Evidence on the Male Reproductive Toxicity of Perfluorodecanoic Acid (PFDA) and Its Salts

Developmental and Reproductive Toxicant Identification Committee

December 14, 2021

Reproductive Toxicology and Epidemiology Section
Reproductive and Cancer Hazard Assessment Branch
Office of Environmental Health Hazard Assessment, CalEPA

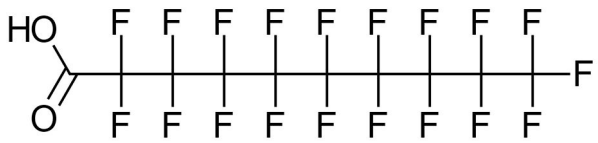
CalEPA
California Environmental Protection Agency

Outline

- Introduction
- Pharmacokinetics of PFDA
- PFDA and its salts: Male reproductive toxicity data
  - Animal studies
  - Human studies
  - Mechanistic data
  - Key characteristics of male reproductive toxicants and endocrine-disrupting chemicals
  - Summary

Introduction: PFDA

- PFDA (and its salts) is a perfluorinated organic compound with surfactant properties.
- A per- and polyfluoroalkyl substance (PFAS).



PFDA ($C_{10}HF_{19}O_2$)

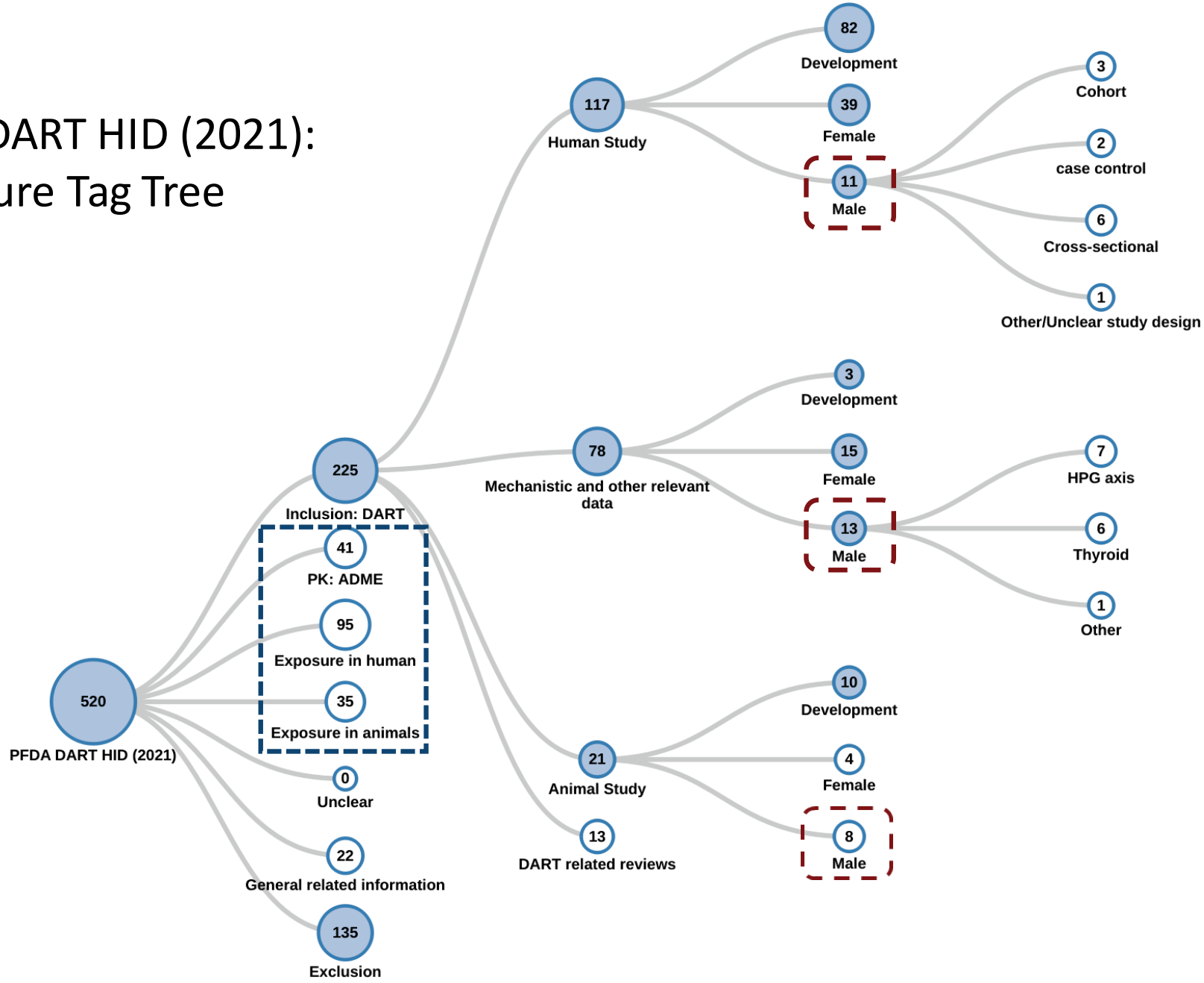
Uses, Occurrence, and Exposure

- Commonly used to make products resistant to stains, grease, soil, and water.
- Used in cosmetic products: Has been detected in creams, concealers, foundations, and body lotions.
- No data available on production or emissions
- PFDA is pollutant of air, water, soil, and wildlife, and persistent in the environment.
- Detection frequency in Biomonitoring California studies (2010 – 2019):
    - PFDA: 65.9% - 100%

Literature search and screening

- OEHHA conducted literature searches on the developmental and reproductive toxicity of PFDA and its salts.
- OEHHA used the HAWC (Health Assessment Workspace Collaborative) as a tool for multi-level screening of literature search results.
- Focused on literature relevant to male reproductive toxicity. These citations underwent Level 2 (full text) screening.

PFDA DART HID (2021): Literature Tag Tree



