

A REVIEW OF PERFLUOROOCCTANOIC ACID
CARCINOGENICITY AND APPLICATION
TO HUMAN RISK

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Submitted to the faculty of the University Graduate School
in partial fulfillment of the requirements
for the degree
Master of Science
in the Department of Pharmacology and Toxicology
Indiana University

May 2010

Accepted by the Faculty of Indiana University, in partial
fulfillment of the requirements for the degree of Master of Science.

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ACKNOWLEDGEMENTS

I would like to thank the people who have influenced and inspired me. Without their help I could not have succeed in my academic career and this thesis would not have been possible.

I specifically want to thank my advisor, Prof. James E. Klaunig, for his guidance throughout my graduate career. His Introduction to Toxicology class inspired me to seek my Master's degree where his life stories about his experiences as a Toxicologist captivated my interests and propelled me to continue my education. I could not have accomplished my goal without his support and recommendation to the graduate program.

I would also like to thank Dr. Lisa M. Kamendulis for her support and for serving as my co-advisor. She has always been accessible and has regularly taken time out of her schedule to offer her assistance and her ear to listen to my concerns. Her lectures will be remembered as engaging and motivational and her hard work and dedication have served as an example worthy of emulation.

Dr. Barbara Hocevar also deserves special thanks as she has served on my thesis committee and has given valuable guidance and direction to me without which this thesis could not have been completed.

My deepest gratitude goes to my wife, Jannie Stone, for her unwavering love and support throughout this entire process; I could not have achieved success without her. She is my soul mate, partner and friend and has never left my side. I am also indebted to my father, Kenneth Stone, for his example of Christian faith and work ethic. He worked tirelessly to support the family and spared no effort to provide the best possible home.

Words cannot describe my gratitude for my mother, Janet Stone, who is simply the best mother a child could be given by God. I remember many sacrifices my mother has made for me, often going without simply to provide for me. I remember her constant support and encouragement and the strength of her Christian faith.

Furthermore, my children Sam Stone, Lindsay Stone and Misty Johnson have completed my life. I am grateful for the love and joy they have given me and their encouragement which has motivated me to continue. I also want to express my gratitude to God for giving me my grandchildren, Ronni, Cole and Camryn Johnson, whose smile alone brightens my day and makes me forget my struggles. I also want to thank of my sister, Karen, for being the best sister a brother could ask for. She has helped our family out more times than I can remember.

Last but not least, my greatest thanks belong to God. He has walked with me throughout my life and has stood by me during my most challenging times. God, you have truly blessed me and made my life joyful and complete. I owe everything to you.

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LIST OF ABBREVIATIONS

APFO	Ammonium Perfluorooctanoate
CAR	Constitutive Androstane Receptor
CAT	Catalase
CCK	Cholestcystokinin
CHO	Chinese Hamster Ovary
DMSO	Dimethyl Sulfoxide
EPA	Environmental Protection Agency
ER	Estrogen Receptor
ERK	Extracellular Receptor Kinase
FSH	Follicle-Stimulating Hormone
GR	Glutathione Reductase
GJIC	Gap Junctional Intracellular Communication
HPT	Hypothalamic Pituitary Testicular
LCT	Leydig Cell Tumors
LH	Luteinizing Hormone
MOA	Mode of Action
ND	None Detected
8-OHdG	8-Hydroxydeoxyguanosine
PACT	Pancreatic Acinar Cell Tumors
PB	Phenobarbital
PC-PLC	Phosphatidylcholine-specific Phospholipase C

PFCs	Perfluorochemicals
PFDA	Perfluorodecanoic Acid
PFOA	Perfluorooctanoic Acid
PFOS	Perfluorooctane Sulfonate
PHA	Provisional Health Advisories
PP	Peroxisome Proliferator
PPAR α	Peroxisome Proliferator-Activated Receptor Alpha
ROS	Reactive Oxygen Species
SOD	Superoxide Dismutase
WY	Wyeth 14,643

I. INTRODUCTION

Perfluorooctanoic acid (PFOA) is a synthetic organic chemical that consists of an 8 carbon alkyl chain with a terminal carboxyl group in which the carbon-hydrogen bonds have been replaced with carbon-fluorine bonds except at the terminal carboxyl end. This perfluoralkyl carboxylate is a contemporary synthetic chemical that does not occur naturally in the environment [1] and has only seen widespread use within the last 50 years. Ammonium Perfluorooctanoate (APFO) is the ammonium salt derivative of PFOA and is used in the manufacturing process and readily disassociates into PFOA in the human body and environment. The data on APFO/PFOA can be used interchangeably since, in the presence of water, APFO readily dissociates into the PFOA anion [2].

PFOA is used in the manufacture of fluoropolymers and fluoroelastomers and is present as a component of some of the top-antireflective coating materials in use today. Fluoropolymers have properties that are useful in the manufacturing and textile industry such as fire resistance and their ability to repel oil, stain, grease and water which makes them ideal for creating a non-stick surface for cookware and protective coatings on clothing and carpeting and are also valuable to the aerospace industry as well. Fluoroelastomers are a family of synthetic rubbers that can be repeatedly stretched and still return to their original shape, such as Viton. Sinclair et al. found measurable PFOA released from several brands of nonstick cookware when heated which suggests that residual PFOA from the manufacturing process may remain on the surface and can be off-gassed when heated at normal cooking temperatures. This study also found that

PFOA was present in the vapors released from prepackaged microwave popcorn bags that are impregnated with PFOA to improve oil and fire resistance during microwaving. [3] Since there has been recent progress in understanding the developmental, tumorigenic and other adverse effects seen in laboratory animals exposed to PFOA, the purpose of this review is to provide an overview of the tumorigenic modes of action of PFOA that result in the tumor triad seen in rodents and to determine the relevance of these studies to human risk.

A. Environmental Persistence

The hydrogen-fluorine bonds found in PFOA are extremely stable and as a result PFOA is resistant to hydrolysis, photolysis, biodegradation, and metabolism leading to a high degree of environmental persistence and bioaccumulation. In January 2006, the Environmental Protection Agency (EPA) initiated the 2010/15 PFOA Stewardship Program in which the eight major companies in the industry committed voluntarily to reduce facility emissions and product content of PFOA and related chemicals on a global basis by 95 percent no later than 2010, and to work toward eliminating emissions and product content of these chemicals by 2015.

B. Environmental Presence

The environmental persistence of PFOA has culminated in the ubiquitous presence of PFOA in the environment with bioaccumulation in many species worldwide, including humans. We will discuss the results of recent studies that have shown the presence of PFOA in human, wildlife and the environment.

1) PFOA in Humans

Recent studies of human populations in the United States have demonstrated the presence of PFOA in similar concentrations in the serum of children, adults and the elderly in the general population as well as in higher concentrations in individuals that were occupationally exposed to PFOA as summarized in Table 1.1. A study by Olsen et al. of 645 serum samples collected in 2001 from adults in the cities of Los Angeles, Boston, Minneapolis-St Paul, Charlotte, Portland, and Hagerstown found PFOA levels in serum that ranged from 1.4 ng/ml to 56.1 ng/ml with a geometric mean for all samples of 4.6 ng/ml [4]. In this study the geometric mean PFOA levels were 4.9 ng/ml for males and 4.2 ng/ml for females. Serum collected in 2001 from 238 elderly adults (age 65-96 years) in Seattle also demonstrated PFOA concentrations in the range of 1.4 ng/ml to 16.7 ng/ml with a geometric mean of all samples of 4.2 ng/ml. Olsen et al. [5] also measured PFOA in serum collected from 598 children ages 2-12 at concentrations ranging from 1.9 ng/ml- 56.1 ng/ml in samples collected in 1994 and 1995. The geometric mean for all participants was 4.9 ng/ml with a geometric mean of 5.2 ng/ml for males and 4.7 ng/ml for females. In a 2000 study by Olsen et al. of PFOA levels in the serum of occupationally exposed workers, Olsen determined that PFOA concentrations were over 300 times higher in the serum of workers at a 3M Decatur Alabama plant than that of the general population [6]. Olsen reported a geometric mean of 1130 ng/ml from a sample of 263 Decatur employees. Serum samples collected from 2,094 members of the general US population in 2003-2004 for the National Health and Nutrition Examination Survey (NHANES) also indicated the presence of PFOA at a mean concentration of 3.9 ng/ml. This is a decrease from the mean PFOA concentration of 5.0 ng/ml found in the serum of

1562 participants in the 1999-2000 NHANES. It is also important to note that PFOA was quantifiable in 99.7% of the U.S. population tested in this study [7].

