AOP ID and Title:

AOP 536: Estrogen receptor agonism leading to reduced survival and population growth due to renal failure
Short Title: ER agonism leads to reduced survival/population growth

Authors

Status

Author status	OECD status	OECD project	SAAOP status
Under development: Not open for comment. Do not cite			

Abstract

This adverse outcome pathway details the linkage between binding and activation of estrogen receptor as a nuclear transcription factor, primarily in oviparous male vertebrates, and a decrease in population growth. Estrogen receptors are ligand-dependent transcription factors that regulate gene transcription through estrogen response elements, allowing for the normal biological functions of estrogens (Klinge, 2001). However, various chemicals/classes of chemicals have been shown to act as ER agonists with the most potent being estradiol (E2) and ethinylestradiol (EE2) (Aarts et al., 2013). Numerous compounds including polycyclic aromatic hydrocarbons, chlorinated chemicals (e.g. PCBs), plasticizers (e.g. phthalates), and phenolic industrial chemicals (e.g., alkylphenols, parabens), and plant sterols also interact with ERα in vitro, with the potential to produce in vivo estrogenic effects (Ng et al., 2014; Pillon et al., 2005). A well characterized response to ER agonists involves hepatic production of vitellogenin (VTG; egg yolk precursor protein). Induction of *vtg* mRNA can result in elevated plasma VTG, particularly in oviparous male vertebrates, and can potentially cause downstream issues such as renal failure and morbidity. This AOP is relevant to both sexes, although more so to males as they do not produce VTG under normal conditions and have no mechanism for readily excreting the lipoprotein (Sumpter & Jobling, 1995). While many aspects of the biology underlying this AOP are largely conserved across oviparous vertebrates our, focus on KER between increased plasma VTG and increased renal pathology was on freshwater fish. Therefore, caution should be used in applying the whole of the AOP beyond freshwater fish species.

Background

The key events in this AOP are well defined in the literature, particularly the early events. While this AOP was initially entered into the wiki over ten years ago it was only entered as a set of place-holder pages for which a full weight of evidence assembly had not been conducted. Following studies conducted on estrogenic PFAS, described below, there was motivation to redevelop and update the AOP.

Houck et al. (2021) used an in vitro high throughput platform to screen and categorize more than 140 structurally diverse PFAS based on their pathway-specific bioactivities including estrogenic activity. Villeneuve et al. (2023) confirmed the estrogenic activity of four diols identified by Houck et al. (2021) in 4 day *in vivo* experiment that evaluated expression of ER responsive genes in male fathead minnows. This led to the motivation to further evaluate FC10-diol, which showed the strongest response, using both male and female fathead minnows in a 21-day study. This study design allowed for the measurement of nearly all the key events within this AOP and allowed for linking activation of the ER to impacts on survival and reproduction in fish.

Summary of the AOP

Events

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

Sequence	Type	Event ID	Title	Short name
	MIE	111	Agonism, Estrogen receptor	Agonism, Estrogen receptor
	KE	307	Increase, Vitellogenin synthesis in liver	Increase, Vitellogenin synthesis in liver
	KE	220	Increase, Plasma vitellogenin concentrations	Increase, Plasma vitellogenin concentrations
	KE	252	Increase, Renal pathology due to VTG deposition	Increase, Renal pathology due to VTG deposition
	KE	351	Increased Mortality	Increased Mortality
	KE	360	Decrease, Population growth rate	Decrease, Population growth rate

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Agonism, Estrogen receptor	adjacent	Increase, Vitellogenin synthesis in liver	High	Low
Increase, Vitellogenin synthesis in liver	adjacent	Increase, Plasma vitellogenin concentrations	High	Moderate
Increase, Plasma vitellogenin concentrations	adjacent	Increase, Renal pathology due to VTG deposition	Moderate	Low
Increase, Renal pathology due to VTG deposition	adjacent	Increased Mortality	Moderate	Low
Increased Mortality	adjacent	Decrease, Population growth rate	Moderate	Moderate

Overall Assessment of the AOP

Domain of Applicability

Sex: Although this AOP is applicable to both sexes it is far more relevant for males. This is in part because females have an excretion pathway for vitellogenin - namely deposition into oocytes which are then released into the environment. Males lack that mechanism, and thus are more likely to accumulate protein that can cause kidney pathologies.

