Evidence on the Male Reproductive Toxicity of Perfluorononanoic Acid (PFNA) 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

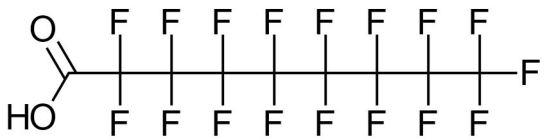
CalEPA
California Environmental Protection Agency

Outline

- Introduction
- Pharmacokinetics of PFNA
- PFNA 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: PFNA

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



PFNA ($C_9HF_{17}O_2$)

Uses, Occurrence, and Exposure

- Commonly used to make products resistant to stains, grease, soil, and water.
- Used as processing aids in fluoropolymer manufacture, e.g., ammonium PFNA comprises approximately 74% of the processing aid Surflon S-111.
- Used in cosmetic products. PFNA has been detected in creams, concealers, foundations, and body lotions.

- Production:
  - PFNA (1975 to 2004) = 800 - 2300 tons

- Emissions:
  - PFNA and ammonium PFNA (1975 to 2014) = 70 - 1400 tons

Uses, Occurrence, and Exposure (continued)

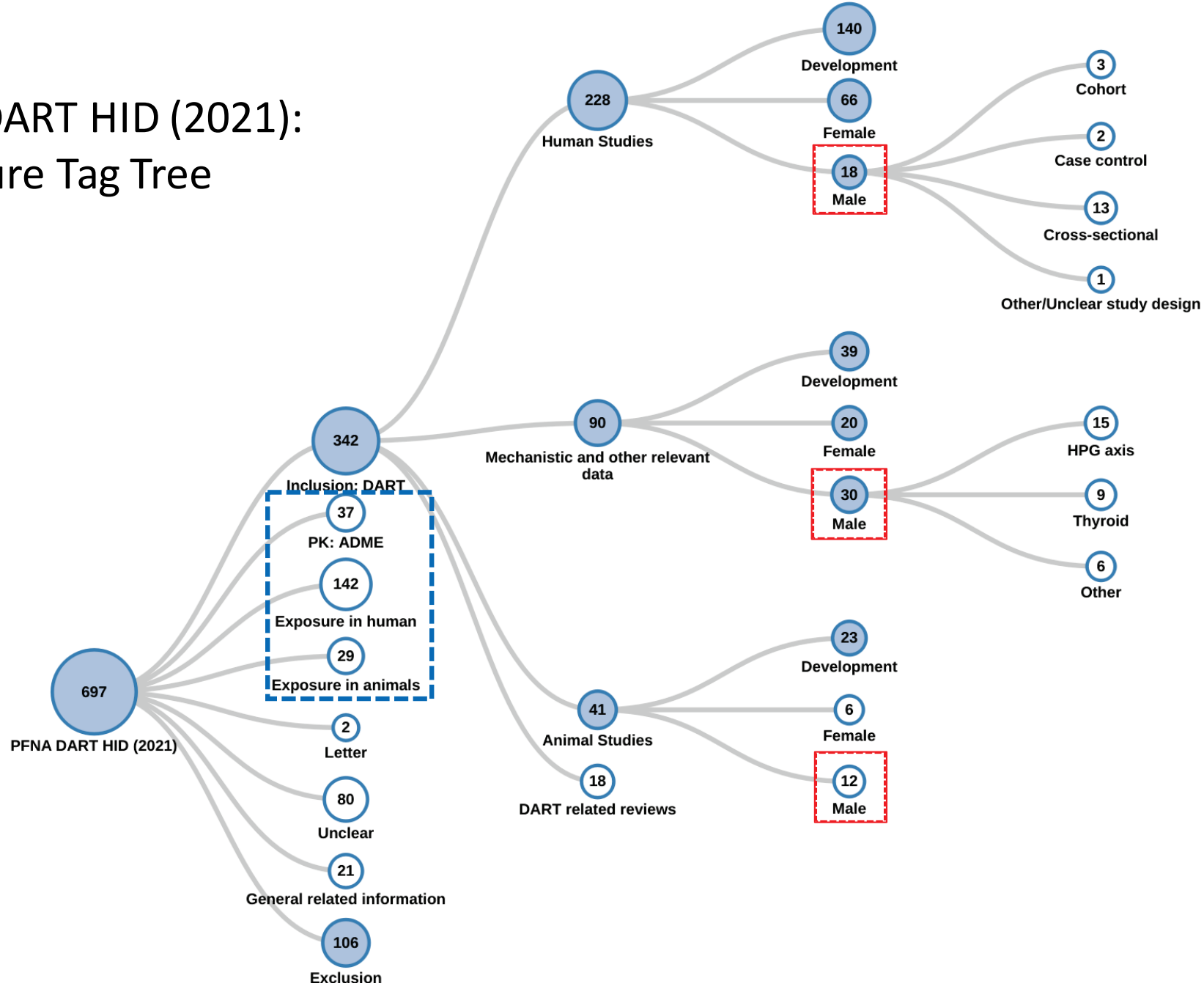
- PFNA is a pollutant of air, water, soil, and wildlife, and persistent in the environment.

- Detection frequency in Biomonitoring California studies (2010 – 2019):
  - PFNA: 92% - 100%

Literature search and screening

- OEHHA conducted literature searches on the developmental and reproductive toxicity of PFNA 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.

