EPA United States Environmental Protection Agency

Case study in the application of "systematic methods" to Adverse Outcome Pathway development: AOPs relevant to Ecological Effects of PFAS

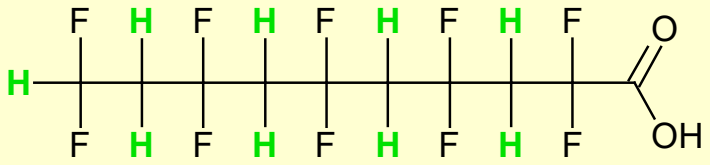
Dan Villeneuve, Gerald Ankley, John Hoang, Kathleen Jensen, Robin Kutsi, Ashley Kittelson, Michael Ellman, Jacob Collins, Jennifer Olker
Great Lakes Toxicology and Ecology Division
Duluth, MN

** Contents of this presentation neither constitute, nor necessarily reflect, U.S. EPA policy.*

Per- and Polyfluoroalkyl Substances (PFAS)

Thousands of PFAS in production of industrial and consumer products.

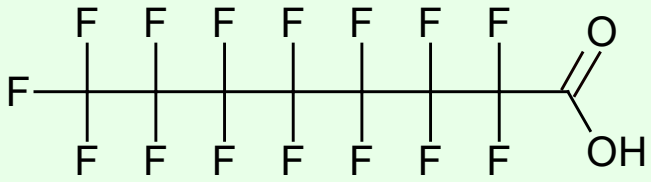
Poly fluorinated = many fluorines



Polyfluorinated carboxylic acid from the production of polyvinylidene fluoride (PVDF) plastic

Newton et al., 2017. Novel polyfluorinated compounds identified downstream of manufacturing facilities near Decatur, AL using high resolution mass spectrometry

Per fluorinated = fully fluorinated



Perfluorooctanoic acid (PFOA ,C-8)

Perfluorooctanesulfonate (PFOS)

Very stable (C-F bond energy 485 kJ/mol)
(C-C 346, C-N 305, C-O 358, C-Cl 327 kJ/mol)

Introduction to PFAS

A class of man-made chemicals that are ubiquitous due to:

- Wide variety of industrial and consumer uses
- Persistence
- High Mobility

They are a concern due to:

- Known or suspected toxicity, especially for PFOS and PFOA
- Bioaccumulation
- Some have very long half lives (several years), especially in humans
- Shorter PFAS tend to be highly mobile, longer PFAS less mobile

Information on PFAS is rapidly evolving.

EPA United States Environmental Protection Agency

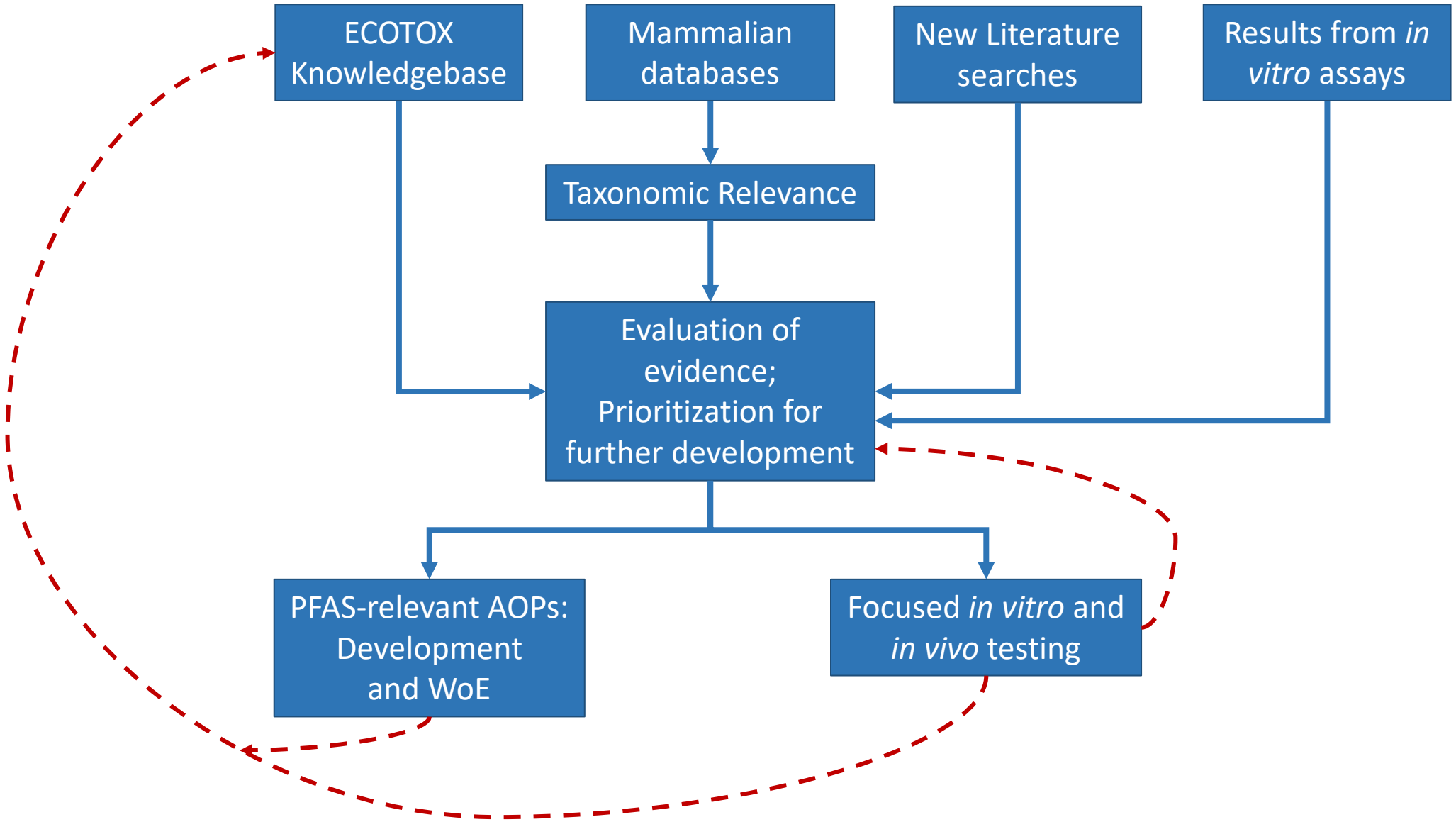
Ecological Effects of PFAS

- Initial emphasis on multiple human health endpoints (e.g., immune suppression, cancer, thyroid disease, etc.)
  - Drinking water and (more recently) dietary exposures

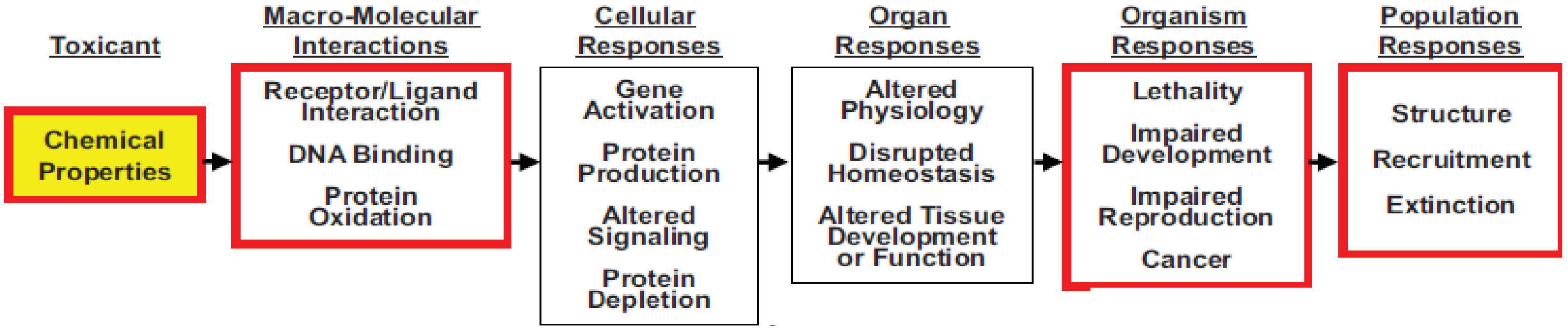
- Increasing attention on potential ecological effects
  - Detected in many ecosystems from point/nonpoint sources
  - Can be highly persistent and bioaccumulative (may biomagnify)
  - Some have significant toxicity potential
  - Large universe (100s/1000s?) of (mostly) poorly understood chemicals