Pharmacokinetics of PFDA

Absorption

- PFDA is well absorbed.

Distribution in humans

- PFDA: brain >>> lung and kidney
- PFDA has been detected in semen, cord serum, fetal tissues, and breast milk.

Estimated half-life	PFDA
Humans	7.1 years
Rodents	36-109 days

Metabolism

- PFDA is not known to be metabolized in animals or humans.

Excretion

- Urine, feces, nails and hair

PFDA and Its Salts: Animal Studies

Animal Studies on PFDA

Reference	Animals & Age	# of animals per group	Dosing methods	Doses (mg/kg)	Assessment
NTP 2019	Rat, SD, adult	10	Daily gavage, for 28 days	0, 0.156, 0.312, 0.625, 1.25, 2.5	24 hrs after last dosing
Olsen & Andersen 1983	Rat, F344, adult	6	Single i.p. injection	0, 50	2, 4, 8, and 16 days after injection
George & Andersen 1986	Rat, F344, adult	6	Single i.p. injection	0, 50	4, 8, 12, 16 and 30 days after injection
Bookstaff et al. 1990	Rat, SD, adult	10	Single i.p. injection	0, 20, 40, 80	7 days after injection
Van Rafelghem et al. 1987b	Mice, CD-1, adult	10	Single i.p. injection	0,150, 200 or 250	28 days after injection
Van Rafelghem et al. 1987b	Hamster, Syrian golden, adult	4	Single i.p. injection	0,50, 100, 200 or 400	16 days after injection
Van Rafelghem et al. 1987b	Guinea pig, Hartley, adult	3	Single i.p. injection	0,125, 150, or 175	14 days after injection

Effects on Reproductive Organ Weights

Rats

- **NTP 2019: SD, 9-11 weeks old SD rats; 28-day oral dosing**
  - Epididymis weight: ↓ dose-dependently; 3.8% (NS), 10.3% ($p<0.05$) and 33.1% ($p<0.05$) reduction at 0.625, 1.25 and 2.5 mg/kg-day, respectively
  - Testis weight: 11% ↓ at 2.5 mg/kg-day ($p<0.01$)

- **Olson & Andersen 1983: adult F344 rats; single i.p. injection (50 mg/kg), assessed on day 16**
  - Testis weight: 33% ↓ ($p<0.05$)