Table 1.1
Summary of Geometric Mean and Range of PFOA Serum Concentrations in United States Populations

Year Samples Taken	Number	Location	Demographic	PFOA Mean (ng/ml)	Range (ng/ml)	Reference
2001	125	Los Angeles, CA	Adult General Population	4.1	2.1-34.1	[4]
2001	109	Boston, Ma	Adult General Population	5.4	1.5-13.9	[4]
2001	100	Mpls-St.Paul, MN	Adult General Population	4.5	1.9-20.0	[4]
2001	96	Charlotte, NC	Adult General Population	6.3	2.1-29.0	[4]
2001	107	Portland, OR	Adult General Population	3.6	2.1-16.7	[4]
2001	108	Hagerstown, MD	Adult General Population	4.2	2.1-52.3	[4]
2001	238	Seattle, WA	Elderly Adult General Population	4.2	1.4-16.7	[8]
1994-1995	598	23 US States	Children General Population	4.9	1.9-56.1	[5]
2000	263	Decatur, AL	Adult with Occupational Exposure	1,130	40-12,700	[6]
1999-2000	1562	US Population	Adult General Population	5.0	*4.7-5.7	[7]
2003-2004	2094	US Population	Adult General Population	3.9	*3.6-4.3	[7]

*95th percentile confidence interval range.

The global presence of PFOAs in the human population has been evidenced by detection of PFOA in the blood of human adults in Columbia, Poland, Belgium, India, Korea, Japan, Sweden, China, Australia, Germany and Spain [9-14]. Results found in a recent study by Jin et al. on the serum levels taken in 1987, 1990, 1999 and 2002 from students, staff and faculty members at China Medical University in Shenyang China are indicative of the exposure to PFOA in the general population that is not occupationally exposed to PFOA [10]. Table 2.1 summarizes the significant increase of PFOA levels seen in the Shenyang China general population from 1987-2002.

Table 1.2
Summary of Serum PFOA Concentrations (ng/ml) in Shenyang China Population

Year Samples Taken	Number	PFOA Geometric Mean	Range
1987	15	0.07	0.01-0.43
1990	33	0.06	0.01-3.4
1999	68	0.83	0.04-6.5
2002	119	3.0	0.24-59.8

The marked increase of PFOA levels present in the blood of occupational exposed workers in the United States was reproduced in a study of exposed workers in Antwerp Belgium. A study performed in 2000 by Olsen et al. [4] found the geometric mean level of PFOA in 255 occupationally exposed Antwerp workers to be 440 ng/ml with a range of 40- 6,240 ng/ml as compared to the range of 4.5-27 ng/ml found in a sample of 20 members of the general Belgium population [11].

2) PFOA in Wildlife

The environmental persistence of PFOA and its global presence has triggered studies for detection of the compound in several species of wildlife across the globe. PFOA has been found in the sera of captive wildlife species Bengal tigers (*Panthera tigris tigris*) and African lions (*Panthera leo* Linnaeus) from Harbin Wildlife Park, Heilongjiang Province, in China [15]. PFOA was found in 175 samples of liver and blood of bluefin tuna (*Thunnus thynnus*), swordfish (*Xiphias gladius*), common cormorants (*Phalacrocorax carbo*), bottlenose dolphins (*Tursiops truncatus*), striped dolphins (*Stenella coeruleoalba*), common dolphins (*Delphinus delphi*), fin whales (*Balenoptera physalus*), and long-finned pilot whales (*Globicephala melas*) from the Italian coast of the Mediterranean Sea and in livers of ringed seals (*Phoca hispida*), gray seals (*Halichoerus grypus*), white-tailed sea eagles (*Haliaeetus albicilla*), in Atlantic salmon (*Salmo salar*) from coastal areas of the Baltic Sea [16]. PFOA has been found in the plasma of 73 loggerhead sea turtles (*Caretta caretta*) and 6 Kemp's ridley sea turtles (*Lepidochelys kempii*) captured from inshore waters of Core Sound, North Carolina (NC), and offshore waters of South Carolina, Georgia, and Florida. [17]. PFOA has even been detected in the serum of the giant panda and the red panda from zoos and animal parks from six provinces in China and has also been detected in all specimens of European Beaver's (*Castor fiber*) liver, in whole blood of Cod (*Gadus morhua*), Velvet Scoter (*Melanitta fusca*), Eider Duck (*Sommateria mollissima*), Long-tailed Duck (*Clangula hyemalis*), Razorbill (*Alca torda*), Red-throated Diver (*Gavia stellata*) sampled in Poland [18, 19]. PFOA has even been shown to be present in increasing levels in East Greenland as demonstrated by a study of a subsample of 128 subadult (3-5 years)

polar bears (*Ursus maritimus*) from 19 sampling years within the period 1984-2006 in which median concentrations showed a significant annual increase of 2.3%. [20]

3) PFOA in the Environment

PFOA contamination has been found in fresh water bodies of the European region. The PFOA levels concentrations were measured in samples of freshwater from several countries including Germany, Italy, Norway, and Sweden and were found to be in the range of <0.65–57 ng/L with approximately 75% of the data falling between the range of non-detectable and 8 ng/L. [21-25].

Levels of PFOA contamination have been found in the surface fresh water of the German Ruhr area and of the river Moehne and selected contaminated tributaries where PFOA concentrations up to 3640 and 33,900 ng/L, respectively were found. Saito [26], Tanaka [22] and So [27] reported that several surface fresh water bodies in Asia, from samples collected in China, Japan and other Asian areas, had PFOA concentrations mostly in the range of 0.10–41.60 ng/L, with peaks up to 456 ng/L. In studies of North American fresh water A number of studies of surface water of the Great Lakes region reported PFOA concentrations of <2–59 ng/L [28-30].

Atmospheric Levels of PFOA in Europe were measured in 2005 by de Voogt [24]. The PFOA levels varied from 0.226–0.828 ng/m³ in March 2005 and from 0.006–0.222 ng/m³ in November. Atmospheric PFOA levels, measured in the towns of Oyamazaki and Morioka located in Kyoto Japan, and were found to be in the range of 0.00159–0.919 ng/m³ with a Geometric mean of 0.2627 ng/m³ found in Oyamazaki and a geometric mean of 0.0020 ng/m³ found in Morioka [31, 32]. High levels of PFOA were

found in samples taken over a 10-week period by the fence of a fluoropolymer manufacturing facility in the United States where Barton [33] reported PFOA concentrations up to 900 ng/m³.

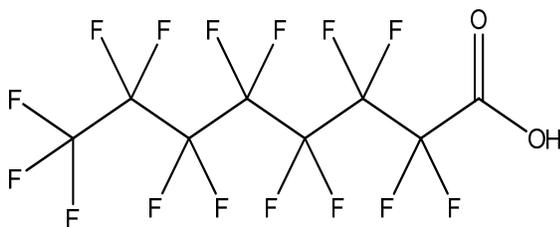
PFOA is also found in the home. House dust from homes (n = 102) and day care centers (n = 10) in Ohio and North Carolina in 2000-2001 were sampled and PFOA was measured at median concentrations of 142 ng/g of dust, with a maximum PFOA concentration of 1960 ng/g [34]. PFOA was found in dust samples that were taken from 67 houses in Canada at levels in the range of 1.15–1234 ng/g of dust with a mean concentration of 106 ng/g [35]. Moriwaki [36] reported detection of PFOA in the indoor dust of Japanese houses at levels of 69–3700 ng/g with a mean level of 380 ng/g.

C. Chemical and Physical Properties

The following are the chemical and physical properties of PFOA:

Molecular Formula: C₈H-F₁₅-O₂

Chemical Structure:



Molecular Weight: 414.09 [37]

Boiling Point: 189 deg C [38]

Melting Point: 52-54 deg C [38]

Density/Specific Gravity: 1.792 g/ml @ 20 °C [38]

Dissociation Constants: pKa = 2.80 [39]

Vapor Pressure: 0.15 mm Hg @ 25 °C [40]

II. TOXICITY IN ANIMALS

A. Acute Toxicity

Olson and Anderson [41] calculated the 30 day LD50 of a single IP injected dose of PFOA to be 189 mg/kg (175-208 mg/kg). All rats treated with doses of PFOA of 175 mg/kg or greater died within 5 days of exposure. The LD50 for oral exposure was determined to be 680 mg/kg for male CD rats and 430 mg/kg for female CD rats by Dean and Jessup [42]. Glaza [43] determined that the LD50 for female Spraque-Dawley rats to be in the range of 250-500 mg/kg and the LD 50 for male Spraque-Dawley rats to be greater than 500 mg/kg which supports the results from Dean and Jessup. The LD 50 from the acute toxicity studies performed in rodents are summarized in Table 2.1 below.