- Recent activities worldwide focused on exposure to/possible ecological effects of PFAS
  - NAMs are being applied to rapidly screen PFAS for potential bioactivity
  - Need for AOPs to provide context in terms of apical hazards

Strategy

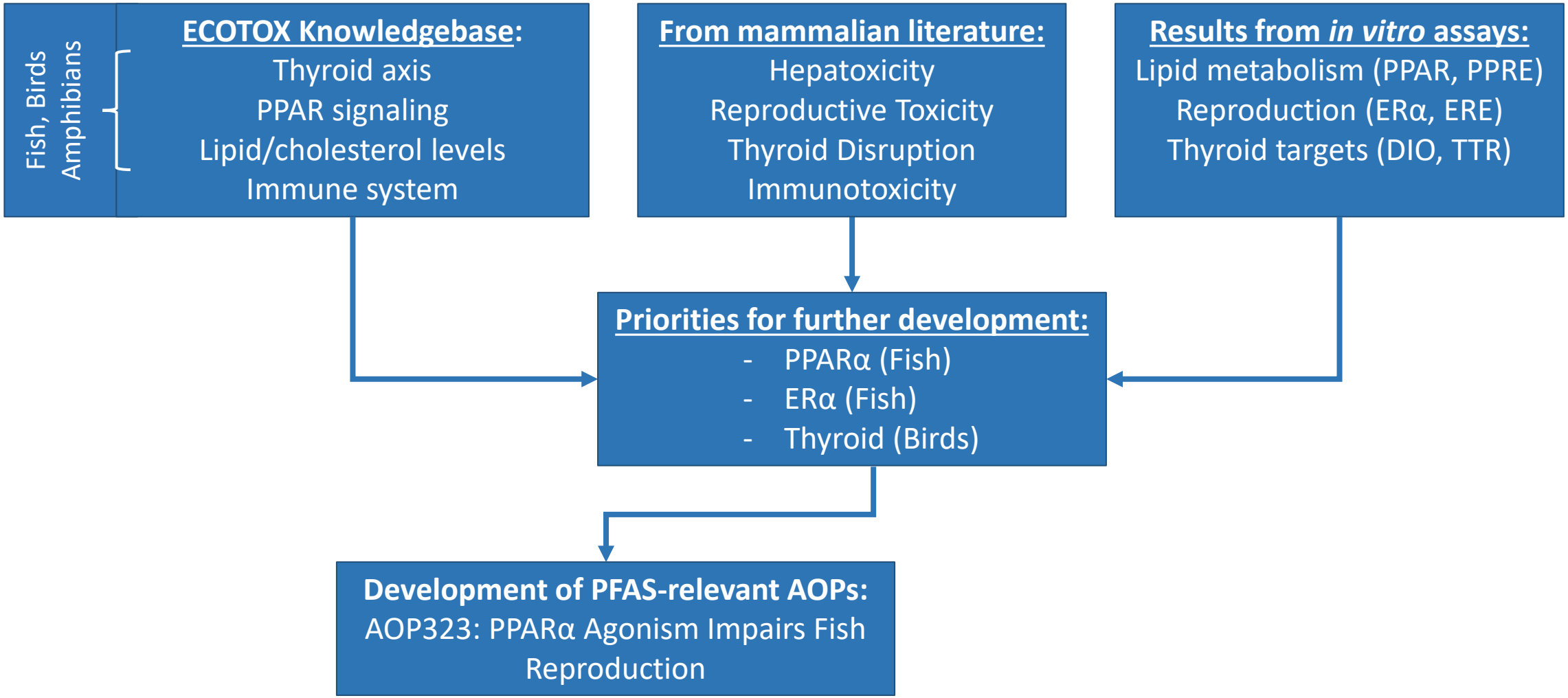

ECOTOX Knowledgebase
Mammalian databases
New Literature searches
Results from *in vitro* assays
Taxonomic Relevance
Evaluation of evidence; Prioritization for further development
PFAS-relevant AOPs: Development and WoE
Focused *in vitro* and *in vivo* testing

Sources of information for AOP development



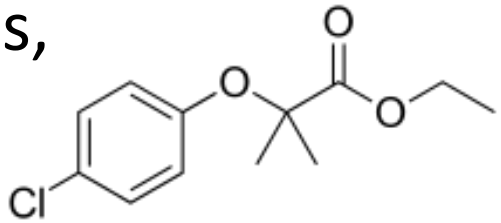
Source					
ECOTOX	?	✓	✓	✓	some
ToxREFDB		?	✓	✓	
HTP data: Attagene, ZFISH, Thyroid assays	✓			✓	
Open Literature	✓	✓	✓	✓	✓

Potential Pathways of PFAS Toxicity

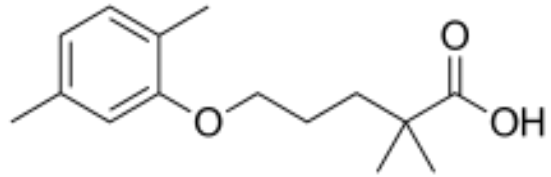


Peroxisome proliferator activated receptor alpha (PPARα)

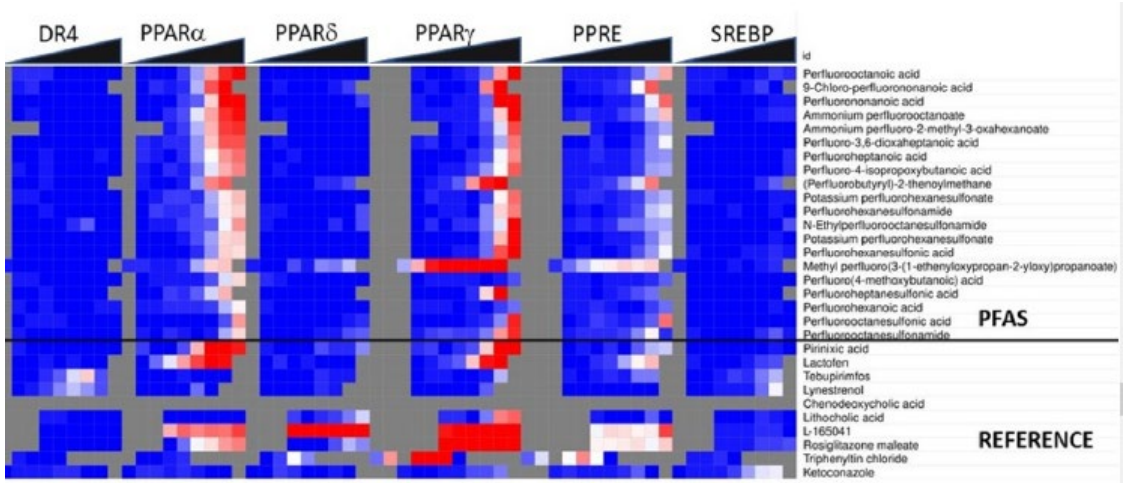
- Nuclear receptor
- Forms heterodimer with RXR & is activated through ligand binding
- Promotes transcription of many genes, including those involved in:
  - Inflammation & Immunity
  - Nutrient metabolism
  - Energy homeostasis
- Fibrate drugs activate PPARα to lower cholesterol
- Evidence multiple PFAS act via PPARα