PFNA DART HID (2021): Literature Tag Tree



Pharmacokinetics of PFNA

Absorption

- PFNA is well absorbed.

Distribution in humans

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

Estimated half-life	PFNA
Humans	3.1 years
Rodents	30-55 days

Metabolism

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

Excretion

- Urine, feces, nails and hair

PFNA and Its Salts
Animal Studies

Outcomes Generally Assessed in Animal Studies for Male Reproductive Toxicity

Outcomes	Parameters
Organ Weights & Histopathology	Absolute organ weights; characterization and incidence of pathological changes in testis and other reproductive organs
Sperm Production and Quality	Testicular spermatid head count; semen or epididymal sperm number, concentration, viability, motility, morphology, DNA integrity, fertilization capacity
Hormonal Evaluation	Serum or testicular levels, biochemical and/or molecular evaluation of production/function (T, DHT, FSH, LH, MIS/AMH, etc.)
Reproductive Performance	Mating index, litter size, live pup numbers, survival rate
Development	Developmental landmarks (AGD, nipple retention, preputial separation), evaluation of testis and accessory glands

Animal Studies on PFNA

Reference	Animals, Age at start of experiment	No. of animals per group	Treatment (Oral Dosing, mg/kg-day)
Feng et al. 2009	SD rats, 8-wks-old	6	0, 1, 3, or 5 for 14 days
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Hadrup et al. 2016	Wistar rats, 7-wks-old	8-10	0, 0.125, 0.25, or 5.0 for 14 days
NTP 2019	SD rats, 10-wks-old	10	0, 0.625, 1.25, 2.5, 5.0 or 10.0 for 28 days
Singh & Singh 2019a	Parkes mice, 25-days-old	14	0, 0.2 or 0.5 for 90 days
Singh & Singh 2019b	Parkes mice, 25-days-old	10	0, 2 or 5 for 14 days
Singh & Singh 2019c	Parkes mice, 25-days-old	10	0, 2 or 5 for 14 days
Singh & Singh 2019d	Parkes mice, pregnant	10	0, 2 or 5, gestational day (GD) 12 - birth.
Das et al. 2015	CD-1 mice, pregnant	8-10	0, 1, 3, 5 or 10, GD 1-17
Zhang et al. 2016	Zebrafish, 5-months-old	30	0, 0.01, 0.1 or 1.0 (mg/L for 180 days via tank water)

Effects on Reproductive Organ Weights

Rats

- **NTP 2019: 9-11 weeks old SD rats; 28-day oral dosing**
  - Epididymis weight: ↓ dose-dependently; 7.2% ($p<0.05$), 13.2% ($p<0.01$), and 34.6% ($p<0.01$) at 0.625, 1.25 and 2.5 mg/kg-day, respectively
  - Testis weight: ↓ 7% ($p<0.05$) and 20% ($p<0.01$) at 1.25 and 2.5 mg/kg-day, respectively
- **Feng et al. 2009 & 2010; Hadrup et al. 2016:** no data on organ weights

Effects on Reproductive Organ Weights (continued)

Mice

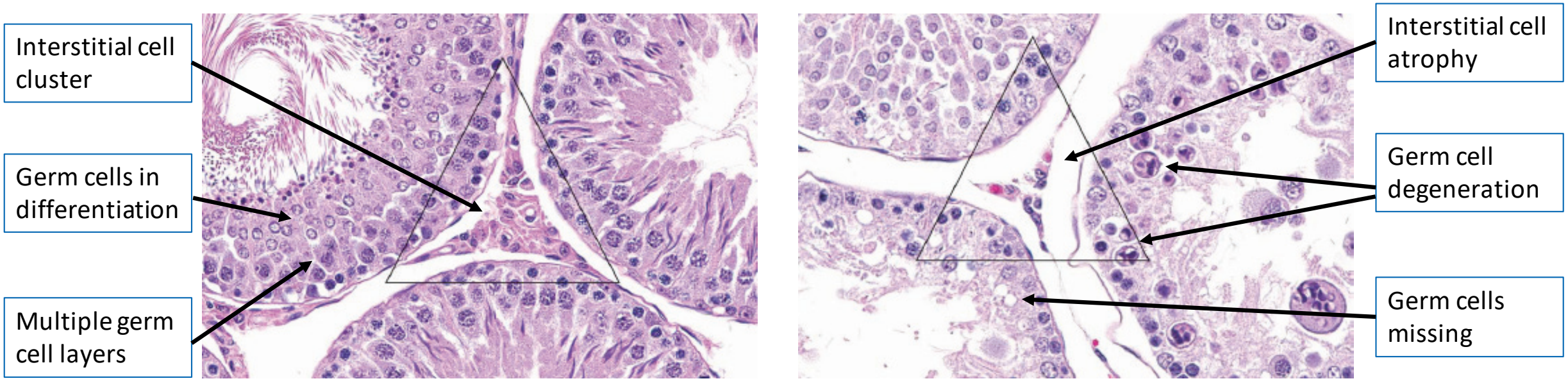
- **Singh & Singh 2019a: 25-days-old; 90-day oral dosing** (assessed at 105 days of age)
  - Testis weight (n = 7/group): 8% reduction at 0.5 mg/kg-day; not statistically significant (NS)
- **Singh & Singh 2019b: 25-days-old; 14-day oral dosing** (assessed at 39 days of age)
  - Testis weight (n = 5/group): 14% and 20% reduction at 2.0 and 5.0 mg/kg-day, respectively; NS