Table 2.1
Summary of PFOA LD50 values

Species	Dose	Exposure Route	Reference
Male Fischer rats	LD 50/30day= 189 mg/kg	IP	[41]
Male CD rats	LD 50= 680 mg/kg	Oral	[42]
Female CD rats	LD 50= 430 mg/kg	Oral	[42]
Male Spraque-Dawley rats	LD 50= >500 mg/kg	Oral	[43]
Female Spraque-Dawley rats	LD 50= 250-500 mg/kg	Oral	[43]

B. Subchronic Toxicity

Subchronic toxicity studies were performed by Goldenthal [44] in rhesus monkeys (2 of each sex per group) at doses of 0, 3, 10, 30 and 100 mg/kg per day by gavage in 0.5% Methocel 7 for a period of 90 days. Table 2.2 contains a summary of the PFOA levels found in the surviving animal's serum and liver tissue at sacrifice.

Table 2.2

Summary of the PFOA levels found in surviving monkeys at sacrifice

Daily Dose (mg/kg)	Serum PFOA ($\mu\text{g/ml}$)		Liver ($\mu\text{g/ml}$)		Total PFOA Liver (μg)	
	Male	Female	Male	Female	Male	Female
0	ND	1	0.05	0.07	3	5
3	53	65	3	7	250	350
3	48	50	ND	ND	ND	ND
10	45	79	9	ND	600	ND
10	71	71	ND	10	ND	750
30	145	Dead	61	Dead	4000	Dead
30	Dead	Dead	Dead	Dead	Dead	Dead
100	Dead	Dead	Dead	Dead	Dead	Dead
100	Dead	Dead	Dead	Dead	Dead	Dead

All animals in the 100 mg/kg per day died during the study with the first death occurring during the second week, with death occurring in all animals by the fifth week. One male from the 30 mg/kg per day group died during week 7 and two females from this group also died during weeks 12 and 13. All the animals that died during the study exhibited marked diffuse lipid depletion in the adrenal glands, slight to moderate bone marrow hypoplasia and atrophy of the lymphoid follicles in the lymph nodes. Of the animals that survived until sacrifice, one male in the 30 mg/kg per day group exhibited similar marked diffuse lipid depletion in the adrenal glands, slight to moderate bone

marrow hypoplasia and atrophy of the lymphoid follicles in the lymph nodes that was present in the animals that died during the study. The absolute and relative weight of the hearts and the absolute brain weight were significantly decreased in females from the 10 mg/kg per day group. It was also noted that the mean weight of the pituitary in males from the 3 mg/kg per day group was significantly increased.

Two subchronic toxicity studies were performed in male cynomolgus monkeys by Thomford in 2001 [45, 46]. The first was a 6 month study in which male cynomolgus monkeys were administered 0, 2 or 20 mg/kg per day orally via a capsule. All animals survived until sacrifice and there were no adverse effects noted in either the gross or clinical studies. This study was then followed by a 26 week study in which male cynomolgus monkeys were given 0, 3, 10 or 30 mg/kg doses per day orally via a capsule. Dosing was discontinued at day 11 because of statistically significant weight loss, decreased food consumption and decreased feces production in the 30 mg/kg per day group. Treatment of this group was then resumed on day 22 at a 20 mg/kg per day dose. At sacrifice significant increases were noted in the mean absolute liver weights and the liver to body weight percentages in all groups receiving PFOA. Thyroid hormones were also found to be decreased beginning on day 35 in the 10 and 30/20 mg/kg per day groups. This study demonstrated a lowest-observed-adverse-effect-level of 3 mg/kg per day.

A 28 day study by was conducted by Christopher and Marias [47] using ChR-CD mice (5/sex per group) administered a daily dietary dose of APFO (the ammonium salt of PFOA used in fluoropolymer manufacturing) ranging from 0 to 30,000 ppm. All animals in the 1000 ppm and higher group died within the first 9 days of treatment, while

death of all the animals with the exception of one male in the 300 ppm occurred within 26 days of treatment. There was a dose-related reduction in the mean body weight of all the treated animals. Liver weights were increased in mice receiving a dose of 30 ppm or greater. Morphological changes in the livers of all treated animals were observed including changes, enlargement and/or discoloration of 1 or more lobes of the liver, a diffuse cytoplasmic enlargement of hepatocytes throughout the liver, with a random distribution of cytoplasmic lipid vacuoles of variable sizes.

The results of a second 28-day study using the same APFO doses as Christopher and Marias were described in a report by Metrick and Marias [48]. All animals receiving a dose that was greater than 10,000 ppm died before the end of the first week of the study with morphological changes noted in the livers of all the animals who received APFO. The morphological changes in the liver noted in the study consisted of panlobular diffuse hypertrophy of the hepatocytes with accompanying focal to multifocal cytoplasmic lipid vacuoles.

A 28-day study of the toxicological effects of oral exposure to PFOA and Perfluorooctane Sulfonate (PFOS) on male Sprague-Dawley rats by gavage has also been performed [49]. Cui used 5 groups of 10 animals per group with the control group receiving no perfluorochemicals (PFCs) and the remaining group receiving doses of 5 mg/kg per day PFOA, 20 mg/kg per day of PFOA, 5 mg/kg per day PFOS and the final group received a dose of 20 mg/kg per day of PFOS. Histological changes in the hepatocytes were observed in all exposed groups. These changes included fatty degeneration, angiectasis in the central vein, congestion in the hepatic sinusoid, acidophilic lesions, focal hemorrhage with necrosis, cytoplasmic vaculation and hepatic

enlargement with inflammatory cellular infiltration. A biodistribution assay was performed to measure the PFOA concentrations in the target organs and the tissue concentrations were found to be in the order of kidney>liver>lung>heart>testicle>spleen and brain.

C. Mutagenicity

There is conflicting evidence on the genotoxic effects of PFOA. Several studies conducted for the United States Environmental Protection Agencies by an independent laboratory concluded that PFOA and APFO did not induce mutations with or without metabolic activation in AMES tests, in human lymphocytes or in Chinese Hamster Ovary (CHO) cells [50, 51]. The same laboratory also tested the in-vivo mutagenic properties of APFO using micronucleus studies on the bone marrow of mice [52, 53] with negative results. Other studies have found that APFO was able to induce both chromosomal aberrations and polyploidy with the presence of metabolic activation in human lymphocytes [54, 55]. A recent study by Yao et al. [56] using human the hepatoma cell line HepG2 found a significant increase in the tail moment in the single cell gel electrophoresis assay in HepG2 cells exposed to PFOA which indicates that PFOA was able to induce DNA strand breaks in HepG2 cells. Yao also found PFOA induced a dose-dependent increase in the frequency that a micronucleus was found in binucleated HepG2 cells, which indicates that chromosome breaks occurred in HepG2 cells after PFOA treatment. Shabalina et al. [57] found DNA breaks in HepG2 cells exposed to PFOA using the TUNEL procedure and propidium iodide staining of cellular DNA.

Although there are conflicting evidence, PFOA has been found to produce a genotoxic effect on human cells in some studies.

D. Carcinogenicity

Carcinogenicity studies of PFOA in rodents demonstrated that PFOA induced a tumor triad which consists of liver adenomas, Leydig cell adenomas and pancreatic acinar cell tumors in male Spraque-Dawley rats.

Sibinski [58] performed a 2 year study of Spraque-Dawley rats exposed to APFO in their diet. Groups of 50 animals from each sex consumed feed containing 0, 30 or 300 ppm APFO; this corresponded to a mean APFO daily dose of 0.0, 1.3 and 14.2 mg/kg in the males and 0.0, 1.6 and 16.1 mg/kg per day in females. The animals were observed daily with body weights and food consumption recorded weekly for the first six months and then bi-weekly for the remainder of the study.

An interim 1 year study was also performed using groups of 15 additional rats per sex fed 0 or 300 ppm APFO with the animals sacrificed at 1 year and the weights of the kidney, liver, testes, adrenal gland and spleen recorded. Pathological examinations which included hematology, serum chemistry and urinalysis were performed on samples from 15 rodents from each group at 3, 6, 12, 18 and 24 months. Post mortem observations were recorded on all animals that died during the study and those that were sacrificed at the 1 year interim and 2 year study end. At the 2 year sacrifice, the organs were weighed for 15 randomly selected animals per sex in all remaining rats in each group. On gross examination, lesions in the liver, testis and ovary were noted.

At the 1 year sacrifice liver hepatomegalocytosis was present in 80% of males consuming feed containing 300 ppm APFO with no hepatomegalocytosis seen in the control group. Portal mononuclear cell infiltration was also present in 87% of the 300 ppm APFO dosed male animals versus 47% of the control male animals and hepatocellular necrosis was evident in 40% of the 300 ppm APFO male animals with no hepatocellular necrosis observed in the control group. Testicular masses were also discovered in 40% of the 300 ppm dosed group at the 1 year sacrifice with no testicular masses observed in the control group.