Clofibrate



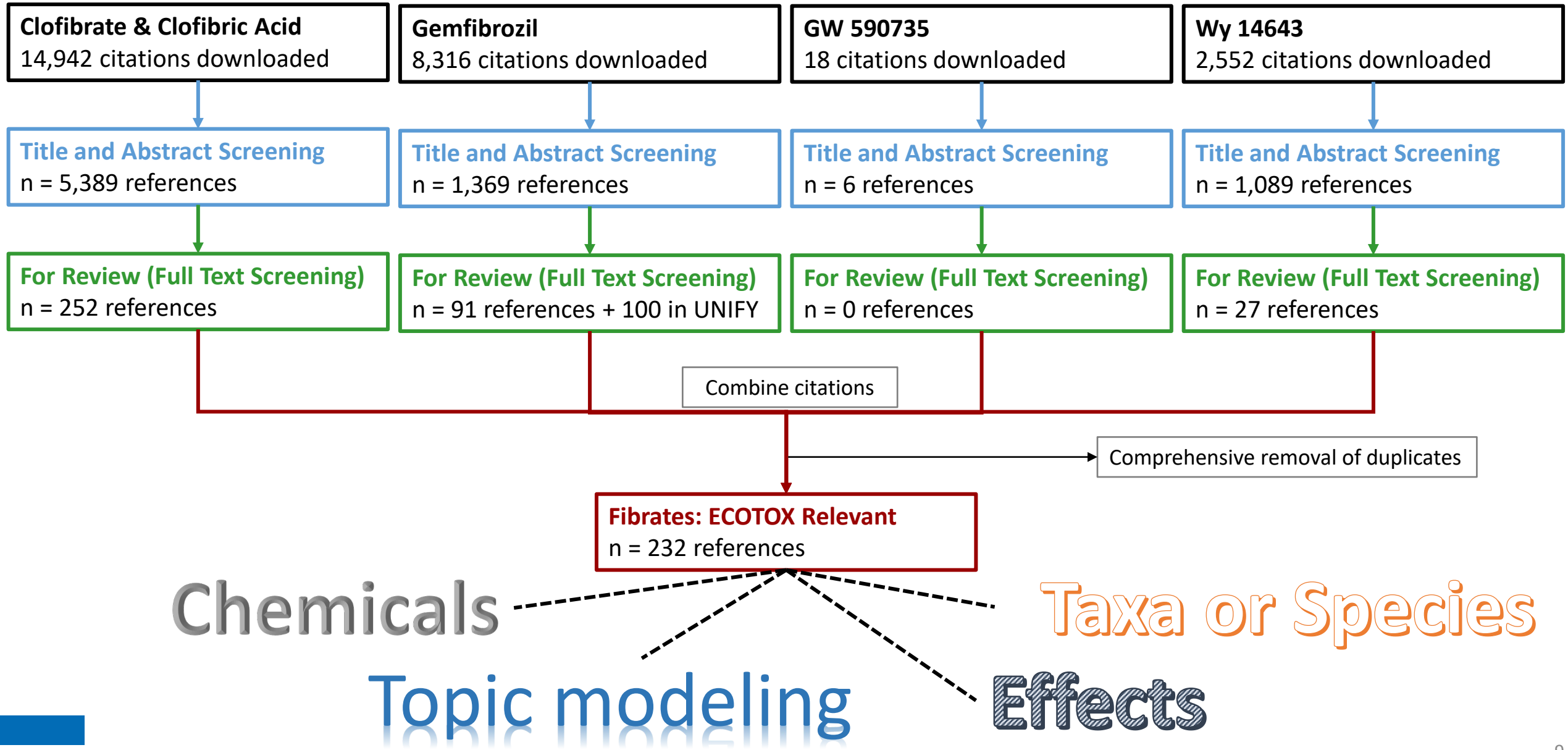
Gemfibrozil



Rakhshandehroo *et al*. 2010. doi:10.1155/2010/612089

Behr *et al*. 2020. doi: 10.1016/j.tiv.2019.104700; Houck et al. 2021. doi: 10.1016/j.tox.2021.152789.

Targeted Literature Search: Fibrates



EPA United States Environmental Protection Agency

AOP Draft

Molecular	Cellular	Tissue	Organ	Organism	Population
MIE: PPARα Agonism	Increase, Peroxisome Proliferation (Histology)	Decrease, Steroid Synthesis: 11-Keto-Testosterone, Estradiol, Testosterone (Radio-immunoassay, ELISA, Ex Vivo Steroidogenesis)	Decrease, Sperm Quality (Histology)	Decrease, Mass & Length	Increase, Sex Ratio of Males to Females
Increase, Expression of PPARα-Regulated Genes (qPCR, Microarray)	Increase, Fatty Acid β-Oxidation (TBARS Assay)		Delayed Oocyte Development (Histology)	Decrease, Egg Production	
	Decrease, Cholesterol (Commercial Assay Kit)			Increase, Male Aggression	

KER by KER Development Strategy Taken

- Google Scholar search for initial background information/key terms curation
- Literature Search via Abstract Sifter (https://doi.org/10.12688/f1000research.12865.1)
  - Objectivity
- Initial scan of abstracts to determine relevance
- Thorough read of relevant papers
- Organized relevant papers into concordance tables
- Evaluated by 3rd party
- Uploaded to AOP wiki

Targeted Literature Search: Fibrates

Clofibrate & Clofibric Acid	Gemfibrozil	GW 590735	Wy 14643
14,942 citations downloaded	8,316 citations downloaded	18 citations downloaded	2,552 citations downloaded

Query terms by KER or KE

- MIE 227: Activation, PPARα
- KER 2073
- KER 2072
- KE 807: Decreased, Cholesterol
- KE 1756: Decreased, 11-Ketotestosterone
- KER 2076
- TBD
- KE 1758: Impaired, Spermatogenesis
- AO: Decreased, Reproduction

PPAR AND activation AND cholesterol

sperm OR spermatogenesis OR spermiogenesis

Activation of PPAR alpha lowers synthesis and concentration of cholesterol by reduction of nuclear SREBP-2

Konig, B.; Koch, A.; Spielmann, J.; Hilgenfeld, C.; Stangl, G. I.; Eder, K.. *Biochemical Pharmacology (2007)*

▼Abstract

To elucidate the mechanisms underlying the cholesterol lowering effects of PPAR alpha agonists we investigated key regulators of cholesterol synthesis and uptake in rats and in the rat hepatoma cell line Fao after treatment with the PPAR alpha agonists clofibrate and WY 14,643, respectively. In rat liver as well as in Fao cells, PPAR alpha activation led to a decrease of transcriptionally active nuclear SREBP-2. mRNA concentrations of the key regulators of SREBP processing, Insig-1 in rat liver and Insig-1 and Insig-2a in Fao cells, were increased upon PPAR alpha activation. Thus we suggest, that the observed reduction of the amount of nuclear SREBP-2 was due to an inhibition of the processing of the precursor protein. Both, in rat liver and in Fao cells, mRNA concentrations of the SREBP-2 target genes HMG-CoA reductase (EC1.1.1.34) and LDL receptor were reduced after treatment with the PPAR alpha agonists. Furthermore, treatment of Fao cells with WY 14,643 reduced cholesterol synthesis. As a result, the amount of total cholesterol in liver, plasma and lipoproteins of clofibrate treated rats and in WY 14,643 treated Fao cells was decreased compared to control animals and cells, respectively. In conclusion, we could show a novel link between PPAR alpha and cholesterol metabolism by demonstrating that PPAR alpha activation lowers cholesterol concentration by reducing the abundance of nuclear SREBP-2. (c) 2006 Published by Elsevier Inc.

Comparison of the Effects of Clofibrate and Silafibrate on Sperm Parameters Quality and Sex Hormones in Male Rats

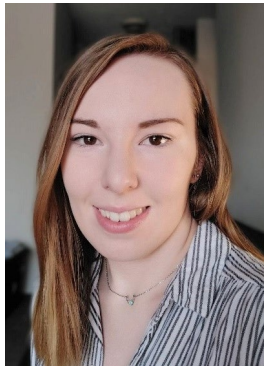
Delashoub, M.; Ziaee, M.; Khorrami, A.; Banan-Khojasteh, S. M.. *Urol J (2018)*

▼Abstract

PURPOSE: Fibrates are drugs widely used for the treatment of hyperlipidemic disorders. Previous studies on a novel analogue of clofibrate, called silafibrate, have shown good lipid lowering effects. This study was designed to assess the role of silafibrate as a peroxisome proliferator-activated receptors (PPARs) agonist on sperm health and spermatogenesis in adult male rats. MATERIAL AND METHODS: Seventy male Wistar rats were randomly allocated into 7 groups: Cl-10, Cl-20, and Cl-40 mg/kg/day (clofibrate); Si-10, Si-20, and Si-40 mg/kg/day (silafibrate); and C, control. After a 28-day treatment, all rats were euthanized. Blood samples were taken for determination of testosterone, total antioxidant capacity, levels of malondialdehyde, and oxidized low-density lipoprotein. Reproductive organs were dissected and spermatozoa collected from the epididymis for analysis. RESULT: Sperm parameters (count, motility, viability, and morphology) and total serum testosterone decreased significantly in clofibrate-treated (20 and