Life stages: This AOP is applicable to all life stages following the differentiation of the liver and kidney. Larvae prior to liver and kidney differentiation should not be included.

Taxonomic: The assumed taxonomic applicability domain of this AOP is oviparous vertebrates that synthesize yolk precursor proteins and have functional kidneys.

Essentiality of the Key Events

Overall, the confidence in the supporting data for essentiality of KEs within the AOP is moderate. There is direct evidence of ER agonism leading to an increase in vitellogenin mRNA expression that is supported in multiple review articles (e.g., Matozzo et al., 2008; Palmer & Selcer, 1996; Verderame & Scudiero, 2017). Similarly, there is direct evidence *vtg* mRNA synthesis precedes increases in plasma VTG. Korte et al. (2000) observed *vtg* mRNA increase in the liver of male fathead minnows within 4 hours followed by plasma VTG increase within 16 hours of treatment. There is indirect evidence that increased plasma VTG can lead to downstream KE, renal pathology, as well as resulting in downstream AOs, mortality and decrease in population growth rate. Studies have shown that when large quantities of VTG are circulating it can lead to hyalin material accumulation in the kidneys which can cause significant pathology (e.g., Folmar et al., 2001; Herman & Kincaid, 1988). Because the kidneys perform a suite of physiological roles that are critical for organismal homeostasis including waste excretion, osmoregulation, and fluid homeostasis (Preuss, 1993), damage to the renal system, including damage caused by circulating VTG, can lead to a loss of renal functions such as decreased glomerular filtration rate or impaired clearance of waste products which can lead to mortality (McKee & Wingert, 2015). As survival rate is an obvious determinant of population size there is indirect evidence linking increased mortality to decrease in population growth rate.

Weight of Evidence Summary

The weight of evidence for each of the KERs comprising the AOP are ranked moderate to high. In particular the biological plausibility at the molecular and cellular level of the early key events is strong. The biological plausibility linking ER activation to increased *vtg* mRNA synthesis is high. Actions of endogenous and exogenous estrogens are mediated by the ER which is part of the nuclear receptor superfamily. Binding to the ligand-binding domain (LBD) of the ER initiates a series of molecular events culminating in the modulation of genes. Transcription of *vtg* is regulated by estrogens and their interaction on ERs and under high estrogen stimulation the fold increase of *vtg* transcripts increases by orders of magnitude (Brock & Shapiro, 1983). Additionally, there is high biological plausibility linking increased *vtg* mRNA synthesis to increased plasma VTG. The liver is the primary source of VTG synthesis and production and after it is synthesized it is secreted into the blood (Wallace, 1985). Vitellogenin transcription and translation results in protein production although there is a delay between expression of *vtg* and actual production/detection of VTG (e.g., Korte et al. 2000). Although a precise quantitative relationship describing all steps of vitellogenesis transcription/translation has not been described there are models and statistical relationships that define quantitative relationships between circulating E2 concentrations and circulating VTG concentrations have been developed (Ankley et al., 2008; Li et al., 2011; Murphy et al., 2009; Murphy et al., 2005).

Some uncertainties regarding the connection between increased VTG availability and the increase in renal pathology remain, resulting in our weight of evidence call as moderate. However, there is evidence that when large quantities of VTG are circulating, hyalin material can accumulate in the kidneys which can cause significant pathology (Folmar et al., 2001; Herman & Kincaid, 1988; Palace et al., 2002). Additionally, numerous studies have documented further renal pathology such as hemorrhages in kidney tubules, hypertrophy of tubular epithelia, accumulated eosinophilic material in renal tissue, and edema in the interstitium between kidney tubules (e.g., Folmar et al., 2001; Hahlbeck et al., 2004; Länge et al., 2001; Mihaich et al.,

