AOP ID and Title:

SNAPSHOT

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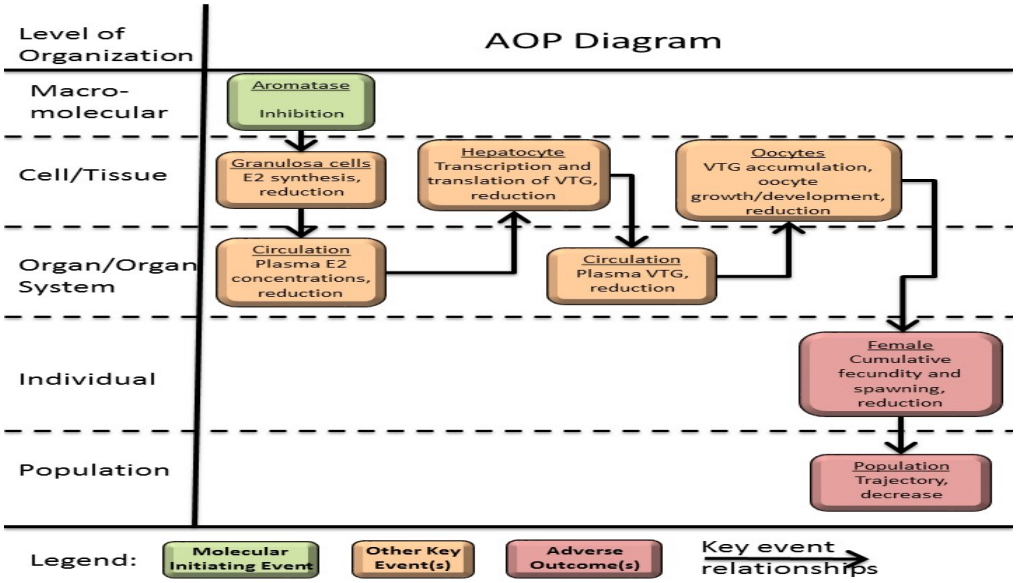
AOP 25: Aromatase inhibition leading to reproductive dysfunction

Short Title: Aromatase inhibition leading to reproductive dysfunction

Graphical Representation



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Status

Author status	OECD status	OECD project	SAAOP status
Open for citation & comment	TFHA/WNT Endorsed	1.12	Included in OECD Work Plan

Abstract

This adverse outcome pathway details the linkage between inhibition of gonadal aromatase activity in females and reproductive dysfunction, as measured through the adverse effect of reduced cumulative fecundity and spawning. Initial development of this AOP draws heavily on evidence collected using repeat-spawning fish species. Cumulative fecundity is the most apical endpoint considered in the OECD 229 Fish Short Term Reproduction Assay. The OECD 229 assay serves as screening assay for endocrine disruption and associated reproductive impairment (OECD 2012). Cumulative fecundity is one of several variables known to be of demographic significance in forecasting fish population trends. Therefore, this AOP has utility in supporting the application of measures of aromatase, or in silico predictions of the ability to inhibit aromatase, as a means to identify chemicals with known potential to adversely affect fish populations and potentially other oviparous vertebrates.

Summary of the AOP

Events

Molecular Initiating Events (MIE), Key Events (KE), Adverse Outcomes (AO)

Sequence	Type	Event ID	Title	Short name
1	MIE	36	Inhibition, Aromatase (https://aopwiki.org/events/36)	Inhibition, Aromatase
2	KE	219	Reduction, Plasma 17beta-estradiol concentrations (https://aopwiki.org/events/219)	Reduction, Plasma 17beta-estradiol concentrations
3	KE	285	Reduction, Vitellogenin synthesis in liver (https://aopwiki.org/events/285)	Reduction, Vitellogenin synthesis in liver
4	KE	309	Reduction, Vitellogenin accumulation into oocytes and oocyte growth/development (https://aopwiki.org/events/309)	Reduction, Vitellogenin accumulation into oocytes and oocyte growth/development
5	KE	3	Reduction, 17beta-estradiol synthesis by ovarian granulosa cells (https://aopwiki.org/events/3)	Reduction, 17beta-estradiol synthesis by ovarian granulosa cells
6	KE	78	Reduction, Cumulative fecundity and spawning (https://aopwiki.org/events/78)	Reduction, Cumulative fecundity and spawning
7	KE	221	Reduction, Plasma vitellogenin concentrations (https://aopwiki.org/events/221)	Reduction, Plasma vitellogenin concentrations
8	AO	360	Decrease, Population trajectory (https://aopwiki.org/events/360)	Decrease, Population trajectory

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Inhibition, Aromatase (https://aopwiki.org/relationships/45)	adjacent	Reduction, 17beta-estradiol synthesis by ovarian granulosa cells	High	Moderate
Reduction, 17beta-estradiol synthesis by ovarian granulosa cells (https://aopwiki.org/relationships/5)	adjacent	Reduction, Plasma 17beta-estradiol concentrations	High	Moderate
Reduction, Plasma 17beta-estradiol concentrations (https://aopwiki.org/relationships/252)	adjacent	Reduction, Vitellogenin synthesis in liver	High	Moderate
Reduction, Cumulative fecundity and spawning (https://aopwiki.org/relationships/94)	adjacent	Decrease, Population trajectory	Moderate	Moderate
Reduction, Vitellogenin accumulation into oocytes and oocyte growth/development (https://aopwiki.org/relationships/337)	adjacent	Reduction, Cumulative fecundity and spawning	Moderate	Moderate
Reduction, Plasma vitellogenin concentrations (https://aopwiki.org/relationships/255)	adjacent	Reduction, Vitellogenin accumulation into oocytes and oocyte growth/development	Moderate	Low
Reduction, Vitellogenin synthesis in liver (https://aopwiki.org/relationships/315)	adjacent	Reduction, Plasma vitellogenin concentrations	High	Moderate
Reduction, Plasma 17beta-estradiol concentrations (https://aopwiki.org/relationships/1386)	non-adjacent	Reduction, Plasma vitellogenin concentrations	High	Moderate

Overall Assessment of the AOP

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
Adult, reproductively mature	

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
medaka	Oryzias latipes	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8090)
zebrafish	Danio rerio	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=7955)
fathead minnow	Pimephales promelas	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=90988)

Sex Applicability

Sex	Evidence
Female	High

- **Sex**: The AOP applies to females only. Males have relatively low gonadal aromatase expression and activity and the androgen 11-KT, rather than the estrogen E2 is a stronger driver of reproductive functions in males. That said, at least in fish, there is a potential autocrine and paracrine for estrogens synthesized in the brain in regulating reproductive behaviors. However, those potential effects are addressed through an alternative AOP that shares the MIE of aromatase inhibition.
- **Life stages**: The relevant life stages for this AOP are reproductively mature adults. This AOP does not apply to adult stages that lack a sexually mature ovary, for example as a result of seasonal or environmentally-induced gonadal senescence (i.e., through control of temperature, photo-period, etc. in a laboratory setting).
- **Taxonomic**: At present, the assumed taxonomic applicability domain of this AOP is class Osteichthyes. In all likelihood, the AOP will also prove applicable to all classes of fish (e.g., Agnatha and Chondrithyes as well). Additionally, all the key events described should be conserved among all oviparous vertebrates, suggesting that the AOP may also have relevance for amphibians, reptiles, and birds. However, species-specific differences in reproductive strategies/life histories, ADME (adsorption, distribution, metabolism, and elimination), compensatory reproductive endocrine responses may influence the outcomes, particularly from a quantitative standpoint.

Essentiality of the Key Events

Support for the essentiality of a number of key events in the AOP was provided by several time-course, stop-reversibility, experiments with fathead minnows exposed to aromatase inhibitors.

1. Villeneuve et al. 2009 and 2013 examined a time-course of key event responses to fadrozole as well as the time-course of recovery following cessation of fadrozole delivery. Once fadrozole was removed from the system, ex vivo E2 production increased, followed by increases in plasma E2 concentrations, and then increases in plasma vitellogenin concentrations. Additionally, while exposure to the chemical was on-going, compensatory up-regulation of CYP19a1a gene expression resulted in increases in ex vivo E2 production, followed by increased plasma E2 and plasma VTG. The essentiality of aromatase inhibition relative to impaired E2 production was further supported by the observation of an "overshoot" in E2 production, relative to controls, shortly after cessation of fadrozole delivery.

2. Similar support was provided in a study by Ankley et al. (2009a). Cessation of prochloraz delivery resulted in rapid recovery of ex vivo E2 production and plasma E2 concentrations, with recovery of vitellogenin concentrations lagging slightly behind. Increased expression of cyp19a1a mRNA during the exposure period aligned with increased ex vivo E2 production, and increased plasma E2, compared to the first day of exposure.

Rationale for essentiality calls:

• Aromatase, inhibition: [Strong] There is good evidence from stop/reversibility studies that ceasing delivery of the aromatase inhibitor leads to recovery of the subsequent key events.

• 17beta-estradiol synthesis by ovarian granulosa cells, reduction: [Strong] In both exposure studies and stop/reversibility studies, when ex vivo E2 production (as measure of this KE) recovers either through compensation or due to removal of the stressor, subsequent KEs have been shown to recover after a lag period.

• plasma 17beta-estradiol concentrations, reduction: [Strong] In both exposure studies and stop/reversibility studies, when plasma E2 concentrations recover either through compensation or due to removal of the stressor, subsequent KEs have been shown to recover after a lag period.

• vitellogenin production in liver (transcription, translation), reduction: [Moderate] This endpoint was not specifically examined in stop/reversibility studies with aromatase inhibitors, but biological plausibility provides strong support for the essentiality of this event.

• plasma vitellogenin concentrations, reduction: [Strong] Shown to recover in a predictable fashion consistent with the order of events in the AOP in stop/recovery studies.

• vitellogenin accumulation into oocytes and oocyte growth/development, reduction: [Weak] Some contradictory evidence regarding the essentiality of this event. No stop/reversibility studies have explicitly considered this key event.

• cumulative fecundity and spawning, reductions: [Moderate] By definition, some degree of spawning is required to maintain population.