KER 2076

- KE 1756: Decreased, 11-Ketotestosterone
- KE 1758: Impaired, Spermatogenesis



Example – evidence for dose-response concordance

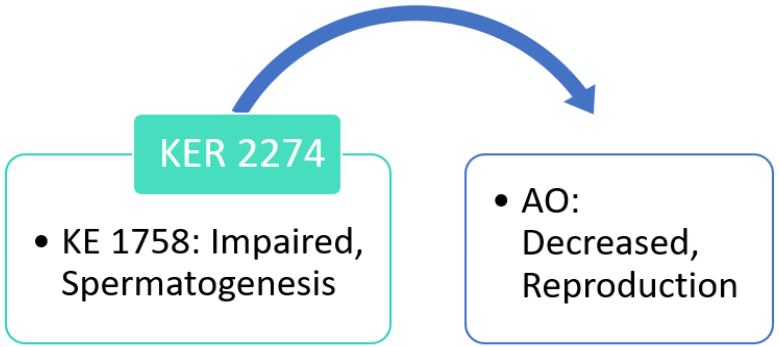
Species	Treatment Type	11-KT Treatment or Effect	Spermatogenesis Effect	11-KT Increase Observed?	Spermatogenesis Start/Increase Observed?	Citation
	pellets containing 30 µg/g body weight of OHA		control to about 50% stage 2 and 50% stage 3			
	Male catfish at beginning of spermatogenesis implanted with pellets containing 30 µg/g body weight of OA	Plasma 11-KT levels reached 2.4 ± 0.3 ng/ml after 2 weeks	Testicular stages changed from about 65% stage 1 and 35% stage 2 in the end control to about 55% stage 2 and 45% stage 3	Yes	Yes	
Atlantic salmon (*Salmo salar*)	Injected with 25 µg OA/g of body weight	After 7 and 14 days, 11-KT plasma levels significantly increased compared to control	5-fold higher number of type A differentiated spermatogonia than control fish after 14 days	Yes	Yes	Melo, M.C. et al. (2015)
Japanese eel (*Anguilla japonica*)	Testes were removed and cultured in medium with varying levels of 11-KT	0.01 ng/ml 11-KT for 15 days	No effect	Yes	No	Miura, T., et al. (1991)
		0.1 ng/ml 11-KT for 15 days	No effect	Yes	No	
		1 ng/ml 11-KT for 15 days	No effect	Yes	No	
		10 ng/ml 11-KT for 15 days	Mitosis occurred in 50-60% of cysts (as effective as 100 ng/ml 11-KT treatment)	Yes	Yes	

KER 2076

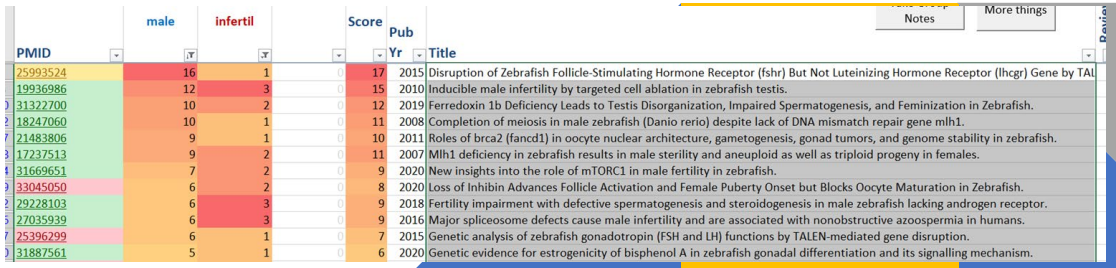
- KE 1756: Decreased, 11-Ketotestosterone
- KE 1758: Impaired, Spermatogenesis

Example – data that did not fit the expected patterns for a causal relationship

Species	Treatment Type	11-KT Effect	Spermatogenesis Effect	Decreased 11-KT Observed?	Decreased Spermatogenesis Observed?	Citation
Nile tilapia (*Oreochromis niloticus*)	Heterozygous mutation of eEF1A1b (eEF1A1b$^{+/-}$) via CRISPR/Cas9	Significantly decreased serum 11-KT at 180 days after hatch	Decreased number of spermatocytes, spermatids and spermatozoa at 180 days after hatch	Yes	Yes	Chen, J. et al. (2017)
Goldfish (*Carassius auratus*)	Anti-androgen vinclozolin (VZ) administered to aquarium at 100 µg/L	Increase in 11-KT level (compared to control)	Slight (not significant) decrease (compared to control) in sperm volume, motility and velocity	No	Yes	Hatef, A. et al. (2012)
	Anti-androgen vinclozolin (VZ) administered to aquarium at 400 µg/L	No significant change in 11-KT level (compared to control)	Slight decrease (compared to control) in sperm volume, motility and velocity; spermatozoa without flagella or with damaged flagella were observed	No	Yes	
	Anti-androgen vinclozolin (VZ) administered to aquarium at 800 µg/L	Decrease in 11-KT level (compared to control); similar level to E2 negative control	Significant decrease (compared to control) in sperm volume, motility, and velocity; spermatozoa without flagella or with damaged flagella were observed	Yes	Yes	
Zebrafish (*Danio rerio*)	*Mettl3* Mutation	Serum concentration significantly decreased	Little or no mature sperm; 24.4% spermatogonia, 56.1% spermatocytes, and 10.4% spermatozoa	Yes	Yes	Xia, H. et al. (2018)



- Search Engine: Google Scholar
  - Search Terms: Impaired spermatogenesis male infertility & ISMI in Fish
    - 41600 Search Results but looked at the first page only to gain familiarity with spermatogenesis
- Search Engine: AbstractSifter
  - Search Terms: Spermatogenesis AND Fish
    - 1587 Initial Results → 9 when filtered with male, infertility, and reduced
    - 11 papers with 1 overlap when filtered with male, infertility, and impaired
  - Search Terms: Spermatogenesis AND Zebrafish
    - 192 Initial Results → 25 papers with 4 overlap when filtered with male and “infertil”



PMID	male	infertil		Score	Pub Yr	Title
25993524	16	1	0	17	2015	Disruption of Zebrafish Follicle-Stimulating Hormone Receptor (fshr) But Not Luteinizing Hormone Receptor (lhcgr) Gene by TAL
19936986	12	3	0	15	2010	Inducible male infertility by targeted cell ablation in zebrafish testis.
31322700	10	2	0	12	2019	Ferredoxin 1b Deficiency Leads to Testis Disorganization, Impaired Spermatogenesis, and Feminization in Zebrafish.
18247060	10	1	0	11	2008	Completion of meiosis in male zebrafish (Danio rerio) despite lack of DNA mismatch repair gene mlh1.
21483806	9	1	0	10	2011	Roles of brca2 (fancd1) in oocyte nuclear architecture, gametogenesis, gonad tumors, and genome stability in zebrafish.
17237513	9	2	0	11	2007	Mlh1 deficiency in zebrafish results in male sterility and aneuploid as well as triploid progeny in females.
31669651	7	2	0	9	2020	New insights into the role of mTORC1 in male fertility in zebrafish.
33045050	6	2	0	8	2020	Loss of Inhibin Advances Follicle Activation and Female Puberty Onset but Blocks Oocyte Maturation in Zebrafish.
29228103	6	3	0	9	2018	Fertility impairment with defective spermatogenesis and steroidogenesis in male zebrafish lacking androgen receptor.
27035939	6	3	0	9	2016	Major spliceosome defects cause male infertility and are associated with nonobstructive azoospermia in humans.
25396299	6	1	0	7	2015	Genetic analysis of zebrafish gonadotropin (FSH and LH) functions by TALEN-mediated gene disruption.
31887561	5	1	0	6	2020	Genetic evidence for estrogenicity of bisphenol A in zebrafish gonadal differentiation and its signalling mechanism.

Other Sources & Example of Initial Organization

- Additional sources were used towards the creation of the weight of evidence for this KER including:
  - Papers recommended by colleagues
  - “Breadcrumb” papers
- Reasons for this included:
  - Papers to provide more information regarding spermatogenesis
  - Lack of papers involving chemical stressors

Spermatogenesis Summary Table(Reduced)