At the 2 year sacrifice megalocytosis was present in 12% of the male rats consuming feed containing APFO at a concentration of 30 ppm and 80% of the males consuming feed with a APFO concentration of 300 ppm. Megalocytosis was also observed in and 2% of the female rats consuming feed containing APFO at a concentration of 30 ppm and in 16% of the females that consumed feed with a APFO concentration of 300 ppm. There was no megalocytosis observed in either the male or female control groups. It was also noted that hepatic cystoid degeneration was observed in 14% and 56% of the 30 ppm and 300 ppm male groups with only 8% observed in the control group. At the 2 year sacrifice a significant increase in the incidence of testicular Leydig cell adenomas (LCT) was reported in the high dose group which was present in 14% of the test animals. 4% of the low dose group also developed LCT with no LCT observed in the control group. Historically, LCT have been observed in 0.82% of control animals in 2-year old Sprague-Dawley rats used in carcinogenicity studies [59]. The effects noted in this study are summarized in Table 2.3.

Table 2.3

Summary of the Effects of APFO Exposure in Male Rats

Observations	Incidence Control Rats (0 ppm APFO)	Incidence Treated Rats (30 ppm APFO)	Frequency Treated Rats (300 ppm APFO)
1 Year Sacrifice Male Rats			
Liver hepatomegalocytosis	NONE	N/A*	80%
Portal mononuclear cell infiltration	47%	N/A*	87%
Hepatocellular necrosis	NONE	N/A*	40%
Testicular masses	NONE	N/A*	40%
2 Year Sacrifice Male Rats			
Liver hepatomegalocytosis	NONE	12%	80%
Hepatic cystoid degeneration	8%	14%	56%
Testicular Leydig Cell Adenomas	NONE	4%	14%

* Only rats treated with 0 and 300 PPM APFO were sacrificed at 1 year.

Biegel et al. [60] performed a 2-year study in which APFO was introduced into the diet of male CD rats. The study was conducted using feed containing 300 ppm APFO. Increased relative liver weights and hepatic beta-oxidation activity were observed in the PFOA treated rats at all time points. Liver adenomas were induced in 13% of the PFOA treated rats at all time points. Liver adenomas were induced in 13% of the PFOA treated group versus 3% of the control group. Leydig cell adenoma was induced in 11% of the testes of the PFOA treated group versus 0% of the control group with Leydig cell hyperplasia present in 46% of the animals exposed to PFOA in their diet. Acinar cell adenoma was induced in 9% of the PFOA treated animals versus 0% of the control group. The frequency of tumor induction is summarized in Table 2.4.

Table 2.4
Summary of the Tumor Induction Frequency of APFO in Rats

Tumor Induced	Incidence Control Rats (0 ppm APFO)	Frequency Control Rats (0 ppm APFO)	Incidence Treated Rats (300 ppm APFO)	Frequency Treated Rats (300 ppm APFO)
Liver				
Adenoma	2/80	3%	10/76	13%
Carcinoma	0/80	NONE	0/76	NONE
Adenoma/carcinoma combined	2/80	3%	10/76	13%
Testes				
Leydig cell hyperplasia	11/80	14%	35/76	46%
Leydig cell adenoma	0/80	NONE	8/76	11%
Pancreas				
Acinar cell hyperplasia	14/80	18%	30/76	39%
Acinar cell adenoma	0/80	NONE	7/76	9%
Acinar cell carcinoma	0/80	NONE	1/76	1%
Adenoma/carcinoma combined	0/80	NONE	8/76	11%

Blood was sampled at 1, 3, 6, 9, 12, 15, 18, and 21 months. Testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin and estradiol levels were quantified in the serum from each of these samples with no significant differences found in serum testosterone, FSH, prolactin, or LH concentrations in the PFOA treated rats when compared to their controls. However, significant increases in serum estradiol concentrations were present in the PFOA treated rats at all months sampled compared to the control groups.

III. MECHANISMS OF CARCINOGENICITY

A. Liver

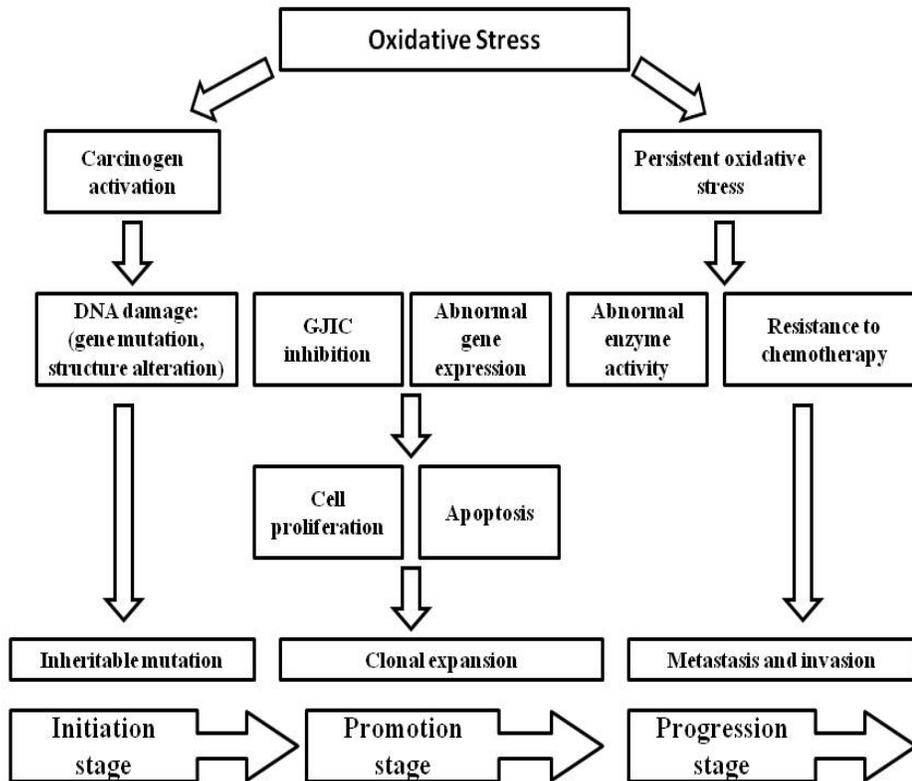
The available published scientific literature provides data suggesting that induction of hepatocellular adenomas by PFOA may result from: (1) generation of reactive oxygen species, (2) increased mitochondrial biogenesis, (3) the activation of Peroxisome Proliferator-Activated Receptor Alpha (PPAR α), (4) influence on molecular pathways such as those mediated by the constitutive androstane receptor (CAR), (5) the inhibition of gap junctional intracellular communication (GJIC), (6) increased estrogen levels and (7) modulation of the immune system. Data in support of these proposed mechanisms are described in the sections that follow.

1) Reactive Oxygen Species

Chemically induced carcinogenesis is thought to consist of a multistage process definable by at least three steps or stages: initiation, promotion, and progression. The oxidative stress that results from the presence of ROS impacts all three stages of the cancer process. During the initiation stage oxidative DNA damage may produce gene mutations and structural alterations of the DNA, resulting in a heritable mutation. During the promotion stage ROS and oxidative stress can contribute to abnormal gene expression and inhibition of GJIC which results in an increase in cell proliferation or a decrease in apoptosis in the initiated cell population. Oxidative stress may also participate in the progression stage of the cancer process by imparting further DNA alterations to the initiated cell population. These changes may result in changes in enzyme activity and

make the lesions resistant to normal growth control [61]. This is exemplified in figure 3.1 taken from Klaunig et al. [61].

Figure 3.1
Summary of the roles of oxidative stress in carcinogenesis



In a study by Liu et al. [62] cultured freshwater tilapia (*Oreochromis niloticus*) hepatocytes were exposed to PFOS or PFOA (at doses of 0, 1, 5, 15 and 30 mg/L for 24h) which resulted in a significant induction of reactive oxygen species (ROS) accompanied by increases in activities of superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR), demonstrating that PFOA produced oxidative stress and induced apoptosis with involvement of caspases in primary cultured tilapia hepatocytes. Panaretakis et al. [63] measured reactive oxygen species (ROS) generation at 1.5, 3, 5

and 24 h after cultures of human HepG2 hepatoma cell lines were exposed to PFOA concentrations of 200 mM and 400 mM. At 3 h, 200 mM of PFOA had elicited detectable levels of H₂O₂ in 91% of the total cell population compared to 12% of the vehicle-treated cells and 400 mM PFOA induced H₂O₂ generation in 98% of the total cell population at the same time point. Superoxide anions, which are another example of ROS species, reached detectable levels in 43% and 71% of the total cell population after treatment with 200 mM and 400 mM PFOA respectively (versus 10% in DMSO-treated cells). This accumulation of ROS is thought to be the result of disruption of the mitochondrial membrane.

