFOA was 1.07 (95 % CI: 0.88, 1.31, 31 cases) while the raw OR for a 1 ppb increase in PFOS was

1.01 (95 % CI: 1.00, 1.02). Fewer cases and controls were included in the adjusted OR model due to missing data for the variables including age, body mass index, pregnancy and breastfeeding history, serum cotinine and menopausal status. For 1 ppb increase in PFOA, the adjusted OR was 1.20 (95 % CI: 0.77, 1.88, 7 cases). For a 1 ppb increase in PFOS, the adjusted OR was 1.03 (95 % CI: 1.00, 1.07). The authors suggest that serum persistent organic pollutants including perfluorinated compounds might be a risk factor for the development of breast cancer in this population; however, the small number of cases and the high correlation between serum PFAA levels limited the study.

In a case-control study of breast cancer among mothers enrolled in the Danish National Birth Cohort from 1996 through 2002, 250 breast cancer cases that occurred through 2010 were matched by age and parity to 233 control subjects without a cancer diagnosis (Bonefeld-Jorgensen et al. 2014). Serum levels of 16 perfluoroalkylated substances (PFAS) including 10 carboxylates and 5 sulfonates were measured for blood samples taken between the 6th and 14th week of pregnancy during enrollment. PFOA and PFOS concentrations were measured in all study subjects and found at relatively higher concentrations than all other PFASs. The mean serum levels reported for control subjects were 5.2 ppb for PFOA and 30.6 ppb for PFOS while serum concentrations for breast cancer cases are not reported. In addition, the authors noted high correlations among the PFASs with a significant correlation coefficient of 0.69 found between PFOA and PFOS concentrations. No significant associations were observed between breast cancer case status and PFOA and PFOS concentrations. Slightly fewer cases and controls were included in the adjusted OR models due to missing data for other variables including age at blood sampling, body mass index before pregnancy, gravidity, oral contraceptive use, age at menarche, alcohol intake and smoking, maternal education, and physical activity. The adjusted OR for a 1 ppb increase in PFOA was 1.00 (95 % CI: 0.90, 1.11, 221 cases) while the adjusted OR for a 1 ppb increase in PFOS was 0.99 (95 % CI: 0.98, 1.01, 221 cases). The authors also categorized the exposure distributions into quintiles and observed no pattern of increasing ORs for higher levels of PFOA and PFOS when compared to the lowest quintile assigned as the referent group. The adjusted OR for PFOA among women in the fifth quintile (PFOA concentration greater than 6.53 ppb) was 0.94 (95 % CI: 0.51, 1.76, 40 cases) while the adjusted OR for PFOS in the highest quintile (PFOS concentration greater than 39.07 ppb) was 0.90 (95 % CI: 0.47, 1.70, 35 cases) with no significant ORs observed for other exposure quintiles. Moreover, the study subjects were stratified by age at breast cancer diagnosis with analyses conducted for cases and matched controls younger than 41 years of age at case diagnosis or older than 40 years of age at case diagnosis. Similar results consistent with the overall analyses were observed in both age strata for PFOA or PFOS. The authors conclude that the results of this study indicate that there is no association between breast cancer occurrence and PFAS concentrations taken during pregnancy.

A case control study in Sweden including 201 cases of prostate cancer compared to 186 population-based controls was reported (Hardell et al. 2014). Serum concentrations of six perfluorinated carboxylates and sulfonates were measured with no

significant differences reported between cases and controls for PFOA and PFOS. The median concentrations of PFOA were 2.0 ppb for prostate cancer cases and 1.9 ppb for control subjects. For PFOS, the median concentrations for prostate cancer cases were 9.0 ppb and 8.3 ppb for control subjects. There was no significant association between prostate cancer and increased exposure defined as having a concentration above the median for any PFAA reported in the study. The OR for having a PFOA concentration above the median was 1.1 (95 % CI: 0.7, 1.7), while for PFOS, the OR for exposure above the median was 1.0 (95 % CI: 0.6, 1.5).

The authors note that they expected heredity to be a risk factor for prostate cancer with cases more likely to report having a first degree relative with prostate cancer (OR = 1.8, 95 % CI: 1.0, 3.1). After stratifying cases and controls by heredity defined as having a first degree relative with prostate cancer and PFOA concentration above the median, the ORs were 1.1 (95 % CI: 0.5, 2.6) for those with heredity and PFOA less than the median, 1.0 (95 % CI: 0.6, 1.5) for those with no heredity and PFOA greater than the median, and 2.6 (95 % CI: 1.2, 6.0) for those with heredity and PFOA greater than the median, compared to a referent group of those without hereditary prostate cancer and PFOA concentration less than the median. A statistical test for interaction between heredity and PFOA concentration greater than the median was not significant ($p = 0.15$). For PFOS, the ORs were 1.2 (95 % CI: 0.6, 2.5) for those with heredity and PFOS less than the median, 0.9 (95 % CI: 0.5, 1.4) for those with no heredity and PFOS greater than the median, and 2.7 (95 % CI: 1.0, 6.8) for those with heredity and PFOS greater than the median, compared to a referent group of those without hereditary prostate cancer and PFOS concentration less than the median. Hardell and colleagues conclude that higher concentrations of PFOA and PFOS without hereditary prostate cancer did not increase the risk of prostate cancer. They suggest that there is an interaction between genes and PFAA exposure based on the observed increased risk for those with hereditary prostate cancer; however, a possible mechanism for this interaction is unknown.