Spermatogenesis Effect
Fertility Effect
Red Text - Red Flag

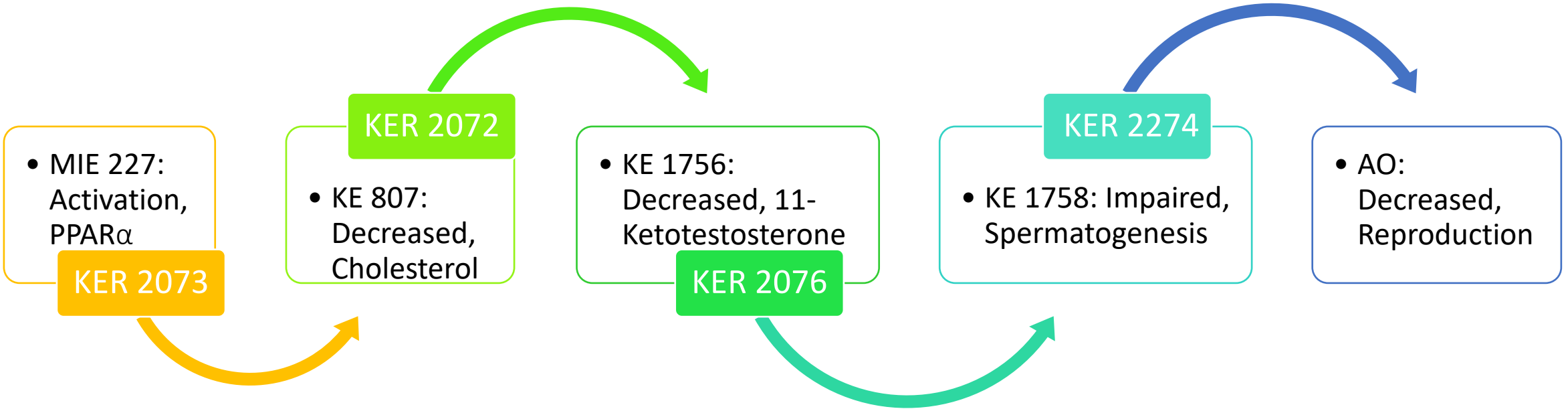
Paper	Exposure/What tests	Effects
Uhrin et al., 2000 Disruption	-mPCI gene targeted by embryonic stem cells using a pPNT vector to disrupt gene function -Used F1 generation of heterozygous mice to create F2 -in vitro fertilization test	- Knockout male mice were infertile; no pregnancy despite normal sexual activity as revealed by # of copulation plugs - More than 95% of sperm from epididymis were morphologically abnormal; most lacked tails and degenerated, and some also had malformed heads - 12.5 motile sperm vs 50.5% and 51.5 % motility from heterozygous and normal sperm - Reduced fertility from in vivo fertilization experiments, 2 oocytes out of 416 (0.5%) recovered from wild-type females were fertilized - 92% (n=415) and 94% (n=420); were recovered from normal and heterozygous - In vitro fertilization experiments in PCL-/- animals are sufficient to explain infertility, even w/o possible additional effects caused by absence of PCI from secretion of sexual glands - Female knockouts reproduced normally and exhibited normal ovaries - Possibly due to abnormal spermatogenesis due to destruction of Sertoli cell barrier, perhaps due to unopposed proteolytic activity - This malfunction or lack of function of Sertoli cells would lead to partially apoptotic spermatocytes, which in turn would lead to malformed sperm accumulating in the seminiferous tubules and in the epididymal duct
Wang et al., 2016 Knockout	-Cre/loxP flp/FRT recombination systems to exons 3 and 4 of BRD7 -breeding assay -sperm counts -sexual hormone assay - normal breeding test -PAS staining	- Causes a complete arrest of spermatogenesis at step 13 of condensing spermatids when looking with periodic acid-schiff staining - post meiotic development of elongating spermatids was disrupted and characterized by abnormal morphology in round spermatids (S1–8) and elongating spermatids (S9–11) (Fig. 4A), and massive degeneration was observed in condensing (S12–13) and condensed spermatids - abnormal spermatids are characterized by an irregular head shape in the CS and CDS, an absent or deformed acrosome - Took sperm from azoospermia males(basically absence of motile sperm in semen) who suffered from spermatogenesis arrest and found a lack of BRD7 present. - When mated KO male mice with WT, no pups were produced or were there any signs of pregnancy - No epididymal sperm was observed in KO mice - Increased proportions of abnormal spermatids - Downregulation of various markers for condensing and condensed spermatids - Association of male infertility resulting from BRD7 disruption with human idiopathic azoospermia where collected 58 samples from azoospermia patients and 35 normal; BRD7 associated in primary

Concordance Table

Concordance Table

Experimental design	Species	Signs of Impaired Spermatogenesis(IS)	Signs of Reduced Reproduction(RR)	IS observed?	RR observed?	Citation	Notes
-Disruption of Protein C inhibitor (PCI) through combining mutant embryonic stem cells with swiss morula embryos to create mutants. -F1 heterozygous mice were then bred to create an F2 that was subsequently used in the study.	Adult Mice (Mus musculus)	-morphologically abnormal sperm -reduced motility(12.5%) compared to control (51.5%) -apoptotic spermatocytes	-reduced in vitro fertilization rate (n=416 blastocysts) (0.5%) vs control (n= 420 blastocysts) (94%) -infertile under standard breeding despite showing signs of normal sexual activity	Yes	Yes	Uhrin et al., 2000	-PCI - inhibitor of anticoagulant serine protease activated protein C and a variety of proteases -PCI is largely present in seminal plasma and is responsible for inhibiting acrosin
Knockout of BRD7 was done through Cre/loxP and flp/FRT recombination and embryonic cells to create a positive clone that was then used to create BRD7-deficient mice	Adult Mice (Mus musculus)	-irregular head shape -deformed acrosome -post meiotic development of elongating spermatids disruption -increased proportion of abnormal spermatids(49.95 ± 7.13% of round spermatids, 67.84 ± 3.51% of elongating spermatids, 80.65 ± 5.8 % of condensing spermatids and 100% of condensed spermatids) -downregulation of various spermatogenic markers	-infertile under standard breeding despite showing signs of normal sexual activity	Yes	Yes	Wang et al., 2016	-BRD7 is a bromodomain gene that inhibits cell growth and cell cycle progression and is a co-factor for p53 -BRD7 has high expression in mice testes
Targeted genetic disruption of fdx1b using a TALEN	Adult Zebrafish (Danio	-reduced sperm count compared to control (p=0.0097%)	-infertile under standard breeding despite being able to cause spawning of eggs(0%	Yes	Yes	Oakes et al., 2019	-fdx1b is an electron-providing cofactor for steroidogenic

AOP 323: PPARα Agonism Impairs Fish Reproduction



Used for OECD case study on KER-by-KER approach to technical review of AOPs

Summary of KER Pilot Study

Development

- KERs developed by different authors
- Each KER took ~2-4 months
- Employed Systematic Review approaches
- Result: Very high quality KERs

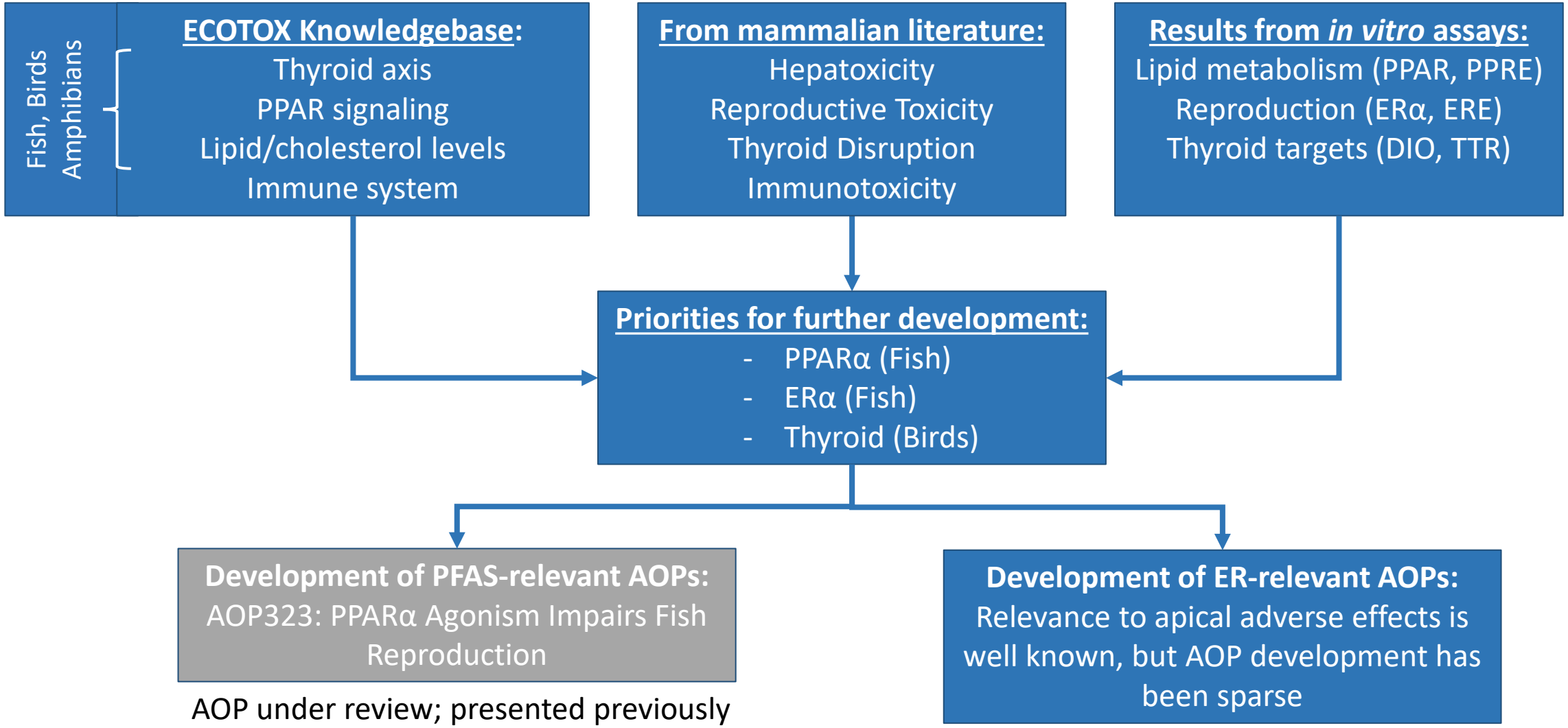
Review

- 17 invitations → 4 reviews received
  - 5 responses with no review, 8 did not respond at all
- Of those that responded, generally, high quality reviews
- Result: Concerns regarding reviewer recruitment confirmed
- ☹