2012; Palace et al., 2002; Zha et al., 2007). In fish exposed to estrogenic compounds there can be excessive production of VTG, which leads to renal failure, and increases mortality in fish (Herman & Kincaid, 1988). Generally, the molecular mass of proteins in glomerular filtrate are lower than albumin but when proteins like VTG are deposited in the kidneys they cannot be resorbed and the excess protein can lead to glomerular rupturing or hemorrhaging (Tojo & Kinugasa, 2012). Ultimately these pathologies can cause acute renal failure resulting in mortality. As survival rate is an obvious determinant of population size and is included in population modeling to calculate long-term persistence of the population (e.g., Miller et al., 2020) there is a moderate weight of evidence linking increased mortality to decrease in population growth rate. Numerous factors have the potential to lead to declining populations (e.g., increased mortality in the reproductive population, excessive mortality in larval population) however there is considerable evidence to support the idea that ER agonism can ultimately lead to decrease in the population growth. A notable example exposed fathead minnows to low concentrations of 17α-ethynylestradiol (EE2) in the Experimental Lakes Area, Canada. Vitellogenin mRNA and plasma was significantly elevated and, after the second season of EE2 additions to the lake, the fathead minnow population collapsed due to loss of the young-of-the-year (Kidd et al., 2007; Palace et al., 2002). Overall, there is considerable evidence to support the idea that ER agonism can ultimately lead to decrease in the population growth rate. Overall weight of evidence is moderate.

Uncertainties, inconsistencies, and data gaps

- Uncertainties related to MIE: Some uncertainty remains regarding which ER subtype(s) regulates vitellogenin gene expression in the liver of fish. In general, the literature suggests a close interplay between several ER subtypes in the regulation of vitellogenesis. Consequently, at present, the AOP is generalized to impacts on all ER subtypes, even though it remains possible that impacts on a particular sub-type may drive the adverse response.
    - Using selective agonists and antagonists for ERα and ERβ, it was concluded that ERβ was primarily responsible for inducing vitellogenin production in rainbow trout and that compounds exhibiting ERα selectivity would not be detected using a vitellogenin ELISA bioassay (Leaños-Castañeda & Van Der Kraak, 2007). However, a subsequent study conducted in tilapia concluded that agonistic and antagonistic characteristics of mammalian, isoform-specific ER agonists and antagonists, cannot be reliably extrapolated to piscine ERs (Davis et al., 2010).
    - Based on RNA interference knock-down experiments Nelson and Habibi (Nelson & Habibi, 2010) proposed a model in which all ER subtypes are involved in E2-mediated vitellogenesis, with ERβ isoforms stimulating expression of both vitellogenin and ERα gene expression, and ERα helping to drive vitellogenesis, particularly as it becomes more abundant following sensitization.
- Uncertainties related to cause of renal pathology: Although the accumulation of hyalin material/lipoprotein within the kidneys has been confirmed to be partially caused by accumulated VTG, some of the accumulated proteins do not respond to VTG antibody (e.g., Folmar et al., 2001). Because male fish will also express other estrogen inducible proteins such as vitelline envelope and zona radiata some renal pathology could be caused by these related proteins rather than VTG (Johan Hyllner et al., 1994; Oppen-Berntsen et al., 1994).
    - Proliferative kidney disease (PKD) in fish caused by the parasite *Tetracapsuloides bryosalmonae* results in significant kidney pathology. However, when PKD infection took place under simultaneous exposure to EE2, kidney pathology was less pronounced even though hepatic vtg was elevated in fish exposed to the estrogen (Bailey et al., 2019; Rehberger et al., 2020).

Quantitative Consideration

Overall, the quantitative understanding for this AOP is low. Presently there is insufficient data to develop a quantitative AOP linking ER activation to mortality and decreased population growth rate. However, a 21-day reproductive study to estrogenic PFAS, FC10-diol, which allowed for the measurement of nearly all the key events within this AOP and allowed for linking activation of the ER to impacts on survival and reproduction in fish (Ankley et al. in prep).

Increase in *vtg* synthesis leading to increase in plasma VTG was scored as having a moderate quantitative understanding due to the well-defined relationship between gene expression and protein synthesis. However, because the delay between expression of *vtg* and production/detection of VTG is not well defined our understanding is still limited.