Large inter-animal variation of testis weight at pubertal age requires large group size

Histopathology: Rats

- **NTP 2019:** ↑ Incidences of germ cell degeneration, interstitial cell atrophy, spermatid retention and epididymal lesions at ≥2.5 mg/kg-day for 28 days ($p < 0.01$)

Testis Histopathology from the study in rats by NTP 2019



Control **PFNA, 2.5 mg/kg-day**

Histopathology (Continued)

Rats (pubertal)

- **Feng et al. 2009:**
  - Germ cell degeneration and sloughing of seminiferous epithelium at 5 mg/kg-day for 14 days
  - ↑ Apoptosis of spermatocytes and spermatogonia at ≥3 mg/kg-day for 14 days ($p<0.01$)
- **Feng et al. 2010:**
  - ↑ Vacuoles between Sertoli cells at ≥3 mg/kg-day for 14 days
  - ↑ Cytoplasmic vacuolization in Sertoli cells at 5 mg/kg-day for 14 days

Mice (pubertal or adult)

- Germ cell degeneration at 0.5 mg/kg-day for 90 days ($p<0.05$) (Singh & Singh 2019a)
- Germ cell degeneration at ≥2 mg/kg-day for 14 days ($p<0.05$) (Singh & Singh 2019b)
- Changes in germ cell population sizes at 5 mg/kg-day for 14 days ($p<0.05$) (Singh & Singh 2019c)

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)	230.2±10.7	192.5±7.1*	220.8±10.8	205.1±9.5
Epididymal sperm count (10^6/cauda epi.)	142.3±9.4	136.2±7.9	116.0±6.3*	98.1±9.0**
Epididymal sperm motility (%)	85.2±0.5	85.9±0.4	86.4±0.75	86.4±0.7

**** p<0.05, **p<0.01***

Mice: Singh & Singh 2019a, 25-days-old, 90-day oral dosing

Doses (mg/kg-day)	0	0.2	0.5
Epididymal sperm count (10^6/cauda epi.)	13.52±1.15	11.63±1.18	8.5±0.4*
Epididymal sperm motility (%)	90.8±2.58	85.4±4.52	66.8±5.82*
Epididymal sperm viability (%)	0.62±0.04	0.48±0.09	0.22±0.02*

**** p<0.05, **p<0.01***

Effects on Testosterone

Serum testosterone (T)

- In rats:
  - ↓ Adult: 2.5 mg/kg-day ($p<0.01$) (NTP 2019)
  - ↓ Pubertal: 5.0 mg/kg-day ($p<0.01$) (Feng et al. 2009)
    - ↑ At 1 mg/kg-day ($p<0.01$), no effect at 3 mg/kg-day
- In mice:
  - ↓ Adult: 0.5 mg/kg-day after 90-day exposure ($p<0.05$) (Singh & Singh 2019a)
  - ↓ Pubertal: 2 & 5 mg/kg-day after 14-day exposure ($p<0.05$) (Singh & Singh 2019b)
- ↑ Zebrafish at 0.01 mg/L ($p<0.01$), no effect at 0.1 or 1.0 mg/L (Zhang et al. 2016)

Intratesticular T

- ↓ Pubertal mice: 2 & 5 mg/kg-day after 14-day exposure ($p<0.05$) (Singh & Singh 2019b)

Effects on Other Hormones

- **Serum Müllerian inhibiting substance (MIS)**
  - ↑ Rats at 5 mg/kg-day ($p<0.05$) (Feng et al. 2010)
- **Serum Estradiol (E2)**
  - ↑ Rats at 5 mg/kg-day ($p<0.01$) (Feng et al. 2009)
- **Serum Inhibin B**
  - ↓ Rats: dose-dependently at 1, 3, & 5 mg/kg-day ($p<0.01$) (Feng et al. 2010)
- **Serum FSH or LH**
  - No effect in rats at 1, 3, or 5 mg/kg-day (Feng et al. 2009)

Effects on Reproductive Performance

- **Singh & Singh 2019a: 90-day dosing in mice**
  - ➢ ↓ litter size at 0.5 mg/kg-day ($p<0.05$)
  - * *Limited information on study design for fertility evaluation*
- **Zhang et al. 2016: 180-day treatment in zebrafish**
  - ➢ ↓ hatching rate (12% at 0.01 and 1.0 mg/L ($p<0.05$), no effect at 0.1 mg/L)
  - ** Both male and female animals were exposed to PFNA*