A study by Yao et al. [56] investigated the genotoxic potential of PFOA in human hepatoma HepG2 cells in culture using single cell gel electrophoresis (SCGE) assay and micronucleus assay. Dichlorofluorescein diacetate was used as a fluorochrome to measure the intracellular generation of ROS with the level of oxidative DNA damage being evaluated by immunocytochemical analysis of 8-hydroxydeoxyguanosine (8-OHdG) in PFOA-treated HepG2 cells. This study found significantly increased levels of ROS and 8-OHdG in the PFOA exposed cells which indicate that human HepG2 cells exposed to PFOA do produce ROS which may play a role in all three stages of the carcinogenesis process.

2) Mitochondrial Biogenesis

PFOA does produce hepatic peroxisome proliferation (PP) in rats, which is discussed later; however, PFOA has been shown to stimulate mitochondrial biogenesis, which is not a characteristic response of PPs [64]. Berthiaume exposed male Spraque-

Dawley rats to 100 mg/kg of the PP chemicals PFOA, PFOS and N-ethyl perfluorooctanesulfonamido ethanol dissolved in dimethyl sulfoxide (DMSO) via a single i.p. injection. The animals were sacrificed 3 days post injection with the liver tissue and mitochondria collected from animals in each treatment group used to examine the effects of each compound on cytochrome oxidase activity, cytochrome content, and mitochondrial DNA copy number to determine their effect on mitochondrial biogenesis. PFOA and PFOS were capable of inducing PP in rats following injection; however, PFOA was the only compound that caused significant decreases in mitochondrial cytochrome oxidase activity with a significant increase in mitochondrial DNA copy number which reveals that a unique mitochondrial biogenesis is occurring in PFOA exposed rats that is not generally produced by other PPs.

Starkov et al. [65] also demonstrated that PFOA and PFOS caused a slight increase in the intrinsic proton leak of the mitochondrial inner membrane, which resembled a surfactant-like change in membrane fluidity. Since mitochondria play a significant role in cell signaling, ROS generation and apoptosis the resulting mitochondrial biogenesis seen in rodents exposed to PFOA may play a role in carcinogenesis.

3) PPAR α Agonism

PPARs are steroid hormone receptors that act as ligand-activated transcription factors. Three PPAR isotypes have been identified, PPAR α , β/δ and γ . These isotypes play a role in lipid metabolism, energy homeostasis and inflammation [66]. PPAR agonism results in an increase in the number and volume fraction of peroxisomes in the

cytoplasm of cells. This proliferation can be visualized utilizing electron microscopy or can be quantitated by measuring the levels of selected peroxisome enzymes such as catalase, cytochrome P-450s (e.g., CYP4A1 and 4A3), and acyl coenzyme A (CoA) oxidase. The response to exposure to PP chemicals, such as PFOA, varies drastically between species with the rat and mouse being the most sensitive. PP has been shown to be present in the hamster, to a lesser degree than the rat and mouse, with relatively no PP present in the guinea pig, monkey, and human when exposed to PPAR agonists at dose levels that produce marked response in the rat and mouse [67-70]. The liver tumors induced by PFOA exposure in rodents appears to occur as a result of PFOA binding to the PPAR α , which results in PP [60, 71, 72]. PFOA is believed to produce these tumors in the liver by binding with and, as a result, activating PPAR α . PPAR α modulates the transcription of genes involved in PP, regulation of the life/death cycle of the cell and lipid metabolism. PPAR α activation produces a proliferation of the peroxisomes, an increase in cell proliferation and a decrease in apoptosis. The decrease in cell death combined with increased stimulation in cell proliferation results in increased cell reproduction. This reproduction promoting environment coupled with the presence of ROS, and their resultant oxidative stress, promotes the reproduction of DNA-damaged cells. As a result preneoplastic foci arise and in the course of time develop into tumors by way of continued clonal expansion [73].

4) CAR Activation

PFOA has also been shown to activate CAR. CAR activation is essential in liver tumor promotion by other nongenotoxic liver carcinogens such as Phenobarbital (PB)

[74]. A DNA microarray analysis of hepatic gene expression in *Car*^{-/-} mice showed that CAR induced or repressed 70 genes [75]. In addition to up-regulation of drug-metabolizing enzymes, CAR down-regulated genes that encode gluconeogenic enzymes, those involved in fatty acid oxidation, and proteins such as angiogenin and fibronectin. Nongenotoxic carcinogens such as PB are known to stimulate cell proliferation and suppress apoptosis, leading to tumor development. While PFOA has been shown to activate PPAR α [71], PFOA also produces liver enlargement in the PPAR α -null knock-out mice [76, 77]. This PPAR α -independent effect suggests other mechanism of carcinogenicity may be present other than PPAR α activation. A study of gene expression changes in PPAR α -null mice exposed to PFOA revealed an up-regulation of certain Cyp2b and Cyp2c genes, such as Cyp2b10, which are markers of CAR activation [78-80]. Another study revealed that two days after a single i.p. administration (50 mg/kg) of (PFOA) and perfluorodecanoic acid (PFDA), mRNA expression of Cyp2b10 was increased 20-fold [81]. It was also shown that there is a strong correlation between the transcript profile of PPAR α independent PFOA genes in PPAR α -null mice exposed to PFOA and those of activators of CAR including Phenobarbital [82] which would suggest that a subset of genes are controlled by CAR in the PPAR α -null mouse exposed to PFOA.

5) GJIC Inhibition

When normal cells are no longer able to control their growth by way of contact inhibition, they can become transformed into tumorigenic cells [83] which may be due to defective intercellular communication [84]. These transformed cells can be benign or

they can develop genetic mutations that lead to a malignant state as demonstrated in multistage carcinogenesis. It has been implied that changes occurring in the cell-to-cell communication by way of gap junction does play a role in the tumorigenic process [85]. A recent study by Upham et al. [86] verified that PFOA does inhibit GJIC and induce hepatomegaly in the livers of rats while the measured level of liver enzymes indicated no cytotoxic response. The inhibition of GJIC was shown to be dependent on the activation of both ERK and phosphatidylcholine-specific phospholipase C (PC-PLC) in the dysregulation of GJIC in an oxidative-dependent mechanism. This study also indicated that PFOA is able to activate the extracellular receptor kinase (ERK). In a previous study, Upham et al. [87] demonstrated that PFOA reversibly inhibited GJIC and this inhibition of GJIC was dependent on the length of the fluorinated tail. Since GJIC plays an essential role in preserving tissue homeostasis, disruption of gap junction function may play a role in the carcinogenicity of PFOA.

6) Estrogenic Signaling

It is known that PFOA can produce PP in rodents, however humans and certain other species, including rainbow trout, guinea pigs, and nonhuman primates, show little to no evidence of PP [70, 88, 89]. A study by Tilton et al. [90] of rainbow trout exposed to PFOA (1,800 ppm or 50 mg/kg/day) and DHEA demonstrated enhanced liver tumor incidence in trout exposed to PFOA at concentrations of 50 mg/kg per day. This carcinogenic effect was independent of PP which was determined by a lack of peroxisomal β -oxidation and catalase activity. Gene profiling also demonstrated that PFOA induced an estrogenic gene signature that strongly correlated with that produced

by 17 β -estradiol. The results from this study suggest that the tumor-promoting activities of PFOA in trout are independent of PP and instead may be related to a mechanism involving estrogenic signaling. A study by Liu et al. [91] demonstrated that PFOA possesses estrogenic activities and additional results of an estrogen receptor inhibition assay indicated that the estrogenic effect of PFCs may be mediated by the estrogen receptor pathway in primary cultured tilapia hepatocytes. A gene expression profile study was conducting using liver tissue obtained from male and female rare minnows (*Gobiocypris rarus*) exposed to PFOA using a custom cDNA microarray containing 1773 unique genes by Wei et al. [92]. The minnows were treated with continuous flow-through exposure to PFOA at concentrations of 3, 10, and 30 mg/L for 28 days. Hepatocyte swelling and vacuolar degeneration with prominent eosinophilic hyaline droplets were observed in the cytoplasm of male and female hepatocytes exposed to 10 mg/L. The livers from the cohort exposed to 10 mg/L PFOA were selected for further hepatic gene expression analysis. The results revealed that a number of genes were either upregulated or downregulated in the livers of the minnows exposed to PFOA with a distinct induction of estrogen-responsive genes identified using a custom cDNA microarray. The fact that other studies [93, 94] have shown that estrogen promotes liver carcinogenesis in rats makes the estrogenic signaling properties of PFOA a viable pathway for tumor promotion in the liver.