Weight of Evidence Summary

Biological plausibility: Biological plausibility refers to the structural or functional relationship between the key events based on our fundamental understanding of "normal biology". In general, the biological plausibility and coherence linking aromatase inhibition through decreases in circulating concentrations of E2 is very solid. The biochemistry of steroidogenesis and the predominant role of the gonad in synthesis of the sex steroids is well established. Similarly, the role of E2 as the major regulator of hepatic vitellogenin production is widely documented in the literature. The direct link between reduced VTG concentrations in the plasma and reduced uptake into oocytes is highly plausible, as the plasma is the primary source of the VTG. However, the direct connection between reduced VTG uptake and impaired spawning/reduced cumulative fecundity is more tentative. It is not clear, for instance whether impaired VTG uptake limits oocyte growth and failure to reach a critical size in turn impairs physical or inter-cellular signaling processes that promote release of the oocyte from the surrounding follicles. In at least one experiment, oocytes with similar size to vitellogenic oocytes, but lacking histological staining characteristic of vitellogenic oocytes was observed (R. Johnson, personal communication). Regulation of oocyte maturation and spawning involves many factors other than vitellogenin accumulation (Clelland and Peng, 2009). At present, the link between reductions in circulating VTG concentrations and reduced cumulative fecundity are best supported by the correlation between those endpoints across multiple experiments, including those that impact VTG via other molecular initiating events (Miller et al. 2007).

Concordance of dose-response relationships: Dose response concordance considers the degree to which upstream events are shown to occur at test concentrations equal to or lower than those that cause significant effects on downstream key events, the underlying assumption being that all KEs can be measured with equal precision. There are a limited number of studies in which multiple key events were considered in the same study. These were considered the most useful for evaluating the concordance of dose-response relationships. In general, effects on downstream key events occurred at concentrations equal to or greater than those at which upstream events occurred (Concordance table: [1] (https://aopwiki.org/wiki/images/4/45/Aromatase_inhibition_dose-response_concordance_table_rev1.pdf)). However, there are exceptions. There are cases where no significant effects on estradiol synthesis by ovarian granulosa cells (ovary explants) were observed, but significant effects on plasma E2 or VTG concentrations were observed. Likewise, there are cases where impacts on plasma VTG were observed at concentrations lower than those reported to reduce plasma E2 concentrations. Based on knowledge of the studies in question, the apparent lack of concordance in some cases is driven by two primary factors. First, differences in the sensitivity and dynamic range of the measurements being made. Second, the effects of compensatory responses along the HPG axis. For instance, although ex vivo E2 production is rapidly affected by exposure to fadrozole, it is also a response that is more rapidly corrected through upregulation of aromatase transcripts (see Villeneuve et al. 2009), meaning that it recovers more quickly than plasma concentrations of E2 or plasma VTG concentrations. Thus, at certain time points, one can get an apparent effect on plasma E2 or T without a measurable impact on E2 production by the gonad tissue, because the upstream insult occurred earlier in time and was subsequently offset by a compensatory response, but the compensation has yet to propagate through the pathway. Sensitivity and dynamic range of the measurement methods is also an issue. Vitellogenin concentrations have a highly dynamic range and can change by orders of magnitude. Other endpoints like plasma steroids are regulated in a narrower range, making differences more difficult to distinguish statistically. Therefore, in our assessment, the deviations from concordance do not call the KERs into question.

The concentration-dependence of the key event responses with regard to the concentration of aromatase inhibitor has been established in vitro and/or in vivo for nearly all key events in the AOP.

1. Concentration-dependent aromatase inhibition: (Villeneuve et al. 2006; Ankley et al. 2005; M et al. 2004; AM et al. 2000; Shilling et al. 1999)
2. Concentration-dependent decreases in E2 production in vitro, ex vivo: (Ankley et al. 2002; Villeneuve et al. 2007; Villeneuve et al. 2009; Ankley et al. 2005; a Marca Pereira et al. 2011; Lee et al. 2006).
3. Concentration-dependent decreases in circulating E2 concentrations: (Ankley et al. 2002; Villeneuve et al. 2009; Ankley et al. 2005; Ankley et al. 2009a; GT et al. 2001)
4. Concentration-dependent decreases in vitellogenin mRNA expression: (Sun et al. 2010; Sun et al. 2011; Zhang et al. 2008)
5. Concentration-dependent decreases in circulating vitellogenin concentrations: (Ankley et al. 2002; Villeneuve et al. 2009; Ankley et al. 2005; Ankley et al. 2009a; Sun et al. 2007; GT et al. 2001; Ralston-Hooper et al. 2013)
6. Concentration-dependent reductions in VTG uptake into oocytes or impaired oocyte development: Concentration-dependence of these effects has not been well demonstrated. The effects, when seen, have typically been documented at the greatest exposure concentration tested, but concentration-dependence of the severity or frequency of the impact was not documented (e.g., (Ankley et al. 2002; Ankley et al. 2005; Sun et al. 2007)
7. Concentration-dependent reductions in cumulative fecundity: (Ankley et al. 2002; Ankley et al. 2005; Sun et al. 2007; Zhang et al. 2008)
8. Declining population trajectory: Modeled population trajectories show a concentration-dependent reduction in projected population size, however, those results are driven by the concentration-dependence of cumulative fecundity. Population-level effects have not been measured directly.

Temporal concordance: Temporal concordance refers to the degree to which the data support the hypothesized sequence of the key events; i.e., the effect on KE1 is observed before the effect on KE2, which is observed before the effect on KE3 and so on. Temporal concordance of the AOP from aromatase inhibition to decreased E2 production, decreased circulating E2, and decreased plasma VTG concentrations has been established (e.g., (Villeneuve et al. 2009; Ankley et al. 2009a; Skolness et al. 2011). Temporal concordance has not been established beyond that key event, in large part due to disconnect in the time-scales over which the events can be measured. For example, most small fish used in reproductive toxicity testing will can spawn anywhere from once daily to several days per week. Given the variability in daily spawning rates, it is neither practical nor effective to evaluate cumulative fecundity at a time scale shorter than roughly a week. Since the impacts at lower levels of biological organization can be detected within hours of exposure, lack of impact on cumulative fecundity before the other key events are impacted cannot be effectively measured. Overall, among those key events whose temporal concordance can reasonably be evaluated, the temporal profile observed is consistent with the AOP.

Consistency: We are aware of no cases where the pattern of key events described was observed without also observing a significant impact on cumulative fecundity. The final adverse outcome is not specific to this AOP. Many of the key events included in this AOP overlap with AOPs linking other molecular initiating events to reproductive dysfunction in small fish.

Uncertainties, inconsistencies, and data gaps: The current major uncertainty in this AOP is whether there is a direct biological linkage between impaired VTG uptake into oocytes and impaired spawning/reduced cumulative fecundity. Plausible biological connections have been hypothesized, but have not yet been tested experimentally.

Quantitative Consideration

Assessment of quantitative understanding of the AOP:

At present, quantitative understanding of the AOP is approaching the point where an in vitro measurement of aromatase inhibition could be used as an input parameter into a series of coupled computational models that could generate quantitative predictions across multiple key events (e.g., circulating E2 concentrations, circulating VTG concentrations, predicted impacts on cumulative fecundity, and effects on population trajectories). A sequence of supporting models has been coupled together and predictions have been made for novel aromatase inhibitors (identified through high throughput in vitro screening), but those predictions have not yet been validated experimentally. The present models are also unable to account for pharmacokinetic considerations (e.g., adsorption, distribution, metabolism/biotransformation, and elimination) and have demonstrated only partial success in simulating compensatory/feedback responses to aromatase inhibition (e.g., (Breen et al. 2013).

Considerations for Potential Applications of the AOP (optional)

- The present AOP can provide potential support for the use of alternatives to the fish short term reproduction assay as a screen for aromatase inhibitors.
- The present AOP can serve as a foundation for tiered testing strategies and IATA related to risk assessments on chemicals identified as aromatase inhibitors.
- The present AOP can be used to guide endpoint selection for effects-based monitoring studies at sites where aromatase inhibition has been identified as a relevant biological activity of interest (e.g., through bioeffects prediction or bioeffects surveillance approaches; see Schroeder et al. 2016).

Schroeder, A. L., Ankley, G. T., Houck, K. A. and Villeneuve, D. L. (2016), Environmental surveillance and monitoring—The next frontiers for high-throughput toxicology. Environ Toxicol Chem, 35: 513–525. doi:10.1002/etc.3309

- A series of computational models aligned with this AOP (i.e., a quantitative AOP construct) can be applied to estimate in vivo bench-mark doses based on in vitro screening results. Case studies evaluating this application are under way.

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Appendix 1

List of MIEs in this AOP

Event: 36: Inhibition, Aromatase (https://aopwiki.org/events/36)

Short Name: Inhibition, Aromatase

Key Event Component

Process	Object	Action
aromatase activity	aromatase	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:25 - Aromatase inhibition leading to reproductive dysfunction (https://aopwiki.org/aops/25)	MolecularInitiatingEvent
Aop:346 - Aromatase inhibition leads to male-biased sex ratio via impacts on gonad differentiation (https://aopwiki.org/aops/346)	MolecularInitiatingEvent

Stressors

Name
Fadrozole
Letrozole
Prochloraz

Biological Context

Level of Biological Organization
Molecular

Cell term

Cell term
granulosa cell

Evidence for Perturbation by Stressor

Overview for Molecular Initiating Event

Characterization of chemical properties: Chemicals are known to inhibit aromatase activity through two primary molecular mechanisms. Steroid-like structures can inhibit the enzyme at its active site, with structures having Δ4 positioned double bonds generally acting as stronger inhibitors than those with Δ5 positioned double bonds (Petkov et al. 2009). Non-steroidal aromatase inhibitors generally act by interfering with electron transfer via the cytochrome P450 heme group of the aromatase enzyme, with greater nucleophilicity of the heteroatom contributing to greater potency as an inhibitor (Petkov et al. 2009). Petkov et al. (Petkov et al. 2009) have provided a detailed analysis of structural categorization of chemicals as potential steroidal or non-steroidal aromatase inhibitors.

Domain of Applicability

Taxonomic applicability: Aromatase (CYP19) orthologs are known to be present among most of the vertebrate lineage, at least down to the cartilaginous fishes. Orthologs have generally not been found in invertebrates, however, CYP19 was detected in the invertebrate chordate,

amphioxus and analysis of conservation of gene order and content suggests a possible origin among primitive chordates (Castro et al. 2005). Fishes generally have two aromatase isoforms, cyp19a1a which is predominantly expressed in ovary and cyp19b, predominantly expressed in brain (Callard et al. 2001). Given that cyp19a1a is dominant isoform expressed in ovary and both isoforms appear to show similar sensitivity to aromatase inhibitors (Hinfray et al., 2006), for the purpose of this key event which focuses on gonadal aromatase activty, distinction of effects on one isoform versus the other are considered negligible. Total activity, without regard to isoform can be considered.