Effects on Development of the Male Reproductive System

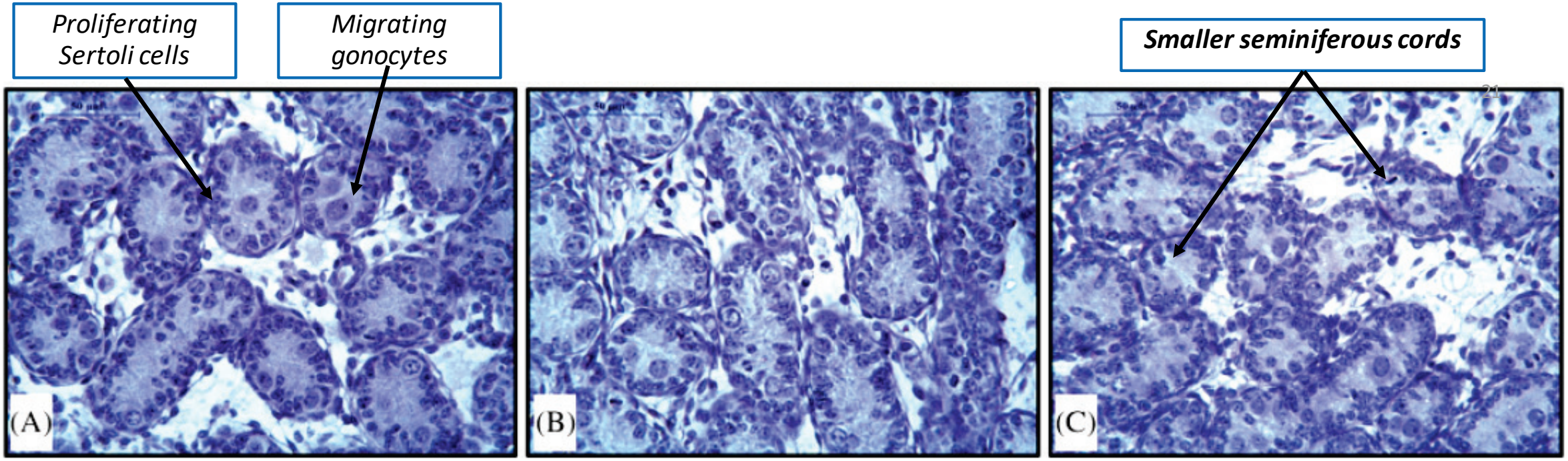
- **Das et al. 2015: CD-1 mice, daily gavage from GD 1 to 17**
  - **Preputial separation:** delayed by 2 days (at 3 mg/kg-day) ($p<0.05$) or 5 days (at 5 mg/kg-day) ($p<0.05$)

- **Singh & Singh 2019d: Parkes mice, daily gavage from GD 12 to birth, evaluation in neonatal mice on postnatal day 3.**
  Endpoints examined: Testis weight, histopathology, testicular PCNA protein, intratesticular testosterone concentration, & regulatory proteins or enzymes involved in steroidogenesis (Results shown on slides 21 & 22)

Effects on Development of the Male Reproductive System (continued)

Singh & Singh 2019d: Parkes mice, daily gavage from GD 12 to birth

- **Histopathology:** ↓ diameter of seminiferous cords, *indicating ↓ number of Sertoli cells per cord*
- **Testis weight:** about 20-30% ↓ at 2 and 5 mg/kg-day (NS), *indicating ↓ number of Sertoli cells*
- **Testicular PCNA protein:** ↓ dose-dependently ($p<0.05$ at 5 mg/kg-day), *indicating ↓ Sertoli cell proliferation*



Control ***PFNA, 2 mg/kg-day*** ***PFNA, 5 mg/kg-day***

Effects on Development of the Male Reproductive System (continued)

Singh & Singh 2019d: Parkes mice, daily gavage from GD 12 to birth

- **Intratesticular testosterone concentration:**
  - ↓ (about 35%) at 5 mg/kg-day ($p<0.05$)
- **Regulatory proteins or enzymes involved in steroidogenesis: :**
  - ↓ StAR protein expression at 2 and 5 mg/kg-day ($p<0.05$);
  - ↓ SF1, CYP11a (P450scc), 3β-HSD, and 17β –HSD at 5 mg/kg-day ($p<0.05$)

Break for Questions from the DARTIC

DARTIC Meeting - December 14, 2021

Male Reproductive Outcomes Examined in Epidemiologic Studies of PFNA

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

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

Male Reproductive Outcomes Examined in Epidemiologic Studies of PFNA

- 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 and prostate-specific antigen (PSA)

Reproductive Hormones

- Testosterone (T)
  - Serum and semen PFNA associated with reduced serum testosterone in four studies
    - 6-9 year old pre-pubescent boys in an area with PFOA-contaminated water (Lopez-Espinoza et al. 2016)
    - 13-15 year old boys (Zhou et al. 2016)
    - *Young men (median age 19 years) (*Non-significant [NS]: β = -0.059 (-0.118, 0.001) nmol/L) (Joensen et al. 2013)
    - Men (strongest association for < 30 year olds) who visited a reproductive medical center (Cui et al. 2010)
  - Other studies reported no associations or inconsistent results (Joensen et al. 2009; Toft et al. 2012, Lewis et al. 2015; Petersen et al. 2018, Ma et al. 2021; Specht et al. 2012).
- No consistent associations with other reproductive hormones or related proteins

Thyroid Hormones

Cross-sectional study of US National Health and Nutrition Examination Survey (NHANES) data

- 16.3% (95% CI 4.0, 30.2) higher serum concentration of thyroid stimulating hormone (TSH) in 12 to < 20-year-olds
- No associations with other serum thyroid hormones (i.e., free and total T3 [triiodothyronine] and T4 [thyroxine]) (Lewis et al. 2015)