- **Bookstaff et al. 1990: adult F344 rats; single i.p. injection (20, 40 or 80 mg/kg), assessed on day 7**
  - Testis weight: ↓ at 80 mg/kg ($p<0.05$)
  - Seminal vesicles and ventral prostate weights: ↓ at all doses ($p<0.05$ or 0.01)

Effects on Reproductive Organ Weights (continued)

Mice, Hamsters, Guinea Pigs (Van Rafelghem et al. 1987b)

- **Adult CF-1 mice: single i.p. injection (150, 200 or 250 mg/kg), assessed on day 16**
  - Testis weight: “a slight reduction in testicular weights” (data not shown)
- **Adult Syrian golden hamsters: single i.p. injection (50, 100, 200 or 400 mg/kg), assessed on day 28**
  - Testis weight: “reduction in testicular weight” (data not shown)
- **Adult Hartley guinea pigs: single i.p. injection (125, 150, or 175 mg/kg), assessed on day 14**
  - Testis weight: “reduction in testicular weight”(data not shown)

Histopathology

Rats

- **NTP 2019:**

Incidence of histopathological lesions in rat testis and epididymis reported by NTP (2019)

PFDA Doses (mg/kg-day)	0	0.156	0.312	0.625	1.25	2.5
Group size	10	10	10	10	10	10
Interstitial cell atrophy	0/10	0/10	0/10	0/10	8/10**	10/10**
Spermatid retention	0/10	0/10	0/10	0/10	0/10	4/10*
Germ cell degeneration	1/10	0/10	0/10	0/10	0/10	4/10
Epididymal lesion	1/10	0/10	0/10	0/10	0/10	4/10

***p<0.05; **p<0.01**

- **George & Andersen 1986:** Germ cell degeneration 16 days after a single (50 mg/kg) i.p. injection
- **Bookstaff et al. 1990:** No degenerative changes in the testis. Epithelial atrophy in seminal vesicles (80 mg/kg) and ventral prostate (40 & 80 mg/kg) 7 days after a single i.p. injection

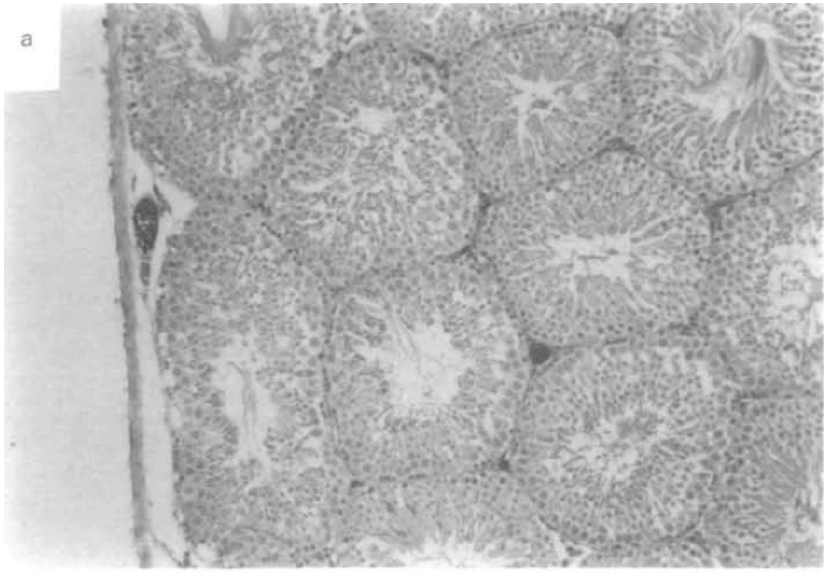
Histopathology (Continued)

Mice, Hamsters, Guinea Pigs

- **Van Rafelghem et al. 1987b:** Mild germ cell degeneration in Syrian golden hamsters (100 mg/kg) and Hartley guinea pigs (150 mg/kg) after a single i.p. injection. No degenerative changes in mouse testis 28 days after a single i.p. injection.