7) Immunomodulation

A healthy and fully functional immune response allows the body to identify and remove abnormal cells, including initiated cells, while a weakened or suppressed immune

system has been associated with increased tumor development [95-97]. A study by Yang et al. [98] demonstrated that exposure of Male C57B1/6 mice to PFOA in the diet resulted in severe thymic and splenic atrophy with the weight of the thymus and spleen rapidly returning to normal within 10 and 5 days, respectively, following withdrawal of PFOA from the diet. It was also noted that these changes in thymus and spleen weight paralleled the changes in total thymocyte and splenocyte counts. Yang also evaluated the effects of PFOA on the adaptive immunity in mice by measuring antigen-specific antibody responses and lymphocyte proliferation in mice immunized with horse red blood cells [77]. Mice were fed a diet of 30 mg PFOA/kg for 15 days after which the immune response was quantified by employing both the plaque-forming cell assay and determination of the antibody titer by ELISA. The results of the study revealed a suppression of the IgM and IgG titers relative to the titers present in the unexposed control animals and reduced plaque formations in the exposed animals compared to the animals in the control group. The results from another study by Yang [99] revealed that while PFOA treatment of wild type mice led to a dramatic atrophy of the thymus and spleen reflecting losses of thymocytes and splenocytes, this effect is not as dramatic in PPAR α -null mice. Although the atrophy of the thymus and loss of thymocytes was not as marked as in the wild type mice, significant thymus atrophy and loss of thymocytes were observed in PPAR α -null mice exposed to PFOA. This weakened response clearly points toward the involvement of both PPAR α -dependent and -independent mechanisms in the immunomodulation process. Note that although immunomodulation is only discussed in this section the immune response is a physiological response of the body and modulation of the immune system affects the body as a whole. As a result the

immunomodulation by PFOA may play a plausible role in tumorigenesis in the liver, testis and pancreas.

B. Testis

Leydig cell adenomas are another type of tumor that is produced by exposure to PFOA. The available published scientific literature provides data suggesting that induction of Leydig Cell adenomas by PFOA may result from: (1) inhibition of testosterone biosynthesis and (2) estradiol modulation. Data in support of these proposed mechanisms are described in the sections that follow.

1) Inhibition of Testosterone Biosynthesis

Inhibition of testosterone biosynthesis may be involved in the induction of Leydig cell tumors by PFOA which has been shown to decrease the production of testosterone in Leydig cells that are exposed to the compound [100]. Results from a study by Gazouli et al. [101] indicate that the inhibition of testosterone production may also be mediated by PPAR α . Decreased testosterone levels can lead to increased luteinizing hormone levels through a negative feedback mechanism called the hypothalamic pituitary testicular (HPT) axis that regulates metabolism. The disruption of the hypothalamus pituitary thyroid axis in the thyroid gland has been shown to produce follicular cell carcinoma in the thyroid [102] and may be applicable to LCT formation in the testis as a disruption of the HPT axis. Several studies have produced sustained hypersecretion of LH in the testis, which are compromised of endocrine controlled tissues, with the disruption of the HPT axis [103-109]. This mechanism was demonstrated in a 1-year study where Vigui-

Martinez et al. [108, 109] fed Sprague-Dawley rats feed containing flutamide, which is an androgen receptor antagonist that blocks the binding of testosterone to the testosterone receptor and results in hypersecretion of LH due to the disruption of the HPT axis, and produced Leydig cell hyperplasia in the animals fed flutamide which progressed to Leydig cell adenoma by the end of the study.

2) Estradiol Modulation

Since PP chemicals have been shown to increase estradiol levels and estradiol modulates growth factor expression in the testes then PFOA may produce Leydig cell tumors (LCT) in the testes as a result of the increased cell hyperplasia produced by estradiol [100, 110]. Administration of estradiol to mice has produced Leydig cell tumors in several studies [111-113] which further supports the plausibility that PFOA can produce these tumors due to the compounds ability to increase estradiol levels. It is also plausible that elevated estradiol levels may act as a mitogen and/or enhance growth factor secretion thereby causing Leydig cell hyperplasia and tumor formation. The transforming growth factor α has been detected in Leydig cells[114] which stimulates cell proliferation and is an example of how enhanced growth factors could be involved in tumor formation.

Although peroxisomes are present in both the liver and testes the limited data on Leydig cell induction by PFOA does not support PP as a plausible MOAs for tumor production in the testes. Two separate studies exposing male rats to known PP chemicals found abundant PP in the liver using biochemical assays and visual examination with electron microscopy with no PP found in the Leydig cells [115, 116]. Currently no other

data exists to support any other alternate MOAs for LCT formation other than the hormonal mechanisms described.

C. Pancreas

Pancreatic acinar cell tumors (PACT) are a third tumor type produced by exposure to PFOA. The available published scientific literature provides data suggesting that induction of pancreatic tumors by PFOA may result from cholestasis.

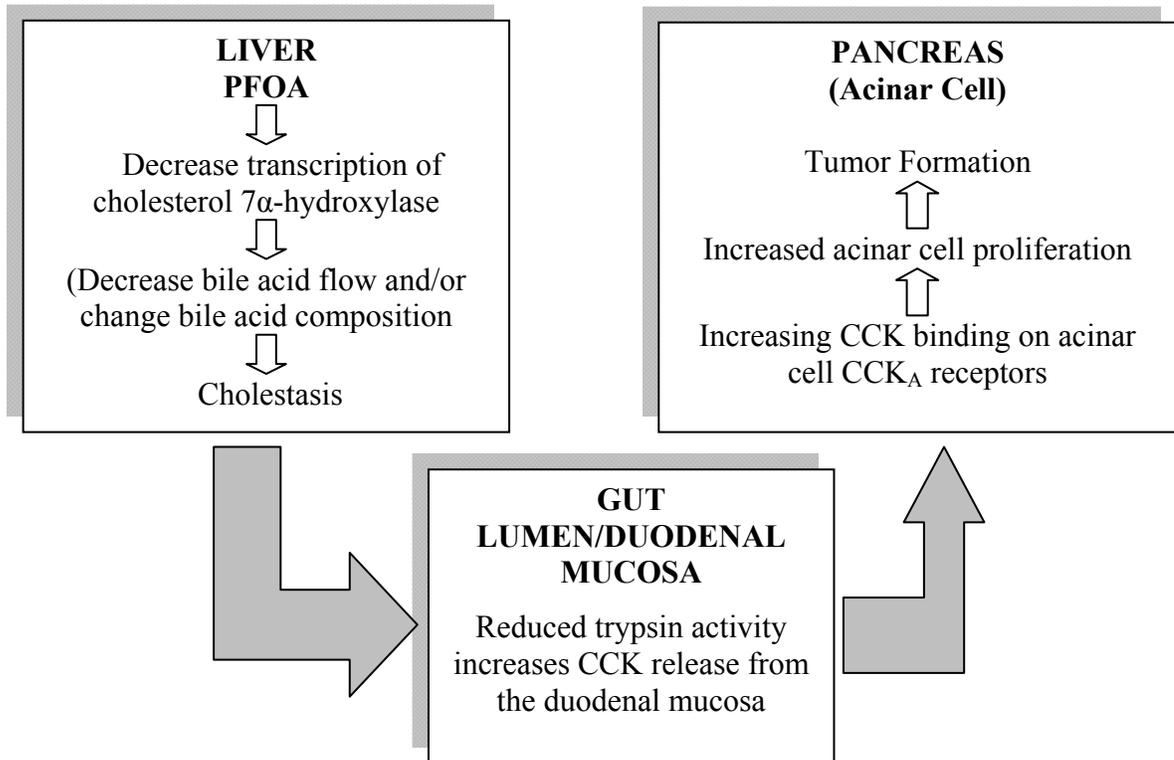
1) Hepatic Cholestasis

Steroids (such as estradiol and testosterone discussed previously), cholecystokinin (CCK) and other growth factors, growth factor receptor over-expression (CCK_A receptor) and dietary fat intake have all been shown to produce PACTs in rats when their levels are altered [117-119]. PACT induction by PFOA and other PP appears to be seen in rats, but not mice, which indicates that PACT tumor formation may be species dependant [67, 68]. A chronic study by Osbourn et al. [120] demonstrated increases in pancreatic weights at 3 months (6% above control) and 6 months (17% above control) with an increase in CCK plasma levels in a 2 year bioassay of rats exposed to the PP chemical WY. The results of the study indicate that chronic exposure to WY causes liver alterations such as cholestasis, which may increase plasma concentrations of CCK. PP chemicals such as PFOA may thus induce PACT as a result of a sustained mild increase in CCK levels produced by hepatic cholestasis. Results from a second study by Osbourn et al. supported the validity of the role of increased CCK levels in PACT

formation [121]. The diagram below (Figure 3.2) depicts the proposed mode of action (MOA) with key events for pancreatic acinar cell tumors (PACTs) induced by PFOA.