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Appendix 1

List of MIEs in this AOP

Event: 111: Agonism, Estrogen receptor

Short Name: Agonism, Estrogen receptor

Key Event Component

Process	Object	Action
estrogen receptor activity	estrogen receptor	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:29 - Estrogen receptor agonism leading to reproductive dysfunction	MolecularInitiatingEvent
Aop:52 - ER agonism leading to skewed sex ratios due to altered sexual differentiation in males	MolecularInitiatingEvent
Aop:53 - ER agonism leading to reduced survival due to renal failure	MolecularInitiatingEvent
Aop:536 - Estrogen receptor agonism leading to reduced survival and population growth due to renal failure	MolecularInitiatingEvent
Aop:537 - Estrogen receptor agonism leads to reduced fecundity via increased vitellogenin in the liver	MolecularInitiatingEvent

Biological Context

Level of Biological Organization

Level of Biological Organization

Molecular

Cell term

Cell term

hepatocyte

Domain of Applicability

Taxonomic applicability: In mammals there are two ER subtypes, ER alpha (ERα) and ER beta (ERβ), which are located on chromosome 6 and 14 and encoded by two different genes (ESR1 and ESR2) (Ascenzi et al., 2006). ERs were conventionally identified as mammal specific, but most vertebrates contain functional ERs. However, although teleost fish have receptors homologous to mammilian ERα, ERβ is divided into ERβ1 and ERβ2 resulting in three distinct ERs (Asnake et al., 2019; Menuet et al., 2004; Menuet et al., 2002). The majority of invertebrates (i.e. mollusks) possess a gene that is the orthologue of the vertebrate ER but in many species it has been demonstrated to only have constitutive transcriptional activity, and is not activated by ligand binding (Balbi et al., 2019). However, ERs in annelids share functional characteristics with vertebrate ERs and its transcriptional activity can be disrupted by known endocrine-disrupting substances (Keay & Thornton, 2009).

This MIE would generally be viewed as relevant to vertebrates, but not invertebrates.

Life stage: This MIE is applicable to all life stages.

Sex: This MIE is applicable to both sexes.

Key Event Description

Site of action: The molecular site of action is the estrogen receptor (ER). ERsare members of the steroid hormone receptor family which belongs to a group of nuclear receptors that are transcriptionally activated by ligands leading to downstream activation of many cellular processes. ERs are composed of three principal domains – N-terminal domain (NTD), DNA binding domain (DBD), and the ligand binding domain (LBD). ER binds to specific DNA sequences known as estrogen response elements (EREs); EREs are generally short sequences located in the promoter region but can also exist in introns or exons (Klinge, 2001). ER-mediated gene transcription is initiated by binding of the DBD to an ERE with two distinct transcriptional activation domains, AF1 and AF2, located on the NTD and LBD respectively (Kumar et al., 2011).

Responses at the macromolecular level: ER's bind to endogenous and exogenous compounds and are activated by endogenous ligands such as estrone (E1), estradiol (E2) and estriol (E3) (Ng et al., 2014). There are numerous compounds (e.g., natural or pharmaceutical estrogens, alkylphenols, organochlorine pesticides, phthalates, etc.) that can act as estrogen agonists or antagonists, and effectively mimic or block the natural effects of estrogens on the ER (Pillon et al., 2005; Schmieder et al., 2014).

ER is part of a multi-protein complex consisting of HSP 90, HSP 70, and immunophilins (Stice & Knowlton, 2008). In this multi-protein complex HSP 90 is the dominant protein and its binding to ER is essential for ER conformational binding of 17β-estradiol (Segnitz & Gehring, 1997). When binding on the LBD receptor occurs ER dissociates from HSP 90 and leads to receptor dimerization which can either be homodimers from the same isoform (ERα-Erα) or heterodimers containing one unit from both isoforms (ERα-Erβ) (Fliss et al., 2000). The translocation of these dimers into the nucleus modulates gene transcription (Aranda & Pascual, 2001).

How it is Measured or Detected

- OECD Test No. 455: Performance-based test guideline for stably transfected transactivation in vitro assays to detect estrogen receptor agonists and antagonists (OECD 2021).
- OECD Test No. 457: BG1Luc Estrogen Receptor Transactivation Test Method for Identifying