Testis histopathology from the study in hamsters by Van Rafelghem et al. 1987b

Cross-sections of seminiferous tubules with multiple layers of germ cells



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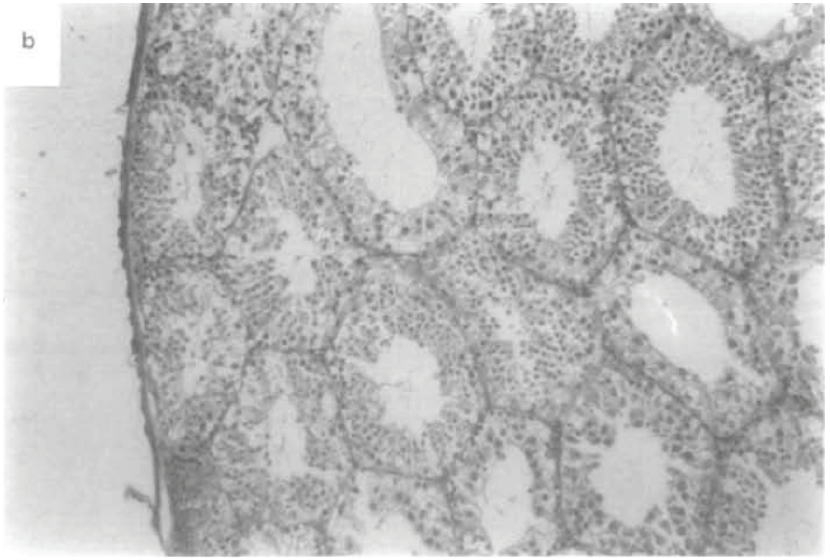

PFDA, 100 mg/kg, single i.p. injection

Germ cell degeneration & diminished layers of germ cells

Effects on Sperm Parameters

Rats: NTP 2019, 10-wks-old, 28-day oral dosing

Doses (mg/kg-day)	0	0.625	1.25	2.5
Testicular spermatid heads (10^6/testis)	230.3±12.5	208.2±8.8	181.6±10.7*	217.0±7.5
Epididymal sperm count (10^6/cauda epi.)	136.3±10.2	120.8±5.5	112.9±7.3	95.7±11.5*
Epididymal sperm motility (%)	85.7±0.7	85.5±1.0	84.1±0.7	76.2±7.8

*** $p<0.05$**

Hormonal Effects

- **Serum or plasma testosterone** (T)
  - ↓ in rats: 15% (NS), 64% ($p<0.05$), and 75% ($p<0.01$) at 0.625, 1.25, & 2.5 mg/kg/day, respectively (NTP, 2019)
  - ↓ in rats: single i.p. injection, 40 & 80 mg/kg ($p<0.05$) (Bookstaff et al. 1990)
  - No effect in zebrafish (Jo et al. 2014)
- **Plasma dihydrotestosterone (DHT)**
  - ↓ in rats: single i.p. injection, 40 & 80 mg/kg ($p<0.05$) (Bookstaff et al. 1990)
- **Plasma ratios of E2/T and E2/11-KT (11-ketotestosterone)**
  - ↑ in zebrafish: 1.0 mg/L ($p<0.05$); no effect on plasma E2, T, or 11-KT (Jo et al. 2014)
- **Serum LH**
  - No effect in rats (Bookstaff et al. 1990)

Break for Questions from the DARTIC

DARTIC Meeting - December 14, 2021

Male Reproductive Outcomes Examined in Epidemiologic Studies of PFDA

Epidemiologic Studies of PFDA and Male Reproductive Toxicity: Methods and Key Issues

- Study designs: cross-sectional, prospective cohort, case-control
- Low PFDA concentrations
- Low sensitivity (small differences between low and high exposures)
- Multiple PFAS and other chemical exposures

Male Reproductive Outcomes Examined in Epidemiologic Studies of PFDA

- Male reproductive function, including:
  - Reproductive and thyroid hormone concentrations
  - Sperm/semen quality
  - Sperm DNA integrity
  - *In vitro* fertilization (IVF) outcomes
- Developmental landmarks (anogenital distance [AGD])
- Prostate cancer

Reproductive Hormones

- Testosterone (T)
  - ↓ 13-15 year old boys (Zhou et al. 2016)
  - ↓ *Young men (median age 19 years) (*NS: β = -0.166 (-0.40, 0.07) nmol/L) (Joensen et al. 2013)
  - ↑ T (NS) in men whose partners had tubal infertility (Ma et al. 2021)
  - No association (Joensen et al. 2009)
- No consistent associations with other reproductive hormones or related proteins