Semen Quality

- Sperm concentration and count
  - Study with highest PFNA concentrations and variability (Ma et al. 2021):
    - Substantial, dose-dependent reduction in sperm concentration in the 2nd and 3rd tertiles; 25% reduction associated with 3rd tertile
    - Reduction (NS) in sperm count (p-trend = 0.05)
  - Other studies with lower PFNA levels: no associations (Joensen et al. 2013; Louis et al. 2015; Pan et al. 2019; Petersen et al. 2018; Toft et al. 2012)
- Sperm morphology and/or motility: inconsistent findings (Joensen et al. 2013; Louis et al. 2015; Ma et al. 2021; Pan et al. 2019; Petersen et al. 2018; Specht et al. 2012; Toft et al. 2012)

Semen Quality (continued)

- Sperm DNA integrity: 2 studies, mixed findings
  - ↑ % sperm with high DNA stainability (Pan et al. 2019)
  - ↑ DNA fragmentation index in one study (Pan et al. 2019), no effect in another (Specht et al. 2012)
- IVF: no effect on rates of clinical pregnancies or live births (Ma et al., 2021)

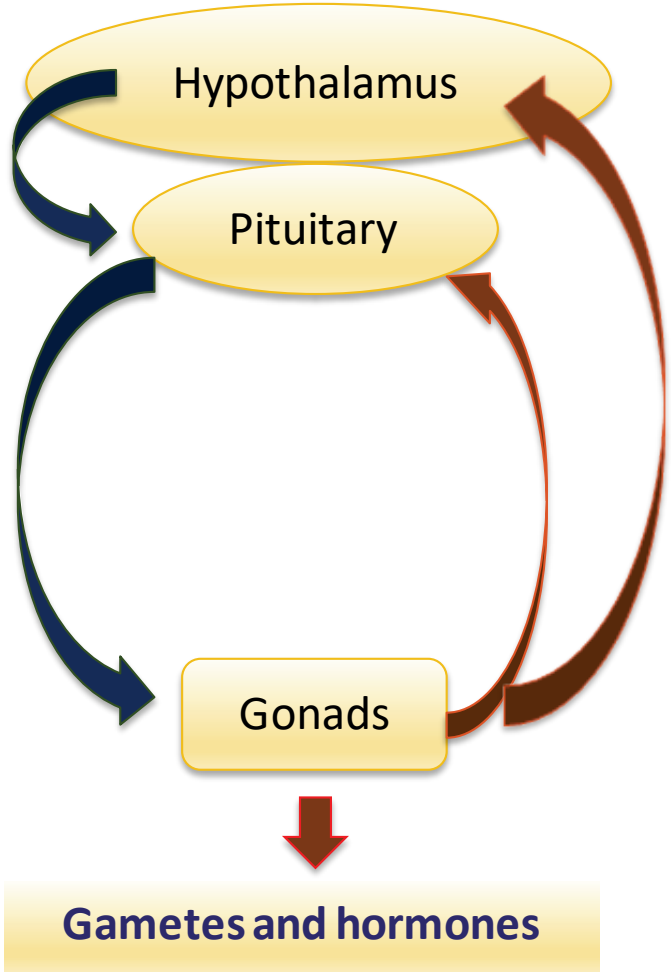
PFNA: Mechanistic considerations and other relevant data

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

Endocrine Control of the Reproductive System: The Hypothalamic-Pituitary-Gonadal-Axis (HPG axis)

Gonadotropin releasing hormone (GnRH)

Luteinizing hormone (LH)
Follicle stimulating hormone (FSH)



Testosterone (T)
Inhibin B
Müllerian inhibiting substance (MIS)
[also known as Anti-Müllerian hormone (AMH)]

Endocrine Effects of PFNA

In vivo

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

In vitro

Rat primary Sertoli cell cultures (Feng et al. 2010)

- Increased MIS mRNA levels at 10, 25, 50, and 75 µM
- Decreased inhibin B mRNA levels at 50 and 75 µM

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

- Concentration-dependent decrease in progesterone production (IC50 16.61 µM)

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

Mice (Singh & Singh 2019c)

- Reduced mRNA of androgen receptor at 2 or 5 mg/kg-day for 14 days

Zebrafish (Zhang et al. 2016)

- Reduced mRNA of:
  - *fshr* and *lhr* at 0.1 and 1.0 mg/L for 180 days
  - *erα* and *erβ* at 0.01 mg/L, and increased at 0.1 mg/L
  - *ar* at 0.1 and 1.0 mg/L
- Increased liver mRNA levels for *erα* and *erβ*

Rainbow trout liver cytosol (Benninghoff et al. 2011)

- Weak competitive binding to *erα*

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

In Vitro

Rat primary Sertoli cell (Feng et al. 2010)

- Reduced mRNA levels of FSH-R (25 and 50 µM)
- No effect on mRNA levels of AR (1 to 75 µM)

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

- No AR agonist activity
- Concentration-dependent antagonistic effects on DHT-induced AR transactivation

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

In Vitro

MVLN cells

- No effects on ER activity (Kjeldsen & Bonefeld-Jørgensen 2013; Juan Li et al. 2020)
- Inhibited the estrogenic response to E2 in a concentration dependent manner (Juan Li et al. 2020)

MCF-7 cells

- Downregulates expression of estrogen responsive genes in the presence of E2 (*TFF1* and *EGR3*) (Juan Li et al. 2020)

HEK-293T cells (Benninghoff et al. 2011)

- Induced hERα gene reporter activity (100 to 1000 nM)

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)