Figure 3.2

Illustration detailing the proposed MOA for PFOA pancreatic acinar cell tumorigenesis

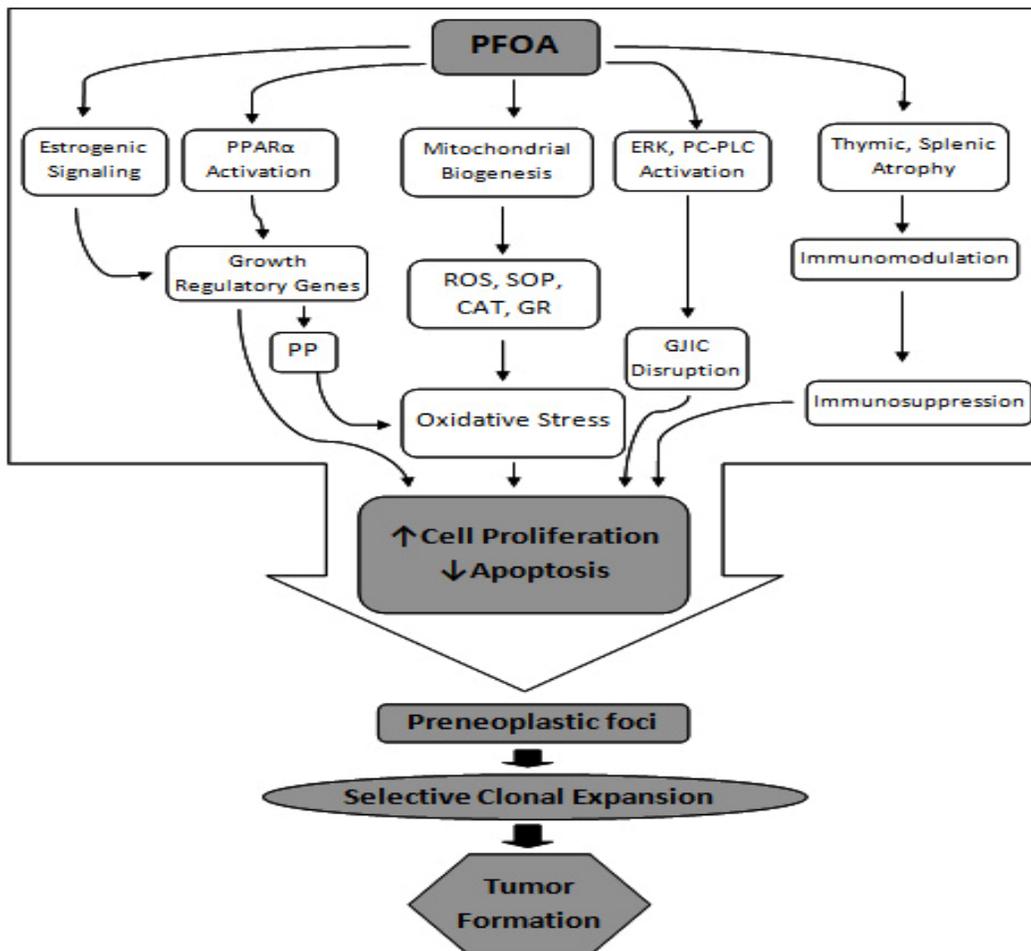


As described above, the experimental evidence suggests that PPAR α agonists such as PFOA induce PACTs in rats by increasing CCK levels secondary to reduced bile acid synthesis and/or alterations in bile acid composition [120, 121]. PPAR α agonists have been shown to decrease transcription of cholesterol 7 α -hydroxylase, the rate-limiting step of bile acid synthesis, via the PPAR α /RXR α heterodimer reducing hepatocyte nuclear factor-4 (HNF-4) binding to the DR-1 sequence, which regulates this gene [122]. The inhibition of cholesterol 7 α -hydroxylase results in reduced bile flow and

altered bile acid composition resulting in cholestasis. This reduction in bile acid flow and/or altered bile acid composition creates a reduction in trypsin activity which increases the CCK release from the duodenal mucosa. This increased release of CCK results in increased activation of the CCK_A receptors which can increase acinar cell proliferation and may lead to tumor formation.

The mechanisms of carcinogenicity by PFOA that have been detailed are summarized in Figure 3.3.

Figure 3.3
Summary of Possible Mechanisms for PFOA Carcinogenicity in Rodents



IV. HUMAN RELEVANCE

As described in the MECHANISMS OF CARCINOGENICITY section, PFOA has been shown to produce cancer in laboratory animals. This section previews studies that have used human sources in an attempt to determine if the possible mechanisms of carcinogenicity hypothesized in animal models are applicable to human risk.

A. Liver

The induction of hepatocellular adenomas by PFOA in rodents may involve any of several pathways including ROS, CAR, GJIC, increased estrogen levels, decreased immune system response and/or PPAR α agonism. Many of these pathways are plausible in human carcinogenesis.

1) ROS Generation

Results of a recent study established that PFOA produces genotoxic effects on human HepG2 cells [56]. This DNA damage is thought to be the result of oxidative stress induced by intracellular ROS, particularly hydrogen peroxide. In this study the fluorochrome 2',7'-dichlorofluorescein was used to measure intracellular generation of ROS and 8-OHdG was used to determine the level of oxidative DNA damage. A separate study by Hu et al. [123] that exposed human Hep G2 cells to PFOA and PFOS at concentrations of 50-200 $\mu\text{mol/l}$ (50–200 $\mu\text{mol/l}$) resulted in the generation of ROS. Panaretakas et al. [63] also demonstrated that human Hep G2 cells treated with 200 and 400 μmol PFOA exhibited a dramatic increase in the cellular content of superoxide

anions and hydrogen peroxide after 3 h. Hypolipidemic drugs, which are known PP, were also shown to induce an overproduction of ROS in human hepatocytes [124]. Many other studies have implicated ROS and oxidative stress in human liver tumors [125-129] which indicates that ROS generation by PFOA may play a role in human carcinogenesis and warrants further study.

2) CAR Activation

As previously discussed, PPAR α -null mice exposed to PFOA developed liver enlargement and a measurable up-regulation of Cyp2b and Cyp2c genes, which are markers of CAR activation. PB is known to stimulate cell proliferation and suppress apoptosis, leading to tumor development via the CAR activation pathway but has typically not been shown to produce liver tumors in humans [130]. Although little evidence suggests that CAR activation by PFOA could result in tumor formation in the liver, CAR expression shows a large amount of genetic polymorphism as demonstrated in a study by Change et al. [131] that revealed a 240-fold variability in hepatic CAR mRNA levels in humans. This highly variable expression among individuals may explain why there are reported cases of liver tumors in humans that have been on long term PB therapy [132, 133] and could represent a plausible tumorigenic pathway for a subset of the human population that highly express CAR and are exposed to high levels of PFOA.

3) GJIC Inhibition

Since carcinogenesis involves a disturbance of homeostasis and cancer cells show uncontrolled growth, it is conceivable that altered GJIC plays an important role in

carcinogenesis. PFOA has been shown to inhibit GJIC and induced hepatomegaly in rat livers [86]. As previously discussed the inhibition of GJIC in rats appears to depend on the activation of the ERK pathway. In studies of human cancer cell lines GJIC was absent or decreased in all human cancer cell lines that were analyzed by Mesnil et al. [134] and was found to be decreased in human hepatocellular carcinoma tissues and cell lines by other investigators as well [135-137]. The inhibition of GJIC via ERK activation has been shown to be a key pathway of in human carcinogenesis [138]. The potential for cross-species effects of PFOA on GJIC was established in a study by Hu et al. where PFOS was shown to inhibit GJIC in rat liver tissue as well as dolphin kidney cells [139]. The ability of perfluorinated chemicals to inhibit GJIC appears to be neither species- nor tissue-specific and can occur both in vitro and in vivo. Currently studies evaluating GJIC inhibition by PFOA in human cell lines are lacking. Future experiments, particularly with human cell lines, on the inhibitory effect of perfluorinated chemicals on GJIC are needed as this may be a plausible MOA for carcinogenesis in humans.

4) Estrogenic Signaling

Although it was discussed previously that PFOA has been shown to promote liver tumors in fish that lack PPAR α through disruptions in estrogenic signaling, more studies are needed to assess the potential for PFOA to mediate carcinogenesis through the estrogenic pathway in other species such as humans. A previous study in primates [140] did not find any evidence of PFOA increasing estrogen levels which would infer that the estrogenic pathway would not be likely involved in human hepatocarcinogenesis,

however studies involving human cell lines are needed to exclude estrogenic signaling as a plausible MOA for PFOA carcinogenesis in humans.

5) Immunomodulation

As discussed previously PFOA and other PP chemicals do exert an effect on the immune system of study animals. The mechanisms are not clear as to why these chemicals are able to modulate the immune system and more research is needed to determine the impact on the human immune system as there does appear to be considerable species variability in the degree of response. The fact that the immunomodulatory effects of PFOA can be present independent of PPAR α activation would imply that it would be possible for PFOA to induce liver carcinogenesis in humans via these effects as well as in other tissues and warrants further investigation.