  (Joensen et al. 2009; Joensen et al. 2013; Pan et al. 2021; Petersen et al. 2018; Zhou et al. 2016)

Semen Quality

- Sperm concentration, count, and morphology
  - Study with highest PFDA concentrations and variability (Ma et al. 2021):
    - ↓ sperm concentration in the 2nd (NS) and 3rd exposure tertiles (24% reduction)
    - ↓ (NS) sperm count in 3rd exposure tertile
    - ↓ (NS) % with normal morphology in the 2nd and 3rd exposure tertiles
  - ↑ % with normal morphology (Louis et al. 2015)
  - Other studies: no associations (Joensen et al. 2013; Louis et al. 2015; Pan et al. 2019; Petersen et al. 2018)

Semen Quality (continued)

- Sperm motility
  - ↓ Progressive motility (Pan et al. 2019; Joensen et al. 2013 (NS))
  - ↓ Straight line velocity (Pan et al. 2019)
- Sperm DNA integrity: 2 studies, mixed findings
  - ↑ % of sperm with high DNA stainability (HDS) and ↑ DNA fragmentation index (DFI) (Pan et al. 2019)
  - No changes in HDS or DFI (Louis et al. 2015)
- IVF: no effect on rates of clinical pregnancies or live births (Ma et al. 2021)

PFDA: Mechanistic considerations and other relevant data

- ***Effects on the hypothalamic-pituitary-gonadal-(liver) axis***
- *Effects on the thyroid*

Endocrine Effects of PFDA

In Vivo

- Effects on reproductive hormones in humans and whole animals were presented earlier.

- In zebrafish, up-regulated transcription levels of cyp19a in male gonads and brain at 1 mg/L (Jo et al. 2014)

In Vitro

JEG-3 cells (Human placental choriocarcinoma cell line) (Kjeldsen & Bonefeld-Jørgensen 2013)

- Decrease in aromatase activity at 10^{-5}M (cytotoxic at 10^{-4}M)

Isolated rat Leydig cells (Boujrad et al. 2000)

- Inhibited hCG-stimulated T secretion at ≥ 10^{-5} M (increased T secretion at <10^{-5}M)

Endocrine Effects of PFDA (continued)

In Vitro

MA-10 cells (mouse Leydig tumor cell line) (Boujrad et al. 2000)

- Decreased hCG-stimulated progesterone secretion in a dose dependent manner
- Decreased hCG-stimulated pregnenolone secretion at all doses
- Decreased mRNA and protein levels of TSPO
- No effect on StAR protein levels or P450scc (cyp11A1) enzyme activity

mLTC-1 cells (mouse Leydig tumor cell line) (Zhao et al. 2017)

- Decreased progesterone production in a concentration dependent manner (IC50 11.52 µM)
  - Significant decrease in mitochondrial membrane potential at 25 and 50 µM.

- **H295R cells** (human adrenocortical carcinoma cell line) (Jo et al. 2014)
  - No significant effects on E2, T, or E2/T ratio (at concentrations of 0.1 to 100 mg/L for 48 h)

PFDA Effects on Sex Hormone Receptors: Expression, Binding, Activity

In Vivo

Zebrafish (Jo et al. 2014)

- Increased mRNA expression of *erα*, and *er2β* at 1 mg/L in male fish brain

Rainbow trout (Benninghoff et al. 2011)

- Concentration dependent increase in plasma Vtg levels in males

In Vitro

Human embryonic kidney cell line (HEK 293T) (Benninghoff et al. 2011)

- Increased hERα reporter activity up to 2.5 fold at 100 to 1000 nM

Trout liver cytosol (Benninghoff et al. 2011)

- Weak competitive binding to ERα

PFDA Effects on Sex Hormone Receptors: Expression, Binding, Activity (continued)

MVLN cells

- Did not induce an estrogenic response (up to 10^{-4}M) (Kjeldsen & Bonefeld-Jørgensen 2013; Juan Li et al. 2020)
- Inhibited the estrogenic response to E2 in a concentration dependent manner; reported EC50 of 20.3 µM (Juan Li et al. 2020)