PFNA Effects on Regulatory Proteins Involved in Steroidogenesis

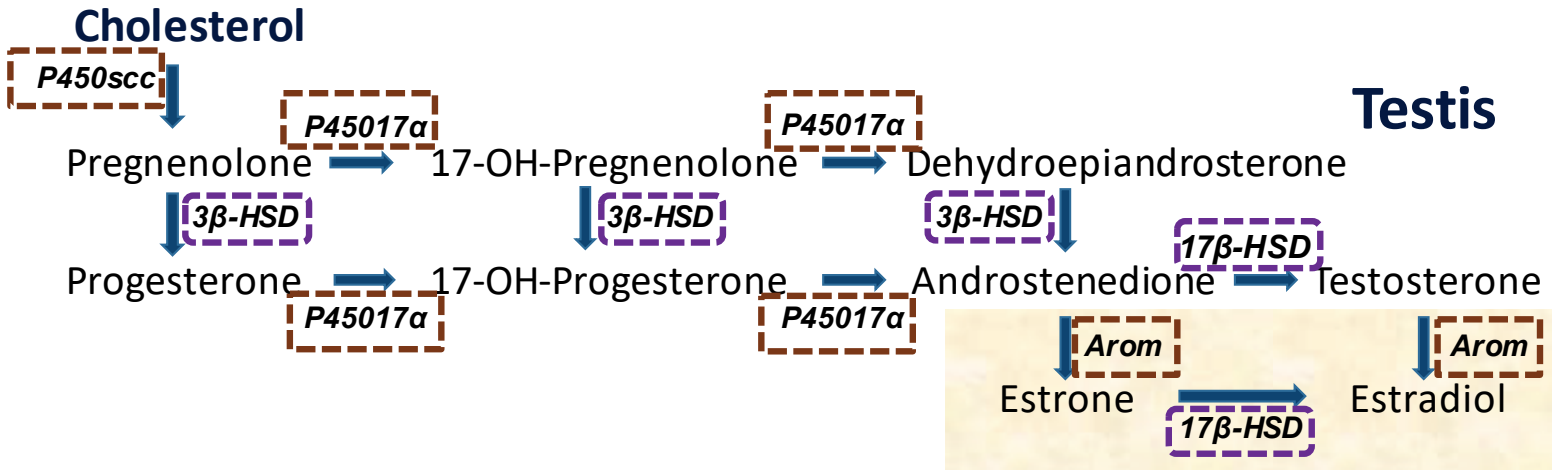
Reduce protein expression of steroidogenic factor 1 (SF1)

- Mice exposed for 14 days 2 and 5 mg/kg-day (Singh & Singh 2019b);
- Mice exposed *in utero* at 5 mg/kg-day (Singh & Singh 2019d)

Steroidogenic acute regulatory protein (StAR)

- Reduced mRNA in mice (Singh & Singh 2019a)
- Reduced StAR protein in mice (Singh & Singh 2019 a, b and d)
- Increased mRNA in zebrafish (Zhang et al. 2016)

Steroidogenesis Pathway



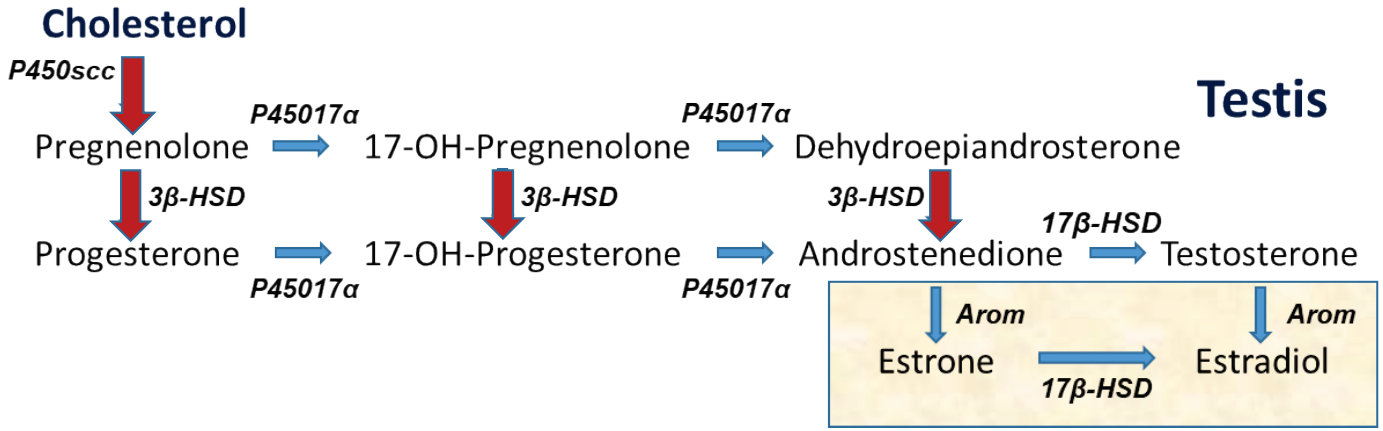
PFNA Effects on Steroidogenic Pathway

Cytochrome P450 family 11 subfamily A (CYP11a)= P450scc

- Reduced mRNA in mice (Singh & Singh 2019a)
- Reduced P450scc protein in mice (Singh & Singh 2019b)
- Increased mRNA in zebrafish (Zhang et al. 2016)

3β-Hydroxysteroid dehydrogenase (3β-HSD)

- Reduced mRNA and protein in mice (Singh & Singh 2019 a and b)
- Reduced mRNA in zebrafish (Singh & Singh 2019a)