RE: KER as Unit of Development and Review

- KER-based approach was very successful from a development perspective
- Continue to promote KER-by-KER approach as a development strategy for AOP authors
- Does not seem feasible as a unit of review; requires more volunteer reviewers

Potential Pathways of PFAS Toxicity

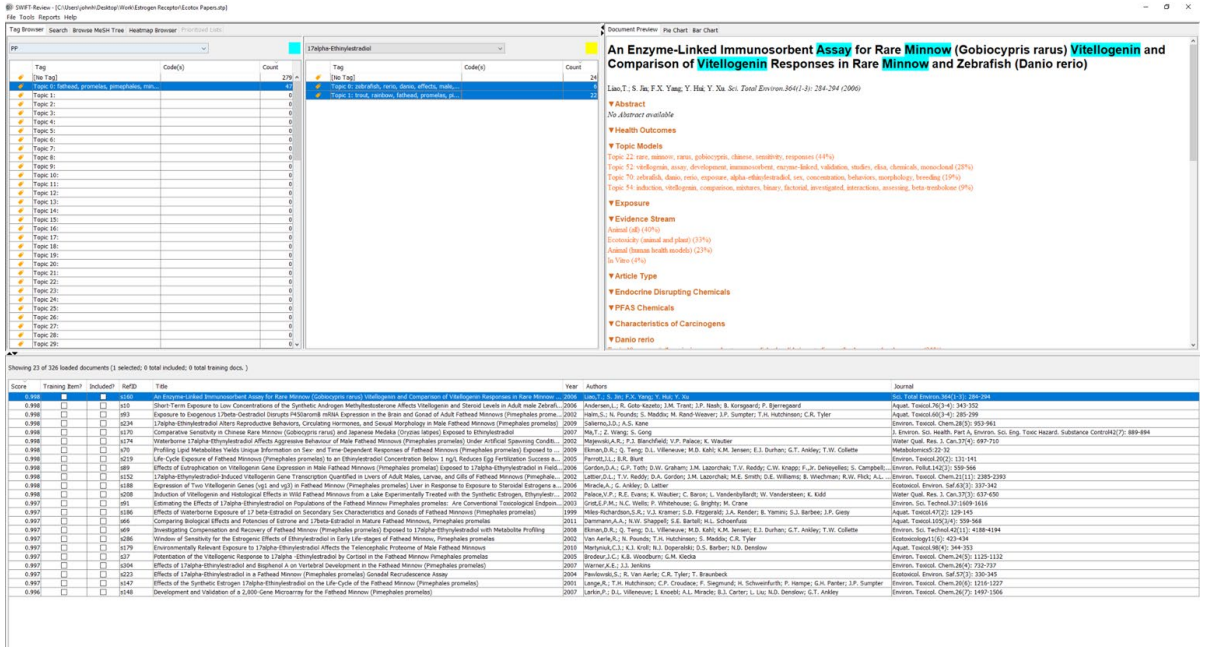


AOP under review; presented previously

Literature Search: ECOTOX & SWIFT-Review

- ECOTOX Knowledgebase Screening:
  - 4 chemicals: 17β-estradiol(E2), 17α-Ethinylestradiol(EE2), estrone(E1), and diethylstilbestrol
  - 4 species: Danio rerio, Oncorhynchus mykiss, Pimephales promelas, Oryzias latipes
  - Initial results lead to 326 references

CAS	Chemical Name	Records	Publicatio	Year Min	Year Max
50282	dChemicals Dashboard	4202	341	1969	2020
53167	EstroneChemicals Dashboard	170	19	1998	2017
56531	DiethylstilbestrolChemicals Dashboard	25	12	1979	2016
57636	17alpha-EthinylestradiolChemicals Dashboard	3721	294	1979	2020



- SWIFT-Review utilized to organize and filter papers
  - 47 initial papers with FHM as a primary filter
  - To hone further, used chemicals as a secondary filter however read all 47 papers by the end

Potential Reproductive Adverse Outcomes

- Within those 47 references, 22 showed some sort of reproductive adverse outcome.
  - 8 papers tested for egg fertility and fertilization and 6 of those papers had a decrease in fertilization/fertility with 0.32 ng EE2/L in a long-term exposure and 10 ng EE2/L for a short-term exposure(21 days).
  - 11 papers tested for egg production and 10 papers reported a decrease or inhibition of egg production with the LOEC inducing this effect being 3.5 ng EE2 /L for a long-term exposure/life-cycle tests(>30 days) and 50 ng E2/L for a short-term exposure(21 days).
  - 15 papers looked for histological changes in male testes and 13 papers reported impaired spermatogenesis with the LOEC being 10 ng EE2/L for a long-term exposure and 0.18 ng E2/L for a short term exposure(14 days).
  - 9 papers tested and reported a female-skewed sex ratio with the LOEC being 0.32 ng EE2/L for the skew to begin and complete feminization at 3.5 ng EE2/L in a life-cycle test setting.