A cross-sectional study compared serum PFOA and PFOS concentrations in 40 cancer patients without a specific diagnostic type to two groups without cancer: 56 employees of a research center in urban Athens, Greece and 86 patients undergoing medical checkups in rural Argolida, Greece (Vassiliadou et al. 2010). The mean serum PFOA levels were 2.3 ppb in the cancer patients, 2.9 ppb in the Athens employees, and 1.9 ppb in the Argolida patients. For PFOS, the mean serum levels were 12.97 ppb in the cancer patients, 14.9 ppb in the urban employees, and 13.6 ppb in the rural patients. Although the results demonstrate that PFASs are detectable in the serum and liver samples from a series of patients with hepatocellular carcinoma (HCC) and hepatitis C viral infection (HCV) as well as liver donors without existing disease, the comparative results indicate no association between PFOA or PFOS and cancer status. The study was limited for a number of reasons including the small sample size, lack of specific cancer types, and no information on potential confounders, selection criteria or participation rates for the study population.

A study compared 66 diseased liver tissues removed prior to liver transplants to 25 healthy liver specimens in Melbourne, Australia (Yeung et al. 2014). Serum and liver concentrations of PFOA and 11 other PFASs were measured. Cases included

those who had undergone liver transplantation for a range of conditions including hepatocellular carcinoma (HCC), cirrhosis due to chronic hepatitis C viral infection (HCV), amyloidosis, and acute liver failure. Among those with HCC, serum concentrations from 24 samples of PFOA were somewhat higher than those for 25 liver donor control samples with mean serum concentration of 2.82 ppb in HCC patients compared to 2.38 ppb in controls; however, mean liver concentrations were 0.59 ppb (ng/g) and 0.62 ppb for these groups, respectively. For PFOS, the mean serum concentrations were 13.3 ppb for those with HCC and 8.48 ppb for controls. Mean liver concentrations of PFOS were 6.24 ppb for HCC samples and 5.22 ppb for controls. The authors suggest that some of the pathologic changes in diseased livers might alter the distribution of PFASs between liver and serum. Overall, the results do not suggest a relationship between PFASs and liver cancer. The study had numerous limitations including a small sample size and the measurement of PFOA and PFOS concentrations after liver specimen removal that preclude its ability to test for an association between HCC and PFOA and PFOS concentrations.

12.3 Reviews and Evaluations

There have been a series of reviews of the carcinogenicity of PFOA. An EPA Draft Risk Assessment (2005) reviewed both the animal and human evidence for a possible relationship between PFOA exposure and cancer risk. Overall, based on no adequate human studies and uncertain human relevance of the tumor triad from rat studies, PFOA was described as having "suggestive evidence of carcinogenicity but not sufficient to assess human carcinogenic potential" under the draft 1999 Guidelines for Carcinogenic Risk Assessment (U.S.EPA 1999). PFOA induces liver tumors, pancreatic acinar cell tumors, and Leydig cell tumors in male rats. There is sufficient evidence to indicate that PFOA is a PPARα agonist and that liver carcinogenicity and toxicity is mediated by binding to the PPARα receptor in the liver. A mode of action analysis demonstrated that the hepatic effects are due to PPARα agonism and that this mode of action is unlikely to occur in humans. There is not sufficient evidence to link the mode of action for both the pancreatic acinar cell tumors and the Leydig cell tumors to PPARα. However, due to the quantitative differences in the expressions of luteinizing hormone and cholecystokinin receptors and other toxicodynamic differences between the rat and the human, tumors induced in the rat by PFOA probably do not represent a significant cancer hazard for man.

A report from the Subcommittee on Classification of Carcinogenic Substances of the Dutch Expert Committee on Occupational Safety of the Health Council (Health Council of the Netherlands 2013) concludes that the available data on PFOA (and its salts) are insufficient to evaluate the carcinogenic properties (Category 3 according to the system of the Health Council of the Netherlands 2010). In reviewing the human information, it was concluded that the available epidemiologic studies were of varying quality with several having significant weaknesses. Several studies report elevated risks for certain types of cancer but overall there was no cancer type that

appeared to be consistently elevated in all studies. The report pointed out that kidney cancer could be a concern as a slight elevation was reported in 2 of the 3 worker cohort studies. With regard to the animal information, the report notes that none of the three tumor types seen in the rodent studies were malignant tumors and that benign tumor development in rodents may be explained in large part by peroxisome proliferation. Thus, it was reported that these tumors appear to be rodent specific and are unlikely to have relevance for liver, pancreatic, and testicular cancers in humans.