Key Event Description

Inhibition of cytochrome P450 aromatase (CYP19; specifically cyp19a1a in fish).

Site of action: The site of action for the molecular initiating event is the ovarian granulosa cells.

While many vertebrates have a single isoform of aromatase, fish are known to have two isoforms. CYP19a1a is predominantly expressed in ovary while cyp19a1b is predominantly expressed in brain (Callard et al. 2001; Cheshenko et al. 2008). For the purposes of this MIE, when applied to fish, the assumed effect is on cyp19a1a. However, given that both isoforms show similar sensitivity to aromatase inhibitors (Hinfray et al. 2006) and catalyze the same reaction, discrimination of specific isoforms is not viewed as critical in relative to determining downstream key events resulting from aromatase inhibition in ovarian granulosa cells.

Responses at the macromolecular level: Aromatase catalyzes three sequential oxidation steps (i.e., KEGG reactions R02501, R04761, R03087 or R01840, R04759, R02351; http://www.genome.jp/kegg/pathway.html (http://www.genome.jp/kegg/pathway.html)) involved in the conversion of C-19 androgens (e.g., testosterone, androstenedione) to C-18 estrogens (e.g., 17β-estradiol, estrone). Aromatase inhibitors interfere with one or more of these reactions, leading to reduced efficiency in converting C-19 androgens into C-18 estrogens. Therefore, inhibition of aromatase activity results in decreased rate of 17β-estradiol (and presumably estrone) production by the ovary.

How it is Measured or Detected

Measurement/detection: Aromatase activity is typically measured by evaluating the production of tritiated water released upon the aromatase catalyzed conversion of radio-labeled androstenedione to estrone (Lephart and Simpson 1991). Aromatase activity can be measured in cell lines exposed in vitro (e.g., human placental JEG-3 cells and JAR choriocarcinoma cells, (Letcher et al. 1999); H295R human adrenocortical carcinoma cells (Sanderson et al. 2000)). Aromatase activity can also be quantified in tissue (i.e., ovary or brain) from vertebrates exposed in vivo (e.g., (Villeneuve et al. 2006; Ankley et al. 2002). In vitro aromatase assays are amenable to high throughput and have been included in nascent high throughput screening programs like the US EPA ToxcastTM program.

References

See Aromatase inhibition leading to reproductive dysfunction (in fish) (https://aopwiki.org/wiki/index.php?title=Aromatase_inhibition_leading_to_reproductive_dysfunction_(in_fish)&action=edit&redlink=1)

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List of Key Events in the AOP

Event: 219: Reduction, Plasma 17beta-estradiol concentrations (https://aopwiki.org/events/219)

Short Name: Reduction, Plasma 17beta-estradiol concentrations

Key Event Component

Process	Object	Action
	17beta-estradiol	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:7 - Aromatase (Cyp19a1) reduction leading to impaired fertility in adult female (https://aopwiki.org/aops/7)	KeyEvent
Aop:25 - Aromatase inhibition leading to reproductive dysfunction (https://aopwiki.org/aops/25)	KeyEvent
Aop:23 - Androgen receptor agonism leading to reproductive dysfunction (in repeat-spawning fish) (https://aopwiki.org/aops/23)	KeyEvent
Aop:122 - Prolyl hydroxylase inhibition leading to reproductive dysfunction via increased HIF1 heterodimer formation (https://aopwiki.org/aops/122)	KeyEvent
Aop:123 - Unknown MIE leading to reproductive dysfunction via increased HIF-1alpha transcription (https://aopwiki.org/aops/123)	KeyEvent
Aop:271 - Inhibition of thyroid peroxidase leading to impaired fertility in fish (https://aopwiki.org/aops/271)	KeyEvent
Aop:289 - Inhibition of 5α-reductase leading to impaired fecundity in female fish (https://aopwiki.org/aops/289)	KeyEvent
Aop:310 - Embryonic Activation of the AHR leading to Reproductive failure, via epigenetic down-regulation of GnRHR (https://aopwiki.org/aops/310)	KeyEvent

Biological Context

Level of Biological Organization
Organ

Organ term

Organ term
blood plasma

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
human	Homo sapiens	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
fathead minnow	Pimephales promelas	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=90988)
Fundulus heteroclitus	Fundulus heteroclitus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8078)

Life Stage Applicability

Life Stage	Evidence
Adult	High

Sex Applicability

Sex	Evidence
Unspecific	Not Specified

Key enzymes needed to synthesize 17β-estradiol first appear in the common ancestor of amphioxus and vertebrates (Baker 2011). Consequently, this key event is applicable to most vertebrates.

Key Event Description

Estradiol synthesized by the gonads is transported to other tissues via blood circulation. The gonads are generally considered to be the primary source of estrogens in systemic circulation.

How it is Measured or Detected

Total concentrations of 17β-estradiol in plasma can be measured by radioimmunoassay (e.g., (Jensen et al. 2001)), enzyme-linked immunosorbent assay (available through many commercial vendors), or by analytical chemistry (e.g., LC/MS; Owen et al. 2014). Total steroid hormones are typically extracted from plasma or serum via liquid-liquid or solid phase extraction prior to analysis.

Given that there are numerous genes, like those coding for vertebrate vitellogenins, choriongenins, cyp19a1b, etc. which are known to be regulated by estrogen response elements, targeted qPCR or proteomic analysis of appropriate targets could also be used as an indirect measure of reduced circulating estrogen concentrations. However, further support for the specificity of the individual gene targets for estrogen-dependent regulation should be established in order to support their use.

A line of transgenic zebrafish employing green fluorescence protein under control of estrogen response elements could also be used to provide direct evidence of altered estrogen, with decreased GFP signal in estrogen responsive tissues like liver, ovary, pituitary, and brain indicating a reduction in circulating estrogens (Gorelick and Halpern 2011).

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Event: 285: Reduction, Vitellogenin synthesis in liver (https://aopwiki.org/events/285)

Short Name: Reduction, Vitellogenin synthesis in liver

Key Event Component

Process	Object	Action
gene expression	vitellogenins	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:25 - Aromatase inhibition leading to reproductive dysfunction (https://aopwiki.org/aops/25)	KeyEvent
Aop:23 - Androgen receptor agonism leading to reproductive dysfunction (in repeat-spawning fish) (https://aopwiki.org/aops/23)	KeyEvent
Aop:30 - Estrogen receptor antagonism leading to reproductive dysfunction (https://aopwiki.org/aops/30)	KeyEvent

AOP ID and Name	Event Type
Aop:122 - Prolyl hydroxylase inhibition leading to reproductive dysfunction via increased HIF1 heterodimer formation (https://aopwiki.org/aops/122)	KeyEvent
Aop:123 - Unknown MIE leading to reproductive dysfunction via increased HIF-1alpha transcription (https://aopwiki.org/aops/123)	KeyEvent
Aop:310 - Embryonic Activation of the AHR leading to Reproductive failure, via epigenetic down-regulation of GnRHR (https://aopwiki.org/aops/310)	KeyEvent

Biological Context

Level of Biological Organization
Tissue

Cell term

Cell term
hepatocyte

Organ term

Organ term
liver

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Pimephales promelas	Pimephales promelas	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=90988)
Fundulus heteroclitus	Fundulus heteroclitus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8078)
Oryzias latipes	Oryzias latipes	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8090)

Life Stage Applicability

Life Stage	Evidence
Adult, reproductively mature	High

Sex Applicability

Sex	Evidence
Unspecific	Not Specified

Oviparous vertebrates. Although vitellogenin is conserved among oviparous vertebrates and many invertebrates, liver is not a relevant tissue for the production of vitellogenin in invertebrates (Wahli 1988)

Key Event Description

Vitellogenin is an egg yolk precursor protein synthesized by hepatocytes of oviparous vertebrates. In vertebrates, transcription of vitellogenin

genes is predominantly regulated by estrogens via their action on nuclear estrogen receptors. During vitellogenic periods of the reproductive cycle, when circulating estrogen concentrations are high, vitellogenin transcription and synthesis are typically orders of magnitude greater than during non-reproductive conditions.

How it is Measured or Detected

Relative abundance of vitellogenin transcripts or protein can be readily measured in liver tissue from organisms exposed in vivo (e.g., (Biales et al. 2007)), or in liver slices (e.g., (Schmieder et al. 2000) or hepatocytes (e.g., (Navas and Segner 2006) exposed in vitro, using real-time quantitative polymerase chain reaction (PCR; transcripts) or enzyme linked immunosorbent assay (ELISA; protein).

References

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Event: 309: Reduction, Vitellogenin accumulation into oocytes and oocyte growth/development (https://aopwiki.org/events/309)

Short Name: Reduction, Vitellogenin accumulation into oocytes and oocyte growth/development

Key Event Component

Process	Object	Action
receptor-mediated endocytosis	vitellogenins	decreased
oocyte growth		decreased
oocyte development		decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:25 - Aromatase inhibition leading to reproductive dysfunction (https://aopwiki.org/aops/25)	KeyEvent
Aop:23 - Androgen receptor agonism leading to reproductive dysfunction (in repeat-spawning fish) (https://aopwiki.org/aops/23)	KeyEvent
Aop:30 - Estrogen receptor antagonism leading to reproductive dysfunction (https://aopwiki.org/aops/30)	KeyEvent
Aop:122 - Prolyl hydroxylase inhibition leading to reproductive dysfunction via increased HIF1 heterodimer formation (https://aopwiki.org/aops/122)	KeyEvent
Aop:123 - Unknown MIE leading to reproductive dysfunction via increased HIF-1alpha transcription (https://aopwiki.org/aops/123)	KeyEvent
Aop:310 - Embryonic Activation of the AHR leading to Reproductive failure, via epigenetic down-regulation of GnRHR (https://aopwiki.org/aops/310)	KeyEvent

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
oocyte

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
fathead minnow	Pimephales promelas	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=90988)
Oryzias latipes	Oryzias latipes	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8090)

Life Stage Applicability

Life Stage	Evidence
Adult, reproductively mature	High

Sex Applicability

Sex	Evidence
Female	High

Oviparous vertebrates and invertebrates. Although hormonal regulation of vitellogenin synthesis and mechanisms of vitellogenin transport from the site of synthesis to the ovary vary between vertebrates and invertebrates (Wahli 1988), in both vertebrates and invertebrates, vitellogenin is incorporated into oocytes and cleaved to form yolk proteins.