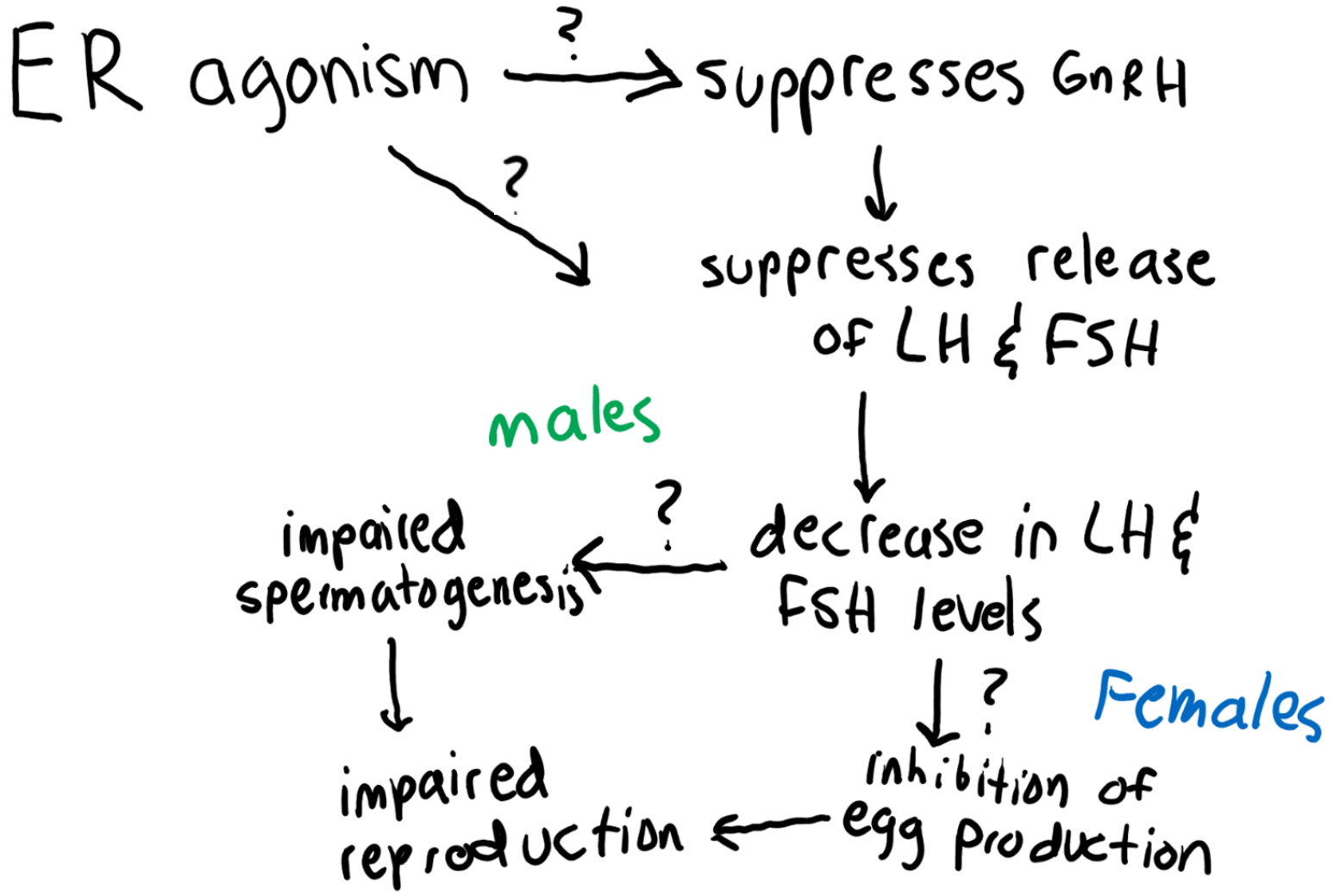
Egg Fertility & Fertilization

Spermatogenesis

Egg Production

Female-Skewed Sex Ratio

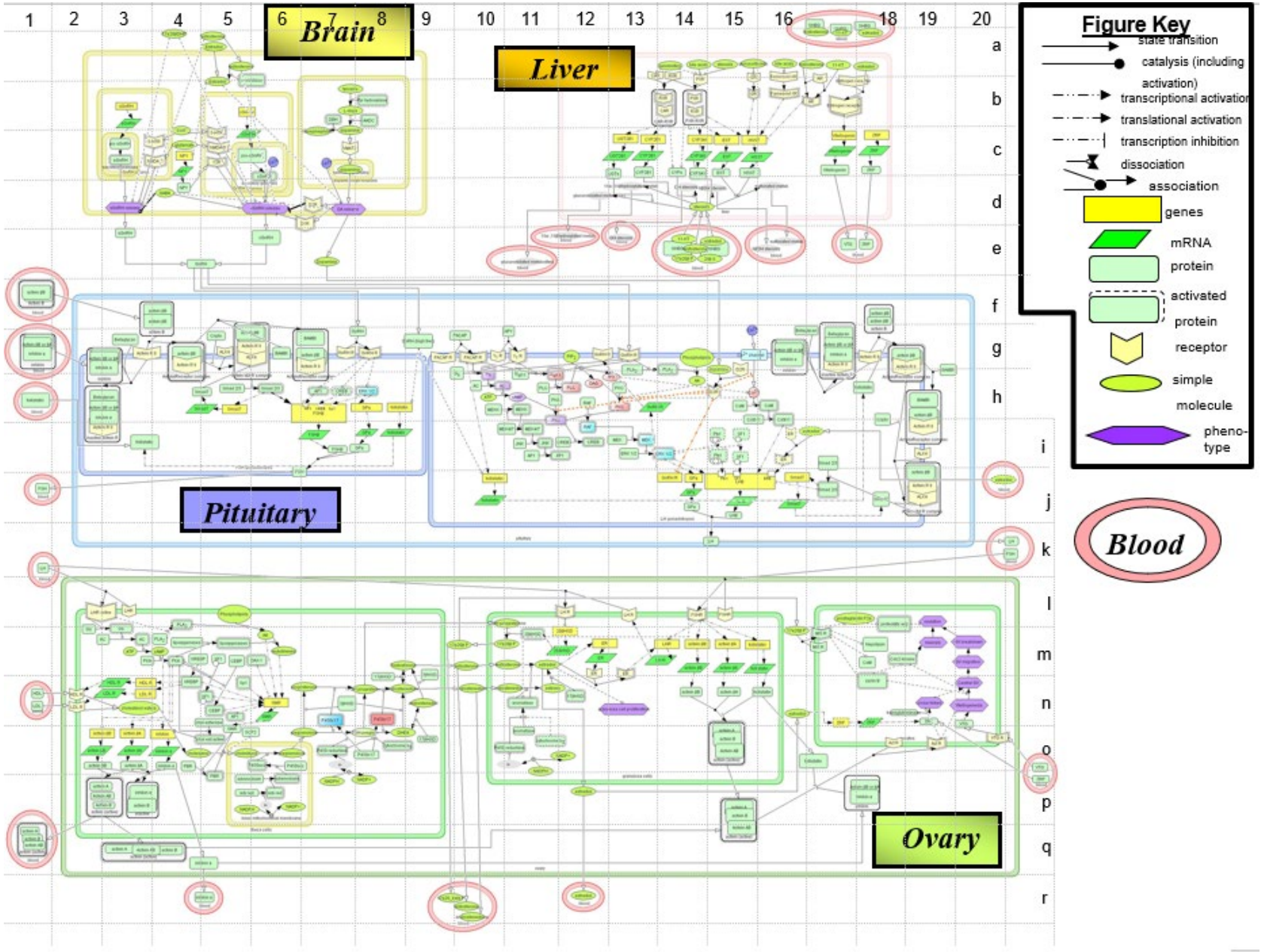
Hypothesis Diagram

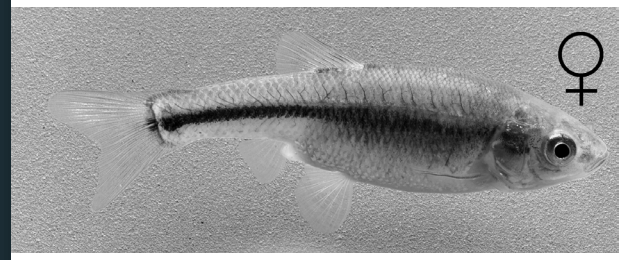


Expert Knowledge

Research team has worked on endocrine toxicity in fish for 20+ years

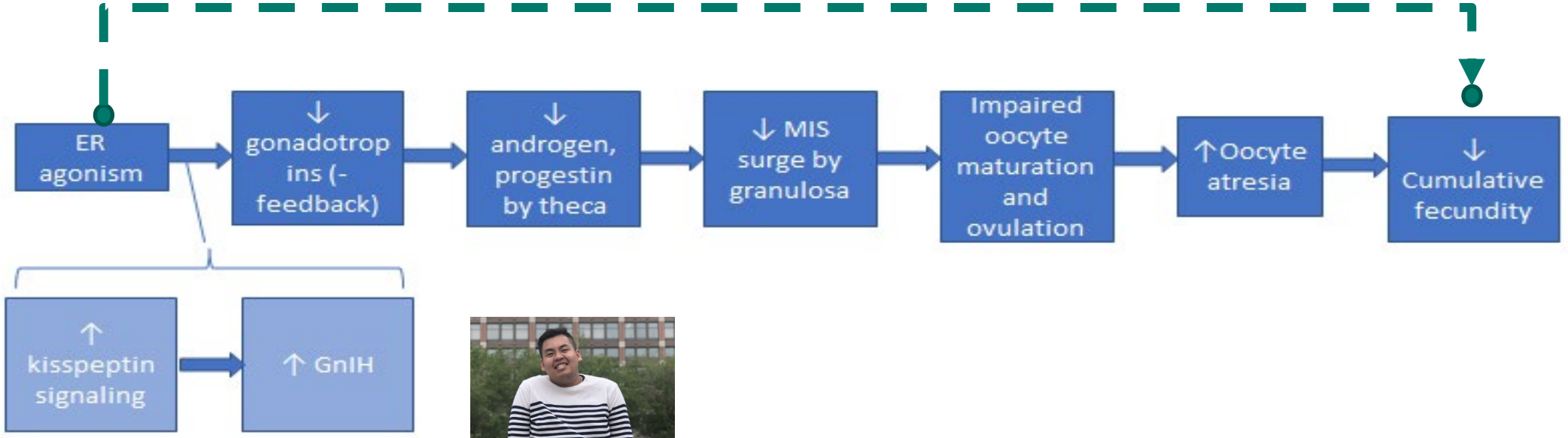
Familiar with the underlying biology





Proposed AOP linking ER agonism to reduced cumulative fecundity (females)

https://aopwiki.org/aops/445



Relationship: 2665

Title

Activation, estrogen receptor alpha leads to Increased, Kisspeptin signalling

Key Event Relationship Description

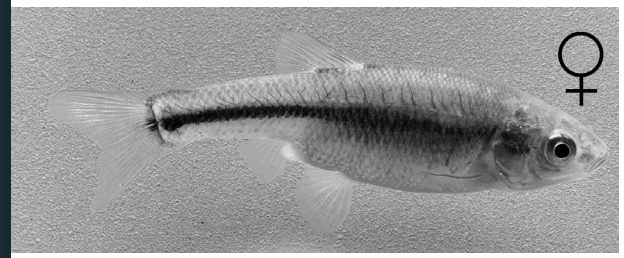
Estrogen receptor alpha (ERα) is a nuclear receptor that can be activated by estrogens, a group of hormones involved in reproductive development. Activation of ERα promotes the transcription and regulation of physiological processes involved with the endocrine system(Christian and Moenter, 2007). Kisspeptins are a family of peptide hormones with varying amino acid lengths derived from the KISS1 gene & neurons (Nejad et al., 2017). Breakthrough research in the 2000s has shown that kisspeptins play a large role in the hypothalamic-pituitary-gonadal axis with gonadotropin circulation(Alcin et al., 2013). In particular, more recent research has shown kisspeptin neurons contain large populations of estrogen receptors, particularly ERα.