6) PPAR α Agonism

PFOA has been shown to act as a strong tumor promoter in rodent livers [141] as well as other PP chemicals [142]. The PP produced by PFOA, which is thought to be the result of PFOA binding to the PPAR α , does not appear to occur in human livers, as humans show little [140] to no evidence of PP. This may be due to the lower PPAR α expression found in humans compared to rodents as reported by Palmer et al. [143] who found a >10-fold lower PPAR α DNA binding activity in human liver lysates than the levels found in mouse liver lysates. There is also evidence that activation of the human PPAR α receptor elicits a different response than activation of the mouse PPAR α receptor. This has been demonstrated in studies using a PPAR α humanized mouse in which

PPAR α -null mice have been genetically manipulated to express human PPAR α at levels comparable to the mouse PPAR α found in wild-type mice. In these studies fibrates altered the expression of genes associated with lipid metabolism in both wild-type and PPAR α -humanized mice, however these compounds did not induce the hepatocyte proliferation in the PPAR α -humanized mouse that is observed in the wild-type mice nor did the compounds induce the liver tumor formation in the PPAR α -humanized that is observed in wild-type mice. This information reinforces the theory that the effects of peroxisome proliferators are species specific and the induction of liver tumors by PFOA due to PPAR α activation is highly unlikely [73].

B. Testis

It has been shown that PFOA does exert an effect on human testicular function as evident in a study by Joensen et al. [144] that analyzed serum samples for levels of 10 different PFAAs and reproductive hormones and assessed semen quality in 105 Danish men from the general population (median age, 19 years). They discovered the presence of measurable quantities of PFOS and PFOA in all subjects with a median of 24.5 and 4.9 ng/mL respectively. A significant decrease in semen production was found in men with high combined levels of PFOS and PFOA compared to those with low levels. Men exposed to high combined levels of PFOS and PFOA were found to produce 6.2 million normal spermatozoa in their ejaculate in contrast to 15.5 million among men with low levels of these PP chemicals in their bloodstream. Not only is it apparent that PFOA exerts an effect on testicular function but it is also plausible that the estradiol modulation

MOA for LCT formation seen in the rat exposed to PFOA may also be relevant in humans.

1) Estradiol Modulation

The ability of PFOA to induce human LCTs via the MOA associated with increased serum estradiol and testicular growth factors discussed previously in rodents may be of relevance to humans. Several authors have reported that estrogen receptor (ER) expression is present in the normal LCs of human adult testis [145-147] which indicates that these cells are a target for estrogens. It has also been shown that the cytochrome P450 aromatase, an enzyme that catalyzes the androgen aromatization into estrogens, has been detected in Leydig cells of normal human testis [148], which further suggests that locally produced estrogens plays a role in steroid production and spermatogenesis [149]. A recent human study by Carpino et al. [150] investigated the expression of aromatase and estrogen receptors (ER, ER β 1, ER β 2) in testes from two patients with LCTs. A strong immunoreactivity for aromatase, ER β 1, and ER β 2, together with a detectable ER immunostaining, was revealed in tumoral tissues. These findings were confirmed by Western blot analysis of tumor extracts. The pattern of ER expression in the LCT cells was also demonstrated to be different from that of control Leydig cells which exhibited only ER β 1 and ER β 2 isoforms. The results of this study reveals that it is plausible that high estrogen production could play a role in the neoplastic transformation of Leydig cells and the exclusive presence of ER in tumoral cells could amplify estradiol-17 β signaling contributing to the tumor cell growth and progression produced by PFOA.

C. Pancreas

The tumorigenic pathways of PACT induction by PFOA in rodents that was discussed previously in this review may not be relevant in humans as the expression of CCK_A receptors in humans are much lower than rodents with data indicating that human pancreatic acinar cells do not respond to CCK receptor activation due to an insufficient level of receptor expression [151]. A study of cynomolgus monkeys exposed to PFOA for 6-months did not produce any effects on CCK levels or evidence of cholestasis [140] and human epidemiological studies of CCK levels in employees exposed to high levels of PFOA did not reveal any increases of CCK levels in workers [152, 153]. These studies also imply a lack of CCK receptor expression in humans. It is also important to note that the majority of human pancreatic cancers are of the ductal type and are not derived from the acinar cells [154] as found in the PACT that is produced in rodents exposed to PFOA. With the incidence of pancreatic cancer in humans increasing every year, it has now become the fourth leading cause of cancer deaths [155], the ability of PFOA to induce pancreatic tumors in humans may warrant further investigation as the exact mechanism of PFOA carcinogenesis is not known, however the PACT induced in the rat would probably not correspond to a significant cancer risk in humans.

V. EPIDEMIOLOGICAL STUDIES

The current epidemiological studies of the carcinogenic effects of human exposure to PFOA are inconclusive. The current data is based on occupational studies most of which was conducted by the 3M company on male workers from their plants. In a 3M mortality study, prostate cancer mortality was the only statistically significant association found for workers exposed to high levels of PFOA in the manufacturing process; however, this was not observed in follow-up studies that utilized more specific exposure measures [156, 157]. A DuPont study did reveal an increase in the incidence of kidney and bladder cancer [158], but this study included very little data on other variables such as exposure to other chemicals in the plant. An epidemiological study by Olsen et al. [153] looking at hormone levels in exposed workers found estradiol to be the only hormone level increased in workers exposed to PFOA and this increase was found only in workers with the highest levels of PFOA in their serum. As discussed in the pancreatic section previously, an epidemiological study was done on CCK levels in employees exposed to PFOA with no increase in pancreatic tumor formation found [152, 153]. There is a pertinent need for collection and analysis of more human data regarding PFOA exposure as there is currently little epidemiological data available, most of which has been collected and analyzed by the industry that manufactures PFOA.

VI. DISCUSSION AND SUMMARY

As previously discussed PFOA, can be detected in the entire population of the United States. The EPA Science Advisory Board has reviewed the information that was available at the time, and suggested that the PFOA cancer data are consistent with the EPA Guidelines for Carcinogen Risk Assessment descriptor “likely to be carcinogenic to humans.” On January 9, 2009, EPA’s Office of Water developed Provisional Health Advisories (PHA) for PFOA and PFOS to protect against potential risk from exposure to these chemicals through drinking water. Provisional Health Advisories serve as informal technical guidance to assist federal, state and local officials in response to an urgent or rapidly developing drinking water contamination. They reflect reasonable, health-based hazard concentrations above which action should be taken to reduce exposure to these contaminants in drinking water. The PHA values are 0.4 µg/L for PFOA and 0.2 µg/L for PFOS. It is striking to note that the PHA value for PFOA in drinking water is 0.4 µg/L when the mean serum concentration in the US population was found to be 3.9 µg/L in 2003-2004 [7] and 3.4 µg/L in 2006 [7, 159]. The EPA and the eight major companies in the industry launched the PFOA Stewardship Program in 2006, in which companies committed to reduce global facility emissions and product content of PFOA and related chemicals by 95 percent by 2010 and to work toward eliminating emissions and product content by 2015. It is evident that the EPA and manufacturers of PFOA are in agreement that PFOA is a persistent environmental contaminant that poses a potential health risk to the human population.

The pathways by which the tumor triad is produced by PFOA exposure in rodents is still not clear. It is evident that PPAR agonism plays a role in liver adenomas yet the

MOA for tumor production in the rodent testes and pancreas is still unknown. Although current data supports that the PPAR agonistic properties of PFOA may not be applicable to human risk, since humans show little PP, several additional rodent tumorigenic pathways presented in this thesis may be applicable to humans. Current research supports that exposure to PFOA results in the generation of ROS in humans and ROS and oxidative stress have been shown to play a role in tumor production in humans. PFOA has been shown to interfere with GJIC by inhibiting GJIC via the ERK pathway which has been shown to be a key pathway of carcinogenesis in humans. The alteration of the immune system by PFOA appears to result in a weakened immune system. A weakened or suppressed immune system has been associated with increased tumor development. Increased estradiol levels were found in an epidemiologic study by Olsen described previously in this thesis and PP chemicals such as PFOA have been shown to increase estradiol levels. This modulation of estradiol has been demonstrated to play a role in LC tumor formation in humans. The activation of the CAR pathway by PFOA has also been discussed in this thesis and a subset of the human population that highly express CAR may be susceptible to tumor formation as a result of CAR activation by PFOA. It is conceivable that each of these pathways may represent pathways of PFOA carcinogenesis in humans.

The global presence and environmental persistence of PFOA, combined with the long half life of PFOA in humans (4.37 years [160]), warrants careful consideration as to the proper handling, use and disposal of this chemical. With little epidemiological studies conducted, most of which have been performed by industries that manufacture PFOA, and given that PFOA is accumulating in not only the occupationally exposed, but

also in the general population is of concern. The ubiquitous presence of PFOA in the environment compounded with the possibility that viable carcinogenic pathways exist in experimental animals that are mirrored in human carcinogenesis reflect a need to further evaluate the carcinogenic potential of PFOA in humans and the need for continued epidemiological studies of the human population.

VII. REFERENCES

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