Key Event Description

Vitellogenin from the blood is selectively taken up by competent oocytes via receptor-mediated endocytosis. Although vitellogenin receptors mediate the uptake, opening of intercellular channels through the follicular layers to the oocyte surface as the oocyte reaches a “critical” size is thought to be a key trigger in allowing vitellogenin uptake (Tyler and Sumpter 1996). Once critical size is achieved, concentrations in the plasma and temperature are thought to impose the primary limits on uptake (Tyler and Sumpter 1996). Uptake of vitellogenin into oocytes causes considerable oocyte growth during vitellogenesis, accounting for up to 95% of the final egg size in many fish (Tyler and Sumpter 1996). Given the central role of vitellogenesis in oocyte maturation, vitellogenin accumulation is a prominent feature used in histological staging of oocytes (e.g., (Leino et al. 2005; Wolf et al. 2004).

How it is Measured or Detected

Relative vitellogenin accumulation can be evaluated qualitatively using routine histological approaches (Leino et al. 2005; Wolf et al. 2004). Oocyte size can be evaluated qualitatively or quantitatively using routine histological and light microscopy and/or imaging approaches.

References

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Event: 3: Reduction, 17beta-estradiol synthesis by ovarian granulosa cells (https://aopwiki.org/events/3)

Short Name: Reduction, 17beta-estradiol synthesis by ovarian granulosa cells

Key Event Component

Process	Object	Action
estrogen biosynthetic process	17beta-estradiol	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:7 - Aromatase (Cyp19a1) reduction leading to impaired fertility in adult female (https://aopwiki.org/aops/7)	KeyEvent
Aop:25 - Aromatase inhibition leading to reproductive dysfunction (https://aopwiki.org/aops/25)	KeyEvent
Aop:23 - Androgen receptor agonism leading to reproductive dysfunction (in repeat-spawning fish) (https://aopwiki.org/aops/23)	KeyEvent
Aop:122 - Prolyl hydroxylase inhibition leading to reproductive dysfunction via increased HIF1 heterodimer formation (https://aopwiki.org/aops/122)	KeyEvent
Aop:123 - Unknown MIE leading to reproductive dysfunction via increased HIF-1alpha transcription (https://aopwiki.org/aops/123)	KeyEvent
Aop:310 - Embryonic Activation of the AHR leading to Reproductive failure, via epigenetic down-regulation of GnRHR (https://aopwiki.org/aops/310)	KeyEvent

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
granulosa cell

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
fathead minnow	Pimephales promelas	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=90988)
Fundulus heteroclitus	Fundulus heteroclitus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8078)

Life Stage Applicability

Life Stage	Evidence
Adult, reproductively mature	High

Sex Applicability

Sex	Evidence
Female	High

Key enzymes needed to synthesize 17β-estradiol first appear in the common ancestor of amphioxus and vertebrates (Markov et al. 2009; Baker 2011). Consequently, it is plausible that this key event is applicable to most vertebrates. This key event is not applicable to invertebrates, which lack the enzymes required to synthesize 17ß-estradiol.

Key Event Description

Like all steroids, estradiol is a cholesterol derivative. Estradiol synthesis in ovary is mediated by a number of enzyme catalyzed reactions involving cyp11 (cholesterol side chain cleavage enzyme), cyp 17 (17alpha-hydroxylase/17,20-lyase), 3beta hydroxysteroid dehyrogenase, 17beta hydroxysteroid dehydrogenase, and cyp19 (aromatase). Among those enzyme catalyzed reactions, conversion of testosterone to estradiol, catalyzed by aromatase, is considered to be rate limiting for estradiol synthesis. Within the ovary, aromatase expression and activity is primarily localized in the granulosa cells (reviewed in (Norris 2007; Yaron 1995; Havelock et al. 2004) and others). Reactions involved in synthesis of C-19 androgens are primarily localized in the theca cells and C-19 androgens diffuse from the theca into granulosa cells where aromatase can catalyze their conversion to C-18 estrogens.

How it is Measured or Detected

Due to the importance of both theca and granulosa cells in ovarian steroidogenesis, it is generally impractical to measure E2 production by isolated granulosa cells (Havelock et al. 2004). However, this key event can be evaluated by examining E2 production by intact ovarian tissue explants either exposed to chemicals in vitro (e.g., (Villeneuve et al. 2007; McMaster ME 1995) or in vivo (i.e., via ex vivo steroidogenesis assay; e.g., (Ankley et al. 2007)). Estradiol released by ovarian tissue explants into media can be quantified by radioimmunoassay (e.g., Jensen et al. 2001), ELISA, or analytical methods such as LC-MS (e.g., Owen et al. 2014).

OECD TG 456 (OECD 2011) (http://www.oecd-ilibrary.org/environment/test-no-456-h295r-steroidogenesis-assay_9789264122642-en) is the validated test guideline for an in vitro screen for chemical effects on steroidogenesis, specifically the production of 17ß-estradiol (E2) and testosterone (T).

The synthesis of E2 can be measured in vitro cultured ovarian cells. The methods for culturing mammalian ovarian cells can be found in the Database Service on Alternative Methods to animal experimentation (DB-ALM): Culture of Human Cumulus Granulosa Cells (EURL ECVAM Protocol No. 92) (http://ecvam-dbalm.jrc.ec.europa.eu/beta/index.cfm/methodsAndProtocols/index?id_prot=266), Granulosa and Theca Cell Culture Systems (EURL ECVAM Method Summary No. 92) (http://ecvam-dbalm.jrc.ec.europa.eu/beta/index.cfm/methodsAndProtocols/index?id_met=535).

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Event: 78: Reduction, Cumulative fecundity and spawning (https://aopwiki.org/events/78)

Short Name: Reduction, Cumulative fecundity and spawning

Key Event Component

Process	Object	Action
egg quantity		decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:29 - Estrogen receptor agonism leading to reproductive dysfunction (https://aopwiki.org/aops/29)	KeyEvent

AOP ID and Name	Event Type
Aop:25 - Aromatase inhibition leading to reproductive dysfunction (https://aopwiki.org/aops/25)	KeyEvent
Aop:23 - Androgen receptor agonism leading to reproductive dysfunction (in repeat-spawning fish) (https://aopwiki.org/aops/23)	KeyEvent
Aop:30 - Estrogen receptor antagonism leading to reproductive dysfunction (https://aopwiki.org/aops/30)	KeyEvent
Aop:122 - Prolyl hydroxylase inhibition leading to reproductive dysfunction via increased HIF1 heterodimer formation (https://aopwiki.org/aops/122)	AdverseOutcome
Aop:123 - Unknown MIE leading to reproductive dysfunction via increased HIF-1alpha transcription (https://aopwiki.org/aops/123)	AdverseOutcome
Aop:271 - Inhibition of thyroid peroxidase leading to impaired fertility in fish (https://aopwiki.org/aops/271)	AdverseOutcome
Aop:310 - Embryonic Activation of the AHR leading to Reproductive failure, via epigenetic down-regulation of GnRHR (https://aopwiki.org/aops/310)	AdverseOutcome
Aop:289 - Inhibition of 5α-reductase leading to impaired fecundity in female fish (https://aopwiki.org/aops/289)	KeyEvent

Stressors

Name
Tris(1,3-dichloropropyl)phosphate - TDCPP

Biological Context

Level of Biological Organization
Individual

Evidence for Perturbation by Stressor

Tris(1,3-dichloropropyl)phosphate - TDCPP

Reduction of cumulative fecundity and spawning following exposure to low levels of TDCIPP (15, 46 and 90 nM) has been reported in 3 different zebrafish studies (Liu et al., 2013; Wang et al., 2015a; Zhu et al., 2015).

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
fathead minnow	Pimephales promelas	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=90988)
Fundulus heteroclitus	Fundulus heteroclitus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8078)
Oryzias latipes	Oryzias latipes	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8090)

Life Stage Applicability

Life Stage	Evidence
Adult, reproductively mature	High

Sex Applicability

Sex	Evidence
Female	High

Cumulative fecundity and spawning can, in theory, be evaluated for any egg laying animal.

Key Event Description

Spawning refers to the release of eggs. Cumulative fecundity refers to the total number of eggs deposited by a female, or group of females over a specified period of time.

How it is Measured or Detected

In laboratory-based reproduction assays (e.g., OECD Test No. 229; OECD Test No. 240), spawning and cumulative fecundity can be directly measured through daily observation of egg deposition and egg counts.

In some cases, fecundity may be estimated based on gonado-somatic index (OECD 2008 (http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2008)22&doclanguage=en)).

Regulatory Significance of the AO

Cumulative fecundity is the most apical endpoint considered in the OECD 229 Fish Short Term Reproduction Assay. The OECD 229 assay serves as screening assay for endocrine disruption and associated reproductive impairment (OECD 2012 (http://www.oecd-ilibrary.org/environment/test-no-229-fish-short-term-reproduction-assay_9789264185265-en)). Fecundity is also an important apical endpoint in the Medaka Extended One Generation Reproduction Test (MEOGRT; OECD Test Guideline 240 (http://www.oecd-ilibrary.org/environment/test-no-240-medaka-extended-one-generation-reproduction-test-meogrt_9789264242258-en); OECD 2015).

A variety of fish life cycle tests also include cumulative fecundity as an endpoint (OECD 2008 (http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2008)22&doclanguage=en)).

References

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- OECD (2015), *Test No. 240: Medaka Extended One Generation Reproduction Test (MEOGRT)*, OECD Publishing, Paris. DOI: http://dx.doi.org/10.1787/9789264242258-en (http://dx.doi.org/10.1787/9789264242258-en)
- OECD. 2012a. Test no. 229: Fish short term reproduction assay. Paris, France:Organization for Economic Cooperation and Development.