MCF-7 cells (Juan Li et al. 2020)

- In cells co-treated with E2, downregulated expression of estrogen regulated genes (*TFF1* and *EGR3*) (at 50 µM)

PFDA Effects on Sex Hormone Receptors: Expression, Binding, Activity (continued)

CHO-K1 cells (Kjeldsen & Bonefeld-Jørgensen 2013)

- No AR agonist activity.
- Concentration-dependent antagonistic effects on DHT-induced AR transactivation (IC50 6×10^{-5}M)

In Silico

- Predicted to bind at the active site of human, mouse, and trout ERα (Benninghoff et al. 2011)
- Predicted to bind to the surface of the E2 activated form of hERα (Juan Li et al. 2020)

PFDA: Mechanistic considerations and other relevant data

- *Effects on the hypothalamic-pituitary-gonadal-(liver) axis*
- ***Effects on the thyroid***

PFDA Effects on Thyroid
Implications for Male Reproductive Toxicity

rT3 = reverse triiodothyronine; **TTR** = transthyretin, a serum transport protein that binds T4

- **Rat –** 28-day study: Adverse effects on male reproductive outcomes only at doses which also altered thyroid outcomes. (NTP 2019)
- **Rat –** Acute studies:
  - Single dose 75 mg/kg PFDA i.p. reduced total T3, T4, and rT3; Addition of supplemental T4 only partially restored total T4 in animals given PFDA. (Langley & Pilcher, 1985; Gutshall et al. 1988 & 1989)
  - Single doses of 20, 40 or 80 mg/kg PFDA i.p. reduced serum T4; 80 mg/kg PFDA led to increased serum T3 and decreased T3 uptake. (Van Rafelghem et al. 1987a)
- **In vitro**:
  - PFDA decreased proliferation in T3-dependent rat pituitary GH3 cells (Long et al. 2013)
  - PFDA inhibited 46% of ^{125}I-labeled T4 to human TTR, and displaced labeled T4 from binding sites on rat serum albumin. (Weiss et al. 2009; Gutshall et al. 1989; Ren et al. 2016)
- **In silico** – Molecular docking model found PFDA fit binding pocket of TTR. (Ren et al. 2016)

PFDA: Summary of Mechanistic Data

Effects on HPG axis:

- Alters hormone levels
  - ↓ T and DHT; no effect on LH in rats; ↑ plasma ratios of E2/T and E2/11-KT in zebrafish
  - ↓ hCG-stimulated pregnenolone, progesterone, and T secretion *in vitro* (rat and mouse Leydig cells)
- Affects gene and/or protein expression of steroidogenic factors and a steroidogenic enzyme:
  - ↓ mRNA and protein levels of TSPO *in vitro* (mouse Leydig tumor cells)
  - ↑ mRNA of aromatase in male zebrafish
  - ↓ Aromatase activity *in vitro* (human placental carcinoma cell line)
- Interacts with estrogen and androgen receptors
- Affects gene and protein expression of some hormone receptors:
  - ↑ era and erb in zebrafish brain

Effects on Thyroid homeostasis

- Interferes with thyroid hormone binding, serum levels, and function

PFDA: ***Key characteristics of male reproductive toxicants and endocrine-disrupting chemicals***

PFDA: KCs of Male Reproductive Toxicants and EDCs

Male Reproductive Toxicants

1. Alters germ cell development, function, or death

2. Alters somatic cell development, functions, or death

3. Alters production and levels of reproductive hormones

4. Alters hormone receptor levels/function

5. Is genotoxic

6. Induces epigenetic alterations

7. Induces oxidative stress

8. Induces inflammation

Endocrine Disrupting Chemicals (EDCs)