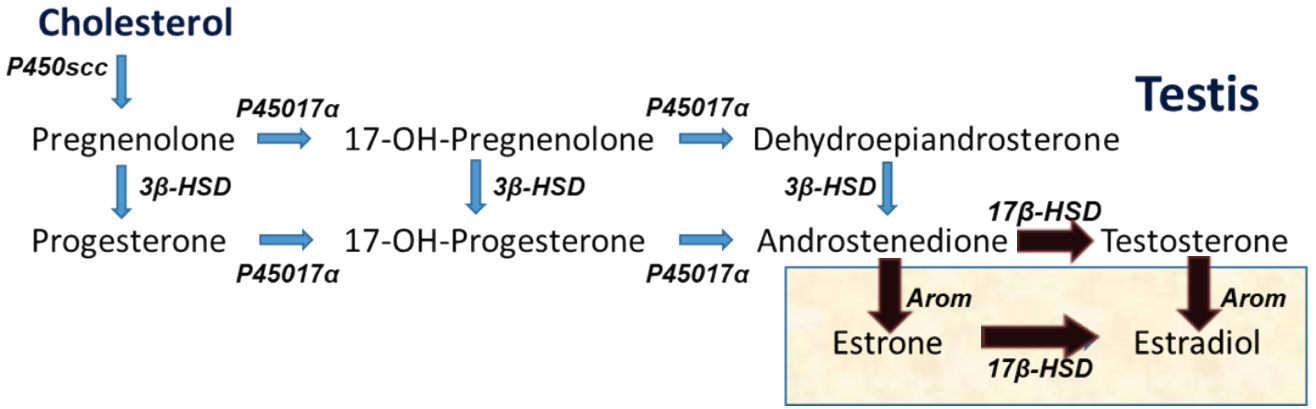
PFNA Effects on Steroidogenic Pathway (continued)

Cytochrome P450 family 17 (CYP17)= P450 17α

- Unclear in rats and no effects in zebrafish (Zhang et al. 2016)

17β-Hydroxysteroid dehydrogenase (17β-HSD)

- Reduced mRNA in mice (Singh & Singh 2019b)
- Reduced protein in mice (Singh & Singh 2019a)
- Increased mRNA in zebrafish (Zhang et al. 2016)



Cytochrome P450 family 19 subfamily A (*cyp19a*)= Aromatase

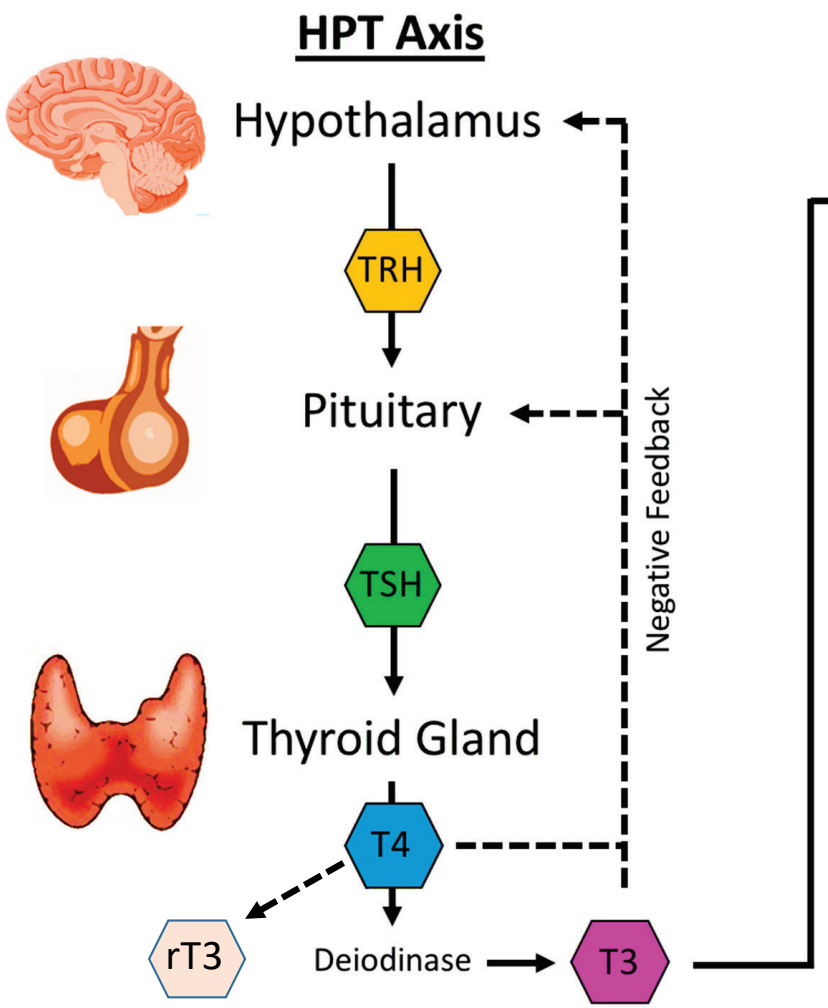
- Increased mRNA in zebrafish (Zhang et al. 2016)
- No effects on aromatase activity in JEG-3 cells (Kjeldsen & Bonefeld-Jørgensen 2013)