Event: 221: Reduction, Plasma vitellogenin concentrations (https://aopwiki.org/events/221)

Short Name: Reduction, Plasma vitellogenin concentrations

Key Event Component

Process	Object	Action
	vitellogenins	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:25 - Aromatase inhibition leading to reproductive dysfunction (https://aopwiki.org/aops/25)	KeyEvent
Aop:23 - Androgen receptor agonism leading to reproductive dysfunction (in repeat-spawning fish) (https://aopwiki.org/aops/23)	KeyEvent
Aop:30 - Estrogen receptor antagonism leading to reproductive dysfunction (https://aopwiki.org/aops/30)	KeyEvent
Aop:122 - Prolyl hydroxylase inhibition leading to reproductive dysfunction via increased HIF1 heterodimer formation (https://aopwiki.org/aops/122)	KeyEvent

AOP ID and Name	Event Type
Aop:123 - Unknown MIE leading to reproductive dysfunction via increased HIF-1alpha transcription (https://aopwiki.org/aops/123)	KeyEvent
Aop:271 - Inhibition of thyroid peroxidase leading to impaired fertility in fish (https://aopwiki.org/aops/271)	KeyEvent
Aop:289 - Inhibition of 5α-reductase leading to impaired fecundity in female fish (https://aopwiki.org/aops/289)	KeyEvent
Aop:310 - Embryonic Activation of the AHR leading to Reproductive failure, via epigenetic down-regulation of GnRHR (https://aopwiki.org/aops/310)	KeyEvent

Biological Context

Level of Biological Organization
Organ

Organ term

Organ term
blood plasma

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
fathead minnow	Pimephales promelas	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=90988)
Oryzias latipes	Oryzias latipes	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8090)
Danio rerio	Danio rerio	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=7955)

Life Stage Applicability

Life Stage	Evidence
Adult, reproductively mature	High

Oviparous vertebrates synthesize yolk precursor proteins that are transported in the circulation for uptake by developing oocytes. Many invertebrates also synthesize vitellogenins that are taken up into developing oocytes via active transport mechanisms. However, invertebrate vitellogenins are transported in hemolymph or via other transport mechanisms rather than plasma.

Key Event Description

Vitellogenin synthesized in the liver is secreted into the blood and circulates to the ovaries for uptake.

How it is Measured or Detected

Vitellogenin concentrations in plasma are typically detected using enzyme linked Immunosorbent assay (ELISA; e.g., (Korte et al. 2000; Tyler et al. 1996; Holbech et al. 2001; Fenske et al. 2001). Although less specific and/or sensitive, determination of alkaline-labile phosphate or Western blotting has also been employed.

References

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- Wahli W. 1988. Evolution and expression of vitellogenin genes. Trends in Genetics. 4:227-232.

List of Adverse Outcomes in this AOP

Event: 360: Decrease, Population trajectory (https://aopwiki.org/events/360)

Short Name: Decrease, Population trajectory

Key Event Component

Process	Object	Action
population growth rate		decreased
population growth rate	population of organisms	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:23 - Androgen receptor agonism leading to reproductive dysfunction (in repeat-spawning fish) (https://aopwiki.org/aops/23)	AdverseOutcome
Aop:25 - Aromatase inhibition leading to reproductive dysfunction (https://aopwiki.org/aops/25)	AdverseOutcome
Aop:29 - Estrogen receptor agonism leading to reproductive dysfunction (https://aopwiki.org/aops/29)	AdverseOutcome
Aop:30 - Estrogen receptor antagonism leading to reproductive dysfunction (https://aopwiki.org/aops/30)	AdverseOutcome
Aop:100 - Cyclooxygenase inhibition leading to reproductive dysfunction via inhibition of female spawning behavior (https://aopwiki.org/aops/100)	AdverseOutcome
Aop:122 - Prolyl hydroxylase inhibition leading to reproductive dysfunction via increased HIF1 heterodimer formation (https://aopwiki.org/aops/122)	AdverseOutcome
Aop:123 - Unknown MIE leading to reproductive dysfunction via increased HIF-1alpha transcription (https://aopwiki.org/aops/123)	AdverseOutcome
Aop:155 - Deiodinase 2 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/155)	AdverseOutcome
Aop:156 - Deiodinase 2 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/156)	AdverseOutcome
Aop:157 - Deiodinase 1 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/157)	AdverseOutcome
Aop:158 - Deiodinase 1 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/158)	AdverseOutcome
Aop:159 - Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	AdverseOutcome
Aop:101 - Cyclooxygenase inhibition leading to reproductive dysfunction via inhibition of pheromone release (https://aopwiki.org/aops/101)	AdverseOutcome

AOP ID and Name	Event Type
Aop:102 - Cyclooxygenase inhibition leading to reproductive dysfunction via interference with meiotic prophase I /metaphase I transition (https://aopwiki.org/aops/102)	AdverseOutcome
Aop:63 - Cyclooxygenase inhibition leading to reproductive dysfunction (https://aopwiki.org/aops/63)	AdverseOutcome
Aop:103 - Cyclooxygenase inhibition leading to reproductive dysfunction via interference with spindle assembly checkpoint (https://aopwiki.org/aops/103)	AdverseOutcome
Aop:290 - DNA methyltransferase inhibition leading to reduced fecundity associated population decline (https://aopwiki.org/aops/290)	AdverseOutcome
Aop:291 - DNA methyltransferase inhibition leading to transgenerational DNA methylation associated population decline (https://aopwiki.org/aops/291)	AdverseOutcome
Aop:292 - Inhibition of tyrosinase leads to decreased population in fish (https://aopwiki.org/aops/292)	AdverseOutcome
Aop:310 - Embryonic Activation of the AHR leading to Reproductive failure, via epigenetic down-regulation of GnRHR (https://aopwiki.org/aops/310)	AdverseOutcome
Aop:16 - Acetylcholinesterase inhibition leading to acute mortality (https://aopwiki.org/aops/16)	AdverseOutcome
Aop:312 - Acetylcholinesterase Inhibition leading to Acute Mortality via Impaired Coordination & Movement (https://aopwiki.org/aops/312)	AdverseOutcome
Aop:334 - Glucocorticoid Receptor Agonism Leading to Impaired Fin Regeneration (https://aopwiki.org/aops/334)	AdverseOutcome
Aop:336 - DNA methyltransferase inhibition leading to population decline (#1) (https://aopwiki.org/aops/336)	AdverseOutcome
Aop:337 - DNA methyltransferase inhibition leading to population decline (#2) (https://aopwiki.org/aops/337)	AdverseOutcome
Aop:338 - DNA methyltransferase inhibition leading to population decline (#3) (https://aopwiki.org/aops/338)	AdverseOutcome
Aop:339 - DNA methyltransferase inhibition leading to population decline (#4) (https://aopwiki.org/aops/339)	AdverseOutcome
Aop:340 - DNA methyltransferase inhibition leading to transgenerational effects (#1) (https://aopwiki.org/aops/340)	AdverseOutcome
Aop:341 - DNA methyltransferase inhibition leading to transgenerational effects (#2) (https://aopwiki.org/aops/341)	AdverseOutcome
Aop:289 - Inhibition of 5α-reductase leading to impaired fecundity in female fish (https://aopwiki.org/aops/289)	AdverseOutcome
Aop:297 - Inhibition of retinaldehyde dehydrogenase leads to population decline (https://aopwiki.org/aops/297)	AdverseOutcome
Aop:346 - Aromatase inhibition leads to male-biased sex ratio via impacts on gonad differentiation (https://aopwiki.org/aops/346)	AdverseOutcome

Biological Context

Level of Biological Organization
Population

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
all species	all species		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=0)

Life Stage Applicability

Life Stage	Evidence
All life stages	Not Specified

Sex Applicability

Sex	Evidence
Unspecific	Not Specified

Consideration of population size and changes in population size over time is potentially relevant to all living organisms.

Key Event Description

Maintenance of sustainable fish and wildlife populations (i.e., adequate to ensure long-term delivery of valued ecosystem services) is an accepted regulatory goal upon which risk assessments and risk management decisions are based.

How it is Measured or Detected

Population trajectories, either hypothetical or site specific, can be estimated via population modeling based on measurements of vital rates or reasonable surrogates measured in laboratory studies. As an example, Miller and Ankley 2004 used measures of cumulative fecundity from laboratory studies with repeat spawning fish species to predict population-level consequences of continuous exposure.

Regulatory Significance of the AO

Maintenance of sustainable fish and wildlife populations (i.e., adequate to ensure long-term delivery of valued ecosystem services) is a widely accepted regulatory goal upon which risk assessments and risk management decisions are based.

References

- Miller DH, Ankley GT. 2004. Modeling impacts on populations: fathead minnow (Pimephales promelas) exposure to the endocrine disruptor 17ß-trenbolone as a case study. Ecotoxicology and Environmental Safety 59: 1-9.

Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

Relationship: 45: Inhibition, Aromatase leads to Reduction, 17beta-estradiol synthesis by ovarian granulosa cells (https://aopwiki.org/relationships/45)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Aromatase inhibition leading to reproductive dysfunction (https://aopwiki.org/aops/25)	**adjacent**	**High**	**Moderate**

Evidence Supporting Applicability of this Relationship

Aromatase (CYP19) orthologs are known to be present among most of the vertebrate lineage, at least down to the cartilaginous fishes. Orthologs have generally not been found in invertebrates, however, CYP19 was detected in the invertebrate chordate, amphioxus and analysis of conservation of gene order and content suggests a possible origin among primitive chordates (Castro et al. 2005).

Evidence Supporting this KER

Biological Plausibility

Within the ovary, aromatase expression and activity is primarily localized in the granulosa cells (reviewed in (Norris 2007; Yaron 1995; Havelock et al. 2004) and others). C-19 androgens diffuse from the theca cells into granulosa cells where aromatase can catalyze their conversion to C-18 estrogens. Therefore, inhibition of ovarian aromatase activity can generally be assumed to directly impact E2 synthesis by the granulosa cells.

Empirical Evidence

- Known aromatase inhibitors including fadrozole and prochloraz were shown to cause concentration-dependent inhibition of aromatase activity in fathead minnow ovary homogenates (Villeneuve et al. 2006; Ankley et al. 2005).
- Fadrozole and prochloraz also cause concentration-dependent decreases in E2 production by fathead minnow ovary explants exposed in vitro (Villeneuve et al. 2007).
- Following in vivo exposure to fadrozole or prochloraz, ex vivo E2 production is significantly decreased in a concentration-dependent manner early in the time-course following exposure, although depending on the concentration, compensatory responses may offset the direct impact later in the exposure time-course (Villeneuve et al. 2006; Villeneuve et al. 2009; Ankley et al. 2009a; Skolness et al. 2011).

Uncertainties and Inconsistencies
Based on the limited set of studies available to date, there are no known inconsistencies.