1. Interacts with or activates hormone receptors

2. Antagonizes hormone receptors

3. Alters hormone receptor expression

4. Alters signal transduction in hormone-responsive cells

5. Induces epigenetic modifications in hormone-producing or hormone-responsive cells

6. Alters hormone synthesis

7. Alters hormone transport across cell membranes

8. Alters hormone distribution or circulating hormone levels

9. Alters hormone metabolism or clearance

10. Alters fate of hormone-producing or hormone-responsive cells

Comparison: Animal Data on PFDA & PFNA

	PFDA (Oral Exposure & i.p. Injection)	Humans
Organ weight	↓ Epididymal weight in rats (≥1.25 mg/kg-day, oral) ↓ Testis weight in rats (2.5 mg/kg-day, oral; ≥50 mg/kg, i.p.) ↓ Seminal vesicles weight in rats (80 mg/kg, i.p.) ↓ Ventral prostate weight in rats (≥40 mg/kg, i.p.)	No data
Histopathology	↑ Interstitial cell atrophy in rats (≥1.25 mg/kg-day, oral) ↑ Spermatid retention in rats (2.5 mg/kg-day, oral) ↑ Germ cell degeneration in rats (2.5 mg/kg-day, oral, NS; 50 mg/kg, i.p.), hamsters (100 mg/kg, i.p.) & guinea pigs (150 mg/kg, i.p.) ↑ Epididymal lesions in rats (2.5 mg/kg-day, oral, NS) ↑ Epithelial atrophy of seminal vesicles & ventral prostate in rats (40 mg/kg, i.p.)	No data
Semen/Sperm	↓ Epididymal sperm count in rats (2.5 mg/kg-day, oral)	↓ Sperm concentration (in the study with the highest PFDA levels) ↓ Sperm count & motility
Hormones	↓ Serum testosterone in rats (≥1.25 mg/kg-day, oral; ≥40 mg/kg, i.p.)	↓ T in adolescents and young men
Reproductive performance	No data	No effect in 1 study
Development of male reproductive system	No data	Inconsistent findings in two studies (AGD)

Comparison: Animal Data on PFDA & PFNA

	PFDA (Oral Exposure & i.p. Injection)	PFNA (Oral Exposure)
Organ weight	↓ Epididymal weight in rats (≥1.25 mg/kg-day, oral) ↓ Testis weight in rats (2.5 mg/kg-day, oral; ≥50 mg/kg, i.p.) ↓ Seminal vesicles weight in rats (80 mg/kg, i.p.) ↓ Ventral prostate weight in rats (≥40 mg/kg, i.p.)	↓ Epididymal weight in rats (≥ 0.625 mg/kg-day) ↓ Testis weight in rats (≥ 1.25 mg/kg-day) and mice (NS) (2.0 and 5.0 mg/kg-day)
Histopathology	↑ Interstitial cell atrophy in rats (≥1.25 mg/kg-day, oral) ↑ Spermatid retention in rats (2.5 mg/kg-day, oral) ↑ Germ cell degeneration in rats (2.5 mg/kg-day, oral, NS; 50 mg/kg, i.p.), hamsters (100 mg/kg, i.p.) & guinea pigs (150 mg/kg, i.p.) ↑ Epididymal lesions in rats (2.5 mg/kg-day, oral, NS) ↑ Epithelial atrophy of seminal vesicles & ventral prostate in rats (40 mg/kg, i.p.)	↑ Interstitial cell atrophy in rats (2.5 mg/kg-day) ↑ Spermatid retention in rats (2.5 mg/kg-day) ↑ Germ cell degeneration in rats (2.5 mg/kg-day) & mice (0.5 mg/kg-day) ↑ Epididymal lesions in rats (2.5 mg/kg-day) ↑ Sertoli cell changes in rats (≥3 mg/kg-day)
Semen/Sperm	↓ Epididymal sperm count in rats (2.5 mg/kg-day, oral)	↓ Epididymal sperm counts in rats (≥1.25 mg/kg-day) & mice (0.5 mg/kg-day) ↓ Epididymal sperm motility and viability in mice (0.5 mg/kg-day)
Hormones	↓ Serum testosterone in rats (≥1.25 mg/kg-day, oral; ≥40 mg/kg, i.p.)	↓ Serum testosterone in rats (2.5 mg/kg-day) & mice (0.5 mg/kg-day) ↓ Intratesticular testosterone in mice (≥2 mg/kg-day)
Reproductive performance	No data	Interpretation limited by study design
Development of male reproductive system	No data	Delayed preputial separation; ↓ Intratesticular T level, steroidogenic proteins, PCNA levels (likely ↓ Sertoli cell proliferation) in mice