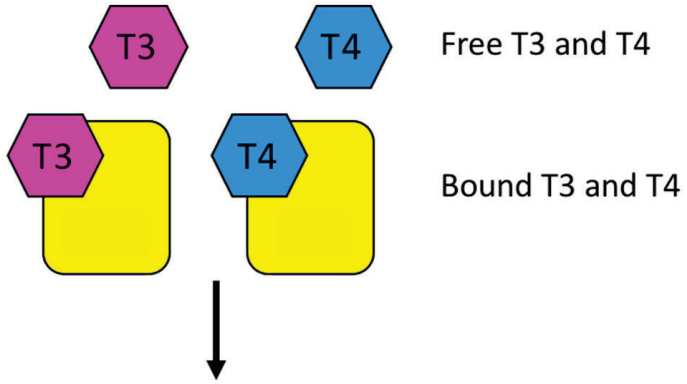
PFNA: Mechanistic considerations and other relevant data

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

Thyroid Hormones in Development and Function of the Male Reproductive System

T3 = tri-iodo-L-thyronine or triiodothyronine; **T4 =** tetra-iodo-L-thyronine or thyroxine





Effects of T3 on Testicular Function

- T3 binds directly to receptors on testicular Sertoli cells, which activates gene transcription, protein synthesis, and Sertoli cell proliferation and differentiation. Possible role in initiating sperm development.
- T3 may also be involved in stimulating basal testosterone generation.
- Short-term hypothyroidism in post-pubertal males can have adverse effects on semen quality.

Image adapted from: Gabrielson et al. Sex Med Rev 2019; 7:57-70.

PFNA Effects on Thyroid
Implications for Male Reproductive Toxicity

TTR = transthyretin, a serum transport protein that binds T4; **TSH** = thyroid stimulating hormone

- **Human** – No significant association between PFNA levels and serum testosterone. Significant increase in TSH only for adolescents. (Lewis et al. 2015)
- **Rat** – Adverse effects on male reproductive outcomes only at doses which also altered thyroid outcomes. (NTP 2019)
- **Zebrafish** – PFNA induced disruption of thyroid hormone transport, metabolism, synthesis and function. (Y Lui et al. 2011)
- **In vitro**:
  - PFNA bound to TTR and inhibited T4 binding (Weiss et al. 2009; Ren et al. 2016)
  - PFNA decreased proliferation in T3-dependent rat pituitary GH3 cells (Long et al. 2013)
- **In silico** – Molecular docking model found PFNA fit binding pockets of TTR and thyroxine-binding globulin. (Ren et al. 2016)

PFNA: Summary of Mechanistic Data

Effects on HPG axis

- Alters hormone levels
  - ↓ Testosterone, ↑ Serum E2, ↑ MIS *in vivo*; ↓ Progesterone production *in vitro*
- Induces changes in gene and/or protein expression of a number of enzymes and factors involved in steroidogenesis
- Interacts with estrogen and androgen receptor
- Affects gene and/or protein expression of some hormone receptors:
  - ↓ Testicular AR; ↓ FSHR and LHR in rodents
  - ↓ era, erb, ar; ↓ fshr, lhr, ↑ liver era and erb in zebrafish

Effects on Thyroid homeostasis

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

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

Key Characteristics

Proposed Key Characteristics of Male Reproductive Toxicants as an Approach for Organizing and Evaluating Mechanistic Evidence in Human Health Hazard Assessments

Xabier Arzuaga,[1] Martyn T. Smith,[2] Catherine F. Gibbons,[1] Niels E. Skakkebæk,[3] Erin E. Yost,[4] Brandiese E. J. Beverly,[5] Andrew K. Hotchkiss,[4] Russ Hauser,[6] Rodrigo L. Pagani,[7] Steven M. Schrader,[8] Lauren Zeise,[9] and Gail S. Prins[7,10]

OPEN

Consensus on the key characteristics of endocrine-disrupting chemicals as a basis for hazard identification

Michele A. La Merrill[1], Laura N. Vandenberg[2], Martyn T. Smith[3], William Goodson[4], Patience Browne[5], Heather B. Patisaul[6], Kathryn Z. Guyton[7], Andreas Kortenkamp[8], Vincent J. Cogliano[9], Tracey J. Woodruff[10], Linda Rieswijk[3,11], Hideko Sone[12], Kenneth S. Korach[13], Andrea C. Gore[14], Lauren Zeise[15] and R. Thomas Zoeller[16]*

Key Characteristics (KCs) of Male Reproductive Toxicants and Endocrine Disrupting Chemicals (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

PFNA: 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

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6. Induces epigenetic alterations

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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

Summary: Animal & Human Data on PFNA

	Animals *in vivo (Oral Exposure)*	Humans
Organ weight	↓ 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)	No data
Histopathology	↑ 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)	No data
Semen/Sperm	↓ 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)	↓ Sperm concentration (in the study with the highest PFNA levels)
Hormones	↓ Serum testosterone in rats (2.5 mg/kg-day) & mice (0.5 mg/kg-day) ↓ Intratesticular testosterone in mice (≥2 mg/kg-day)	↓ T in boys, adolescents, young men, and men <30 years)
Reproductive performance	Interpretation limited by study design	No effect in 1 study
Development of male reproductive system	Delayed preputial separation; ↓ Intratesticular T level, steroidogenic proteins, PCNA levels (likely ↓ Sertoli cell proliferation) in mice	Inconsistent findings in two studies (AGD)