References

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Relationship: 5: Reduction, 17beta-estradiol synthesis by ovarian granulosa cells leads to Reduction, Plasma 17beta-estradiol concentrations (https://aopwiki.org/relationships/5)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Aromatase (Cyp19a1) reduction leading to impaired fertility in adult female (https://aopwiki.org/aops/7)	**adjacent**	**High**	
Aromatase inhibition leading to reproductive dysfunction (https://aopwiki.org/aops/25)	**adjacent**	**High**	**Moderate**
Androgen receptor agonism leading to reproductive dysfunction (in repeat-spawning fish) (https://aopwiki.org/aops/23)	**adjacent**	**High**	**Low**
Prolyl hydroxylase inhibition leading to reproductive dysfunction via increased HIF1 heterodimer formation (https://aopwiki.org/aops/122)	**adjacent**	**High**	**Moderate**
Unknown MIE leading to reproductive dysfunction via increased HIF-1alpha transcription (https://aopwiki.org/aops/123)	**adjacent**		

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Embryonic Activation of the AHR leading to Reproductive failure, via epigenetic down-regulation of GnRHR (https://aopwiki.org/aops/310)	**adjacent**	**High**	**Moderate**

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
mouse	Mus musculus	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
rat	Rattus norvegicus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
fathead minnow	Pimephales promelas	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=90988)
Fundulus heteroclitus	Fundulus heteroclitus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8078)

Life Stage Applicability

Life Stage	Evidence
Adult, reproductively mature	High

Sex Applicability

Sex	Evidence
Female	High

Key enzymes needed to synthesize 17β-estradiol first appear in the common ancestor of amphioxus and vertebrates (Baker 2011). While some E2 synthesis can occur in other tissues, the ovary is recognized as the major source of 17β-estradiol synthesis in female vertebrates. Endocrine actions of ovarian E2 are facilitated through transport via the plasma. Consequently, this key event relationship is applicable to most female vertebrates.

Key Event Relationship Description

See plausibility, below.

Evidence Supporting this KER

Updated 03/20/2017.

Biological Plausibility

While brain, interrenal, adipose, and breast tissue (in mammals) are capable of synthesizing estradiol, the gonads are generally considered the major source of circulating estrogens in vertebrates, including fish (Norris 2007). Consequently, if estradiol synthesis by ovarian granulosa cells is reduced, plasma E2 concentrations would be expected to decrease unless there are concurrent reductions in the rate of E2 catabolism. Synthesis in other tissues generally plays a paracrine role only, thus the contribution of other tissues to plasma E2 concentrations can generally be considered negligible.

Empirical Evidence

Include consideration of temporal concordance here

Fish

- In multiple studies with aromatase inhibitors (e.g., fadrozole, prochloraz), significant reductions in ex vivo E2 production have been linked to, and shown to precede, reductions in circulating E2 concentrations (Villeneuve et al. 2009; Skolness et al. 2011). It is also notable that

compensatory responses at the level of ex vivo steroid production (i.e., rate of E2 synthesis per unit mass of tissue) tend to precede recovery of plasma E2 concentrations following an initial insult (Villeneuve et al. 2009; Ankley et al. 2009a; Villeneuve et al. 2013).
- Ex vivo E2 production by ovary tissue collected from female fish exposed to 30 or 300 µg ketoconazole/L showed significant decreases prior to significant effects on plasma estradiol being observed (Ankley et al. 2012).
- Ekman et al. (2011) reported significant reductions in ex vivo E2 production and plasma E2 concentrations in female fathead minnows exposed to 0.05 ug/L 17ß-trenbolone. The effect on plasma E2 was observed at an earlier time point (24 h, versus 48 h for E2 production.
- Rutherford et al. (2015) reported significant reductions in both E2 production and circulating E2 concentrations in female Fundulus heteroclitus exposed to 5alpha-dihydrotestosterone or 17alpha-methyltestosterone for 14 d. The effects were equipotent in the case of 17alpha-methyltestosterone, but in the case of 5alpha-dihyrotestosterone, the effect on plasma E2 could be detected at a lower dose (10 ug/L) than that at which a significant effect on E2 production was detected (100 ug/L).
- In female Fundulus heteroclitus exposed to 17alpha-methyltestosterone for 7 or 14 d, both E2 production and plasma E2 were impacted at the same exposure concentrations (Sharpe et al. 2004).

Mammals

- MEHP /DEHP, mice, ex vivo DEHP (10 -100 µg/ml); MEHP (0.1 and 10 µg/ml) dose dependent reduction E2 production (Gupta et al., 2010)
- DEHP, rat, in vivo 300-600 mg/kg/day, dose dependent reduction of E2 plasma levels (Xu et al., 2010)

Evidence for rodent and human models is summarized in Table 1.

Compound class	Species	Study type	Dose	E2 production/levels	Reference
Phthalates (DEHP)	rat	ex vivo	1500 mg/kg/day	Reduced/increased E2 production in ovary culture	(Laskey & Berman, 1993)
Phthalates (MEHP)	rat	in vitro	From 50 µM	Reduced E2 production (concentration and time dependent in Granulosa cell)	(Davis, Weaver, Gaines, & Heindel, 1994)
Phthalates (MEHP)	rat	in vitro	100-200µM	reduction E2 production (dose dependent)	(Lovekamp & Davis, 2001)
Phthalates (DEHP)	rat	in vivo	300-600 mg/kg/day	reduction E2 levels dose dependent	(Xu et al., 2010),
Phthalates (MEHP)	human	in vitro	IC(50)= 49- 138 µM (dependent on the stimulant)	reduction E2 production (dose dependent)	(Reinsberg, Wegener-Toper, van der Ven, van der Ven, & Klingmueller, 2009)
Phthalates (MEHP/DEHP)	mice	ex vivo	DEHP (10 -100 µg/ml); MEHP (0.1 and 10 µg/ml)	reduction E2 production (dose dependent)	(Gupta et al., 2010)

Table 1. Summary of the experimental data for decrease E2 production and decreased E2 levels. IC50- half maximal inhibitory concentration values reported if available, otherwise the concentration at which the effect was observed.

Uncertainties and Inconsistencies

Based on the limited set of studies available to date, there are no known inconsistencies.

References

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Relationship: 252: Reduction, Plasma 17beta-estradiol concentrations leads to Reduction, Vitellogenin synthesis in liver (https://aopwiki.org/relationships/252)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Aromatase inhibition leading to reproductive dysfunction (https://aopwiki.org/aops/25)	**adjacent**	**High**	**Moderate**
Androgen receptor agonism leading to reproductive dysfunction (in repeat-spawning fish) (https://aopwiki.org/aops/23)	**adjacent**	**High**	**Moderate**
Prolyl hydroxylase inhibition leading to reproductive dysfunction via increased HIF1 heterodimer formation (https://aopwiki.org/aops/122)	**adjacent**	**High**	**Moderate**
Unknown MIE leading to reproductive dysfunction via increased HIF-1alpha transcription (https://aopwiki.org/aops/123)	**adjacent**		
Embryonic Activation of the AHR leading to Reproductive failure, via epigenetic down-regulation of GnRHR (https://aopwiki.org/aops/310)	**adjacent**	**High**	**Moderate**

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
fathead minnow	Pimephales promelas	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=90988)

Life Stage Applicability

Life Stage	Evidence
Adult, reproductively mature	Not Specified

Sex Applicability

Sex	Evidence
Female	High

Key enzymes needed to synthesize 17β-estradiol first appear in the common ancestor of amphioxus and vertebrates (Baker 2011). However, non-oviparous vertebrates do not require vitellogenin. Consequently, this KER is applicable to oviparous vertebrates.

Key Event Relationship Description

See Plausibility below.

Evidence Supporting this KER

Updated 2017-03-17.

Biological Plausibility
Vitellogenin synthesis in fish is localized in the liver and is well documented to be regulated by estrogens via interaction with estrogen receptors (Tyler et al. 1996; Tyler and Sumpter 1996; Arukwe and Goksøyr 2003). The vitellogenin gene contains estrogen repsonsive elements in its promoter region and site directed mutagenesis has shown these to be essential for estrogen-dependent expression of vitellogenin (Chang et al. 1992; Teo et al. 1998). Liver is not regarded as a major site of E2 synthesis (Norris 2007), therefore the majority of E2 in liver comes from the circulation.

- Estrogen regulates expression of the vitellogenin gene in the amphibian Xenopus laevis (Skipper and Hamilton, 1977).

Empirical Evidence

- Empirical support for estrogen-dependent regulation of vitellogenin synthesis:
  - Many studies have demonstrated that exposure of hepatocytes to estrogens in vitro or in vivo induce vitellogenin mRNA synthesis (e.g., see reviews by (Navas and Segner 2006; Iguchi et al. 2006)).
  - In female fathead minnows exposed to 17β-trenbolone, significant reductions in plasma E2 concentrations preceded significant reductions in plasma VTG (Ekman et al. 2011).
  - Intra-arterial injection of the estrogen 17α ethynyl estradiol into male rainbow trout causes vitellogenin induction with about a 12 h lag time before increasing from basal levels (Schultz et al. 2001).
- Specific empirical support for reductions in plasma E2 leading to reductions in hepatic vitellogenin synthesis:
  - In a number of time-course experiments with aromatase inhibitors (e.g., fadrozole, prochloraz), decreases in plasma estradiol concentrations precede decreases in plasma vitellogenin concentrations (Villeneuve et al. 2009; Skolness et al. 2011; Ankley et al. 2009b). Recovery of plasma E2 concentrations also precedes recovery of plasma VTG concentrations after cessation of exposure (Villeneuve et al. 2009; Ankley et al. 2009a; Villeneuve et al. 2013).
  - It was demonstrated in Danio rerio that in vivo exposure to the aromatase inhibitor letrozole significantly reduced the expression of mRNA transcripts coding for vtg1, vtg2, and erα, all of which are known to be regulated by estrogens (Sun et al. 2010). However, similar effects were not observed in primary cultured hepatocytes from Danio rerio, indicating that letrozole's effects on vtg transcription were not direct.
  -

Uncertainties and Inconsistencies
Based on the limited set of studies available to date, there are no known inconsistencies.