Evidence Collection Strategy

The majority of papers used in evidence supporting the key event relationship were found through AbstractSifter, a Microsoft Excel-based application that extracts papers from PubMed. AbstractSifter ranks abstracts based on their relevance through key search and filter terms. Initial papers were found through the search engine, Google Scholar, utilizing the search terms "Kisspeptin" and "estrogen". This search yielded 11600 search results but only papers found on the first page of results were further examined. These papers were used to help curate search and filter terms used in Abstract Sifter. An additional search using CSU Long Beach's One Search engine with key terms "GPR54" and "Kisspeptin" was also done in support of further curating search and filter terms for Abstractsifter. In this search, 3395 papers were initially found and only papers on the first page of the search were initially read. In AbstractSifter, 2 different searches were done to curate a subset of 71 papers. Search terms for the 2 searches included "kisspeptin AND GPR54" and "danio rerio AND kisspeptin" which yielded an initial set of 521 and 60 results respectively. Filter terms for the 2 searches included "estr AND LH" and "estr" which yielded 58 and 13 papers. Additional sources used towards the weight of evidence were found through sources in papers curated in the AbstractSifter search.

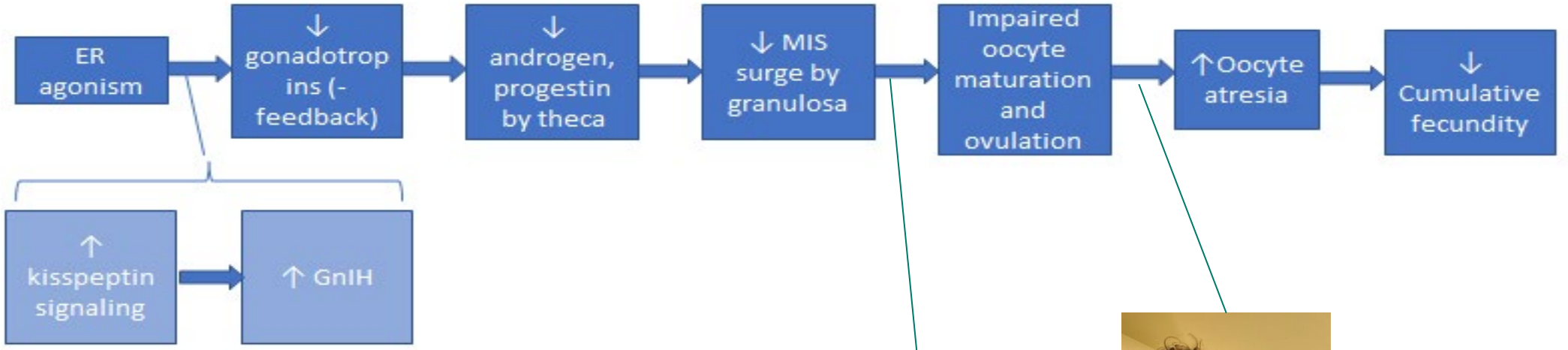
New field in the AOP-Wiki, implemented July 2022.

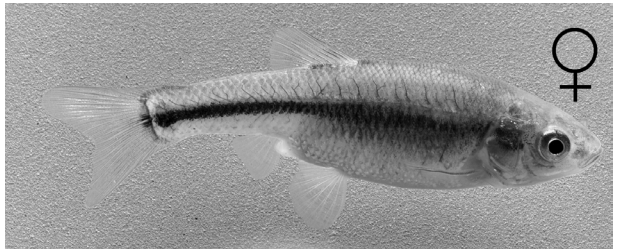
- # AOP Development Strategy
  - Context (**WHY** the AOP was developed)
    - Research question / problem formulation
    - Scope
    - Envisioned use (may be different from end use)
    - Funding source(s) and stakeholders
  - Strategy (**HOW** the AOP was developed)
    - Decisions on the level of resolution at which to describe the pathway
    - Overall data search and identification strategy(ies)



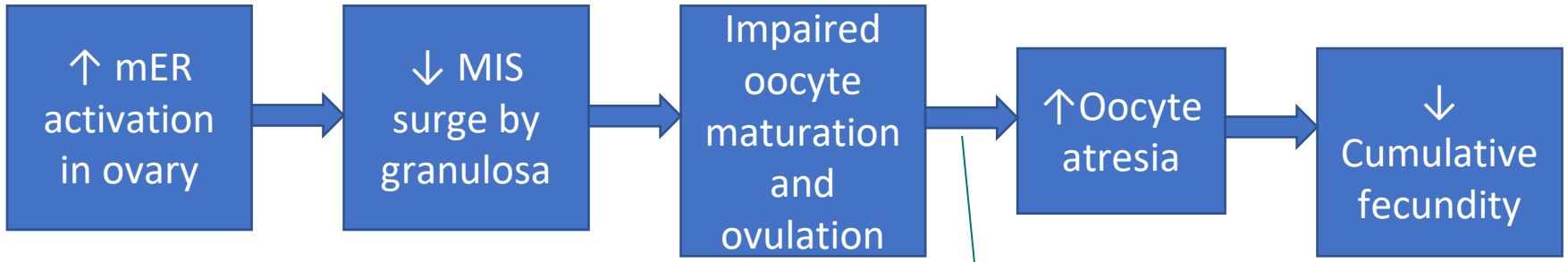
Proposed AOP linking ER agonism to reduced cumulative fecundity (females)

https://aopwiki.org/aops/445

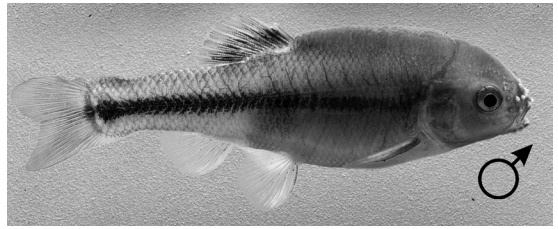




Alternative AOP linking ER agonism to reduced cumulative fecundity (females)



An alternative AOP was revealed through evaluation of literature associated with one of the downstream KERs.



Hypothesized AOPs - Males

ER agonism → ↓ gonadotropins (- feedback) → ↓ androgen, by Leydig cells → ↑ E2-EQ/androgen ratios, Sertoli cells → Impaired spermatogenesis → ↓ Fertility → ↓ viable offspring

Impaired spermiation ?

ER agonism → ↑ vitellogenin synthesis in liver → ↑ plasma vitellogenin → ↑ renal pathology due to VTG deposition → Increased adult mortality → ↓ reproductive output

Next Steps

- Multiple interns engaged in **review and synthesis of literature** related to hypothesized AOP(s)
- **Effects of ER-active PFAS** on multiple intermediate KEs along the pathway still being analyzed
- **Quantitative understanding**:
  - Define total E2 equivalents associated with effects on all measured KEs as a basis for development of response thresholds
  - Estimate the relevance of AOPs for other PFAS and weak xenoestrogens based on in vitro relative potencies.
  - Apply PNEC for FC10-diol for a TTC-like “worst case” evaluation of PFAS mixtures

Acknowledgements

- All co-authors

- Discussions and ideas from OECD EAGMST subgroups that directly or indirectly influenced this research:
  - Handbook, Guidance, and Gardening (HGG)
  - AOP-knowledgebase (AOP-KB)
  - Education, Training, and Communications (ETC)

- ECOTOX-knowledgebase team for contributions to compiling, screening, and coding relevant PFAS papers