In a critical review, Chang et al. (2014) conclude that, taken together, the epidemiologic evidence does not support the hypothesis of a causal association between PFOA or PFOS exposure and cancer risk in humans. The review included the human epidemiologic and animal toxicologic studies covered in this chapter. It is noted that the majority of the relative risk estimates in these papers for both PFOA and PFOS range between 0.5 and 2.0 with the confidence intervals including 1.0. Results suggesting a positive association are counterbalanced by negative associations, no apparent monotonic dose-response, and the lack of concurrence between the animal and human findings. The authors conclude that many of the positive associations reported for PFOA exposure in the community and general population studies were not supported by studies of occupational exposures. Since occupational exposures are often one to two orders of magnitude higher than environmental exposures, this indicates that the positive associations in the community and general populations studies are most likely due to chance, confounding, or bias.

On the basis of limited evidence in humans that PFOA causes testicular and renal cancer, and limited evidence for cancer causality in experimental animals, an IARC working group classified PFOA as possibly carcinogenic to humans (IARC group 2B). The IARC working group noted reports of increased risk of kidney cancer with a statistically significant exposure-response trend in workers in a fluoropolymer production plant in West Virginia, USA and in an exposed community near the plant (Steenland and Woskie 2012; Vieira et al. 2013). In addition, there was an increase of about threefold in the risk of testicular cancer reported in the most highly exposed residents in communities near the same plant (Vieira et al. 2013; Barry et al. 2013). However, the working group considered the evidence regarding mechanisms of PFOA-associated carcinogenesis to be limited due to the inability to exclude chance as an explanation for these findings (Benbrahim-Tallaa et al. 2014).

12.4 Conclusions

Overall, there have been a number of studies investigating cancer and exposure to PFAAs, particularly PFOA. Historically, PFOA and PFOS have been the most widely used members of this chemical class making these substances the subject of the largest proportion of reported studies. Most persons in developed countries have detectable serum concentrations of PFOA ranging from 1 to 10 ppb. PFOS has similar environmental exposure conditions and has reported serum concentrations in general populations that are somewhat higher than those for PFOA. Due to

contaminated drinking water supplies near a DuPont fluoropolymer production facility in West Virginia, residents of six neighboring water districts in West Virginia and Ohio have mean serum concentrations that range from 10 to 300 ppb. Additionally, occupationally exposed cohorts typically have serum concentrations of PFOA and PFOS with an upper range of 3,500 ppb reported in some studies.

The toxicologic evidence for carcinogenicity of PFAAs is limited to four studies evaluating the carcinogenic potential of PFOA (two studies), PFHxA (one study), and PFOS (one study) in rats. Each of these chemicals produced a different response. PFOA causes the tumor triad common to peroxisome-proliferating chemicals including adenomas of the liver, pancreas, and testes. Rats exposed to PFOS developed liver tumors, but a study of PFHxA reported no increase in tumors of any type. Considerable research has been done to elucidate a potential carcinogenic mechanism. There is evidence that the liver is the main target of PFOA exposure due to activation of PPARα. This mechanism contributes to the induction of liver tumors in rats. There is limited evidence that Leydig cell tumors may be induced by a hormonal mechanism mediated by PPARα activation. Thus, one needs to be careful when predicting the presence or absence of carcinogenic activity for other perfluorinated chemicals using the results from the available studies.

Epidemiologic studies have been reported for several levels of population exposure. Limited evidence for associations with kidney and testicular cancer has been reported by studies among community members exposed to drinking water contaminated by PFOA. These associations are not consistently reported such that random chance cannot be excluded as an explanation. Studies of workers exposed to relatively higher levels of PFOA and PFOS have not shown consistent evidence for an association with any specific cancer type. More recent incidence studies among workers from 3M (Raleigh et al. 2014) and DuPont (Barry et al. 2013 supplement) did not report similar or strong associations with specific cancer types including kidney or testicular cancers. Studies of specific tumor types among populations exposed to low levels of PFOA and PFOS have shown equivocal results for a variety of specific cancer outcomes with no consistent associations reported. Based on the evidence reported to date on PFOA and PFOS and considering the relatively low and decreasing exposures to these compounds, the prospect for developing carcinogenic outcomes is remote. For other perfluorinated chemicals, there is not sufficient evidence regarding their potential carcinogenicity, and human exposures are low and appear to be decreasing.

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