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Relationship: 94: Reduction, Cumulative fecundity and spawning leads to Decrease, Population trajectory (https://aopwiki.org/relationships/94)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Aromatase inhibition leading to reproductive dysfunction (https://aopwiki.org/aops/25)	**adjacent**	**Moderate**	**Moderate**
Androgen receptor agonism leading to reproductive dysfunction (in repeat-spawning fish) (https://aopwiki.org/aops/23)	**adjacent**	**Moderate**	**Moderate**
Estrogen receptor antagonism leading to reproductive dysfunction (https://aopwiki.org/aops/30)	**adjacent**	**Moderate**	**Moderate**
Prolyl hydroxylase inhibition leading to reproductive dysfunction via increased HIF1 heterodimer formation (https://aopwiki.org/aops/122)	**adjacent**		
Unknown MIE leading to reproductive dysfunction via increased HIF-1alpha transcription (https://aopwiki.org/aops/123)	**adjacent**		
Embryonic Activation of the AHR leading to Reproductive failure, via epigenetic down-regulation of GnRHR (https://aopwiki.org/aops/310)	**adjacent**	**Moderate**	**Moderate**
Inhibition of 5α-reductase leading to impaired fecundity in female fish (https://aopwiki.org/aops/289)	**adjacent**	**High**	**High**

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
fathead minnow	Pimephales promelas	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=90988)

Life Stage Applicability

Life Stage	Evidence
All life stages	Not Specified

Sex Applicability

Sex	Evidence
Unspecific	Not Specified

Spawning generally refers to the release of eggs and/or sperm into water, generally by aquatic or semi-aquatic organisms. Consequently, by definition, this KER is likely applicable only to organisms that spend a portion of their life-cycle in or near aquatic environments.

Key Event Relationship Description

SEE BIOLOGICAL PLAUSIBILITY BELOW

Evidence Supporting this KER

Updated 03/20/2017

Biological Plausibility

Using a relatively simple density-dependent population model and assuming constant young of year survival with no immigration/emigration, reductions in cumulative fecundity have been predicted to yield declines in population size over time (Miller and Ankley 2004). Under real-world environmental conditions, outcomes may vary depending on how well conditions conform with model assumptions. Nonetheless, cumulative fecundity can be considered one vital rate that contributes to overall population trajectories (Kramer et al. 2011).

Empirical Evidence

- Using a relatively simple density-dependent population model and assuming constant young of year survival with no immigration/emigration, reductions in cumulative fecundity have been predicted to yield declines in population size over time (Miller and Ankley 2004). However, it should be noted that the model was constructed in such a way that predicted population size is dependent on cumulative fecundity, therefore this is a fairly weak form of empirical support.
- In a study in which an entire lake was treated with 17alpha-ethynyl estradiol, Kidd et al. (2007) declines in fathead minnow population size were associated with signs of reduced fecundity.

Uncertainties and Inconsistencies

- Wester et al. (2003) and references cited therein suggest that although egg production is an endpoint of demographic significance, incomplete reductions of egg production may not translate in a simple manner to population reductions. Compensatory effects of reduced predation and reduced competition for limited food and/or habitat resources may offset the effects of incomplete reductions in egg production.
- Fish and other egg laying animals employ a diverse range of reproductive strategies and life histories. The nature of the relationship between reduced spawning frequency and cumulative fecundity and overall population trajectories will depend heavily on the life history and reproductive strategy of the species in question. Relationships developed for one species will not necessarily hold for other species, particularly those with differing life histories.

References

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Relationship: 337: Reduction, Vitellogenin accumulation into oocytes and oocyte growth/development leads to Reduction, Cumulative fecundity and spawning (https://aopwiki.org/relationships/337)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Aromatase inhibition leading to reproductive dysfunction (https://aopwiki.org/aops/25)	**adjacent**	**Moderate**	**Moderate**

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Androgen receptor agonism leading to reproductive dysfunction (in repeat-spawning fish) (https://aopwiki.org/aops/23)	**adjacent**	**Moderate**	**Moderate**
Estrogen receptor antagonism leading to reproductive dysfunction (https://aopwiki.org/aops/30)	**adjacent**	**Moderate**	**Moderate**
Prolyl hydroxylase inhibition leading to reproductive dysfunction via increased HIF1 heterodimer formation (https://aopwiki.org/aops/122)	**adjacent**		
Unknown MIE leading to reproductive dysfunction via increased HIF-1alpha transcription (https://aopwiki.org/aops/123)	**adjacent**		
Embryonic Activation of the AHR leading to Reproductive failure, via epigenetic down-regulation of GnRHR (https://aopwiki.org/aops/310)	**adjacent**	**Moderate**	**Moderate**

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
fathead minnow	Pimephales promelas	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=90988)
Oryzias latipes	Oryzias latipes	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8090)

Life Stage Applicability

Life Stage	Evidence
Adult, reproductively mature	High

Sex Applicability

Sex	Evidence
Female	High

On the basis of the taxonomic relevance of the two KEs linked via this KER, this KER is likely applicable to aquatic, oviparous, vertebrates which both produce vitellogenin and deposit eggs/sperm into an aquatic environment.

Key Event Relationship Description

SEE BIOLOGICAL PLAUSIBILITY BELOW

Evidence Supporting this KER

Biological Plausibility

Vitellogenesis is a critical stage of oocyte development and accumulated lipids and yolk proteins make up the majority of oocyte biomass (Tyler and Sumpter 1996). At least in mammals, maintenance of meiotic arrest is supported by signals transmitted through gap junctions between the granulosa cells and oocytes (Jamnongjit and Hammes 2005). Disruption of oocyte-granulosa contacts as a result of cell growth has been shown to coincide with oocyte maturation (Eppig 1994). However, it remains unclear whether the relationship between vitellogenin accumulation and oocyte growth and eventual maturation is causal or simply correlative.

Empirical Evidence

- At present, to our best knowledge there are no studies that definitively demonstrate a direct cause-effect relationship between impaired VTG accumulation into oocytes and impaired spawning. There is, however, strong correlative evidence. Across a range of laboratory studies with small fish, there is a robust and statistically significant correlation between reductions in circulating VTG concentrations and reductions in cumulative fecundity (Miller et al. 2007). To date, we are unaware of any fish reproduction studies which show a large reduction in circulating VTG concentrations, but not reductions in cumulative fecundity.
- Ankley et al. (2003) reported significant reductions in VTG accumulation in oocytes along with significant reductions in cumulative fecundity, although fecundity was significantly impacted at a lower dose (0.05 ug/L 17beta-trenbolone versus 0.5 ug/L for VTG accumulation).
- Kang et al. (2008) reported significant reductions in both VTG accumulation in occytes and cumulative fecundity in Japanese medaka, with

cumulative fecundity being impacted at slightly lower concentrations (0.047 ug 17alpha-methyltestosterone/L versus 0.088 ug/L).

Uncertainties and Inconsistencies

Based on the limited number of studies available that have examined both of these KEs, there are no known, unexplained, results that are inconsistent with this relationship.

References

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Relationship: 255: Reduction, Plasma vitellogenin concentrations leads to Reduction, Vitellogenin accumulation into oocytes and oocyte growth/development (https://aopwiki.org/relationships/255)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Androgen receptor agonism leading to reproductive dysfunction (in repeat-spawning fish) (https://aopwiki.org/aops/23)	**adjacent**	**Moderate**	**Low**
Aromatase inhibition leading to reproductive dysfunction (https://aopwiki.org/aops/25)	**adjacent**	**Moderate**	**Low**
Estrogen receptor antagonism leading to reproductive dysfunction (https://aopwiki.org/aops/30)	**adjacent**	**Moderate**	**Low**
Prolyl hydroxylase inhibition leading to reproductive dysfunction via increased HIF1 heterodimer formation (https://aopwiki.org/aops/122)	**adjacent**	**Moderate**	
Unknown MIE leading to reproductive dysfunction via increased HIF-1alpha transcription (https://aopwiki.org/aops/123)	**adjacent**		
Embryonic Activation of the AHR leading to Reproductive failure, via epigenetic down-regulation of GnRHR (https://aopwiki.org/aops/310)	**adjacent**	**Moderate**	**Low**

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
fathead minnow	Pimephales promelas	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=90988)
Oryzias latipes	Oryzias latipes	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8090)

Life Stage Applicability

Life Stage	Evidence
Adult, reproductively mature	High

Sex Applicability

Sex	Evidence
Female	High

This KER is expected to be primarily applicable to oviparous vertebrates that synthesize vitellogenin in hepatic tissue which is ultimately incorporated into oocytes present in the ovary.

Key Event Relationship Description

SEE BIOLOGICAL PLAUSIBILITY BELOW

Evidence Supporting this KER

Biological Plausibility
Vitellogenin synthesized in the liver and transported to the ovary via the circulation is the primary source of egg yolk proteins in fish (Wallace and Selman 1981; Tyler and Sumpter 1996; Arukwe and Goksøyr 2003). In many teleosts vitellogenesis can account for up to 95% of total egg size (Tyler and Sumpter 1996).

Empirical Evidence
In some (Ankley et al. 2002; Ankley et al. 2003; Lalone et al. 2013), but not all (Ankley et al. 2005; Sun et al. 2007; Skolness et al. 2013) fish reproduction studies, reductions in plasma vitellogenin have been associated with visible decreases in yolk protein content in oocytes and overall reductions in ovarian stage.

Uncertainties and Inconsistencies
Not all fish reproduction studies showing reductions in plasma vitellogenin have caused visible decreases in yolk protein content in oocytes and overall reductions in ovarian stage. (Ankley et al. 2005; Sun et al. 2007; Skolness et al. 2013).

While plasma vitellogenin is well established as the only major source of vitellogenins to the oocyte, the extent to which a decrease will impact an ovary that has already developed vitellogenic staged oocytes is less certain. It would be assumed that the more rapid the turn-over of oocytes in the ovary, the tighter the linkage between these KEs. Thus, repeat spawning species with asynchronous oocyte development that spawn frequently would likely be more vulnerable than annual spawning species with synchronous oocyte development that had already reached late vitellogenic stages.

References

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Relationship: 315: Reduction, Vitellogenin synthesis in liver leads to Reduction, Plasma vitellogenin concentrations (https://aopwiki.org/relationships/315)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Aromatase inhibition leading to reproductive dysfunction (https://aopwiki.org/aops/25)	**adjacent**	**High**	**Moderate**
Androgen receptor agonism leading to reproductive dysfunction (in repeat-spawning fish) (https://aopwiki.org/aops/23)	**adjacent**	**High**	**Moderate**
Estrogen receptor antagonism leading to reproductive dysfunction (https://aopwiki.org/aops/30)	**adjacent**	**High**	**Moderate**

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Prolyl hydroxylase inhibition leading to reproductive dysfunction via increased HIF1 heterodimer formation (https://aopwiki.org/aops/122)	**adjacent**	**High**	**High**
Unknown MIE leading to reproductive dysfunction via increased HIF-1alpha transcription (https://aopwiki.org/aops/123)	**adjacent**		
Embryonic Activation of the AHR leading to Reproductive failure, via epigenetic down-regulation of GnRHR (https://aopwiki.org/aops/310)	**adjacent**	**High**	**Moderate**

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
fathead minnow	Pimephales promelas	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=90988)

Life Stage Applicability

Life Stage	Evidence
Adult, reproductively mature	Not Specified

Sex Applicability

Sex	Evidence
Unspecific	Not Specified

This KER primarily applies to taxa that synthesize vitellogenin in the liver which is transported elsewhere in the body via plasma (i.e., oviparous vertebrates).

Key Event Relationship Description

See biological plausibility, below.

Evidence Supporting this KER

Updated 03/20/2017.

Biological Plausibility
Liver is the major source of VTG protein production in fish (Tyler and Sumpter 1996; Arukwe and Goksøyr 2003). Protein production involves transcription and subsequent translation. The time-lag between decreases in transcription/translation and decreases in plasma VTG concentrations can be expected to be dependent on vitellogenin elimination half-lives.

Empirical Evidence

- In a number of time-course experiments with aromatase inhibitors, decreases in plasma estradiol concentrations precede decreases in plasma vitellogenin concentrations (Villeneuve et al. 2009; Skolness et al. 2011; Ankley et al. 2009b). Recovery of plasma E2 concentrations also precedes recovery of plasma VTG concentrations after cessation of exposure (Villeneuve et al. 2009; Ankley et al. 2009a; Villeneuve et al. 2013).
- In experiments with strong estrogens, increases in vtg mRNA synthesis precede increases in plasma VTG concentration (Korte et al. 2000; Schmid et al. 2002).
- Elimination half-lives for VTG protein have been determined for induced male fish, but to our knowledge, similar kinetic studies have not been done for reproductively mature females (Korte et al. 2000; Schultz et al. 2001).
- In male sheepshead minnows injected with E2, induction of VTG mRNA precedes induction of plasma VTG (Bowman et al. 2000).
- In male Cichlasoma dimerus exposed to octylphenol for 28 days and then held in clean water, decline in induced VTG mRNA concentrations precedes declines in induced plasma VTG concentrations (Genovese et al. 2012).

Uncertainties and Inconsistencies
There are no known inconsistencies between these KERs which are not readily explained on the basis of the expected dose, temporal, and incidence relationships between these two KERs. This applies across a significant body of literature in which these two KEs have been measured.

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List of Non Adjacent Key Event Relationships

Relationship: 1386: Reduction, Plasma 17beta-estradiol concentrations leads to Reduction, Plasma vitellogenin concentrations (https://aopwiki.org/relationships/1386)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Androgen receptor agonism leading to reproductive dysfunction (in repeat-spawning fish) (https://aopwiki.org/aops/23)	**non-adjacent**	**High**	**Moderate**
Aromatase inhibition leading to reproductive dysfunction (https://aopwiki.org/aops/25)	**non-adjacent**	**High**	**Moderate**
Inhibition of thyroid peroxidase leading to impaired fertility in fish (https://aopwiki.org/aops/271)	**adjacent**		
Inhibition of 5α-reductase leading to impaired fecundity in female fish (https://aopwiki.org/aops/289)	**adjacent**	**High**	**High**
Embryonic Activation of the AHR leading to Reproductive failure, via epigenetic down-regulation of GnRHR (https://aopwiki.org/aops/310)	**non-adjacent**	**High**	**Moderate**

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
fathead minnow	Pimephales promelas	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=90988)
Fundulus heteroclitus	Fundulus heteroclitus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8078)

Life Stage Applicability

Life Stage	Evidence
Adult, reproductively mature	High

Sex Applicability

Sex	Evidence
Female	High

This key event relationship likely applies to oviparous vertebrates only.

- Key enzymes needed to synthesize 17β-estradiol first appear in the common ancestor of amphioxus and vertebrates (Baker 2011).
- Vitellogenesis is common to a range of egg-laying vertebrates and invertebrates. However, in the case of invertebrates, vitellogenins are transported via hemolymph rather than plasma and vitellogenesis is regulated by invertebrate hormones, not estradiol.

Key Event Relationship Description

There is not a direct structural/functional relationship between reduced concentrations of 17ß-estradiol in plasma and reduced plasma VTG concentrations. The relationship is thought to be mediated through additional events of hepatic estrogen receptor activation, vitellogenin protein synthesis in the liver, and subsequent secretion of vitellogenin into the plasma.

Evidence Supporting this KER

Updated 2017-03-17

Biological Plausibility

The mechanisms through which 17ß-estradiol stimulates the transcription and translation of hepatic vitellogenin are well understood.

- In fish, see: Tyler et al. 1996; Tyler and Sumpter 1996; Arukwe and Goksøyr 2003; Teo et al. 1998
- In frogs: Chang et al. 1992; Wangh and Knowland 1975
- In reptiles: Ho et al. 1980
- Ho (1987)
- In birds: Deeley et al. 1975;

17ß-estradiol is not synthesized in significant amounts in the liver. Its synthesis originates in other tissues, principally the gonads. It is then transported to the liver and other tissues via circulation (Norris 2007; Payne and Hales 2004; Miller 1988; Nagahama et al. 1993).

Empirical Evidence

- Under conditions of continuous flow through exposure to 17ß-trenbolone (a non-aromatizable androgen receptor agonist), plasma E2 concentrations were reduced in female fathead minnows after 2, 4, or 8 d of exposure to concentrations of 0.05 ug/L or greater. Plasma VTG concentrations were significantly reduced only after 4 or 8 d of exposure, and at 4 d, only at a concentration of 0.5 ug/L, not 0.05 ug/L (Ekman et al. 2011).
- In the same study by Ekman et al. (2011), once exposure ceased, plasma E2 concentrations returned to control levels within 48 h, while plasma VTG concentrations remained significantly depressed until d. 4, post-exposure.
- Ankley et al. (2003) detected reductions in both plasma E2 and plasma VTG in female fathead minnows following 21 d of continuous exposure to 17ß-trenbolone. At 21 d, plasma E2 concentrations were impacted at concentrations of 0.5 ug/L or greater, while plasma VTG was significantly reduced at 0.05 ug/L or greater.
- Villeneuve et al. (2016) observed significant reductions in both plasma E2 and plasma VTG in female fathead minnows exposed to 0.5 ug/L 17ß-trenbolone for 14 d.
- Jensen et al. (2006) observed significant reductions in both plasma E2 and plasma VTG following exposure to 0.03 ug/L 17alpha-trenbolone for 21 d.
- Following 21 d of continuous exposure to spironolactone, plasma E2 and plasma VTG were both significantly reduced in female fathead minnows. The lowest effect concentration for plasma E2 was 0.5 ug/L, while that for plasma VTG was 5 ug/L (LaLone et al. 2013).
- In female Fundulus heteroclitus exposed to 5alpha-dihydrotestosterone for 14 d, plasma E2 was significantly reduced following exposure to 10 ug/L, while plasma VTG was reduced at 100 ug/L (Rutherford et al. 2015).
- In two experiments in which female Fundulus heteroclitus were exposed to 17alpha-methyltestosterone, both plasma E2 and plasma VTG were significantly reduced. In both cases, plasma E2 was impacted at lower concentrations (0.25 ug/L in a 7 d study; 0.01 ug/L in a 14 d study) than plasma VTG (1 ug/L in the 7 d study; 0.1 ug/L in the 14 d study; Sharpe et al. 2004).
- In two experiments where plasma E2 and plasma VTG were measured in female fathead minnows (Pimephales promelas) in a time-course following continuous exposure the aromatase inhibitor fadrozole, both plasma VTG and plasma E2 were depressed (Villeneuve et. al. 2009;

2013). In both cases, following cessation of exposure, plasma E2 concentrations recovered to control levels before plasma VTG concentrations recovered (Villeneuve et al. 2009; 2013).
- Shroeder et al. (in preparation) reported effects on plasma E2 concentrations within 4 h of initiating exposure to 5 or 50 ug/L fadrozole. Plasma VTG concentrations did not decline until 24 h or later (Schroeder et al. 2009; Villeneuve et al. 2009; 2013).
- In female fathead minnows exposed to 300 ug/L prochloraz, plasma E2 concentrations were significantly reduced after 12 h of exposure, while plasma VTG concentrations were not significantly reduced until 24 h of exposure (Skolness et al. 2011).
- Ankley et al. (2009) reported significant reductions in plasma E2 in female fathead minnows following 24 h of exposure to 30 ug/L prochloraz. In the same study, plasma VTG concentrations did not significantly decline until 48 h of exposure, and then only at 300 ug/L prochloraz.
- In a 21 d exposure to prochloraz, plasma E2 was significantly reduced in females exposed to 300 ug prochloraz/L, while plasma VTG was significantly reduced in females exposed to 100 ug/L (Ankley et al. 2005).

Uncertainties and Inconsistencies
- In several studies, significant decreases in plasma vitellogenin are detected at lower concentrations than those that result in significant decreases in plasma E2. However, detection of differences in plasma VTG is ofen enhanced by the greater dynamic range in the concentrations of the protein that occur in plasma, compared to the dynamic range of steroid hormone concentrations.

Quantitative Understanding of the Linkage

- A computational model developed by Cheng et al. (2016) is capable of simulating altered plasma VTG concentrations associated with changes in plasma E2 concentrations in female fathead minnows. This model has been used to generate a quantitative response-response relationship that can predict steady state plasma VTG concentrations for a given steady state plasma E2 concentration (Conolly et al. 2017).
    - The model and response-response relationship were developed based on data from exposures to the model aromatase inhibitor fadrozole. The validity of the model-based predictions/relationships for other stressors and species has not yet been established.
- Li et al. (2011) also developed a physiologically-based computational model of the adult female fathead minnow (*Pimephales promelas*) hypothalamic-pituitary-gonadal axis. Conceptually, this model could also be applied to derive a quantitative response-response relationship between plasma E2 and plasma VTG concentrations. The Li et al. model was calibrated based on data from exposures to 17alpha-ethynylestradiol and 17ß-trenbolone. Neither its validity for other stressors or speices, nor its agreement with the Cheng et al. (2016) model have been examined in detail.

Response-response relationship

Under long term, steady state exposure conditions, the following equation can be used to estimate the µM concentration of plasma vitellogenin (downstream event) from the µM concentration of plasma 17ß-estradiol.

y=0.2855e^(365.55x) (https://aopwiki.org/system/dragonfly/production/2018/10/18/6jgav91rlv_VTG_E2.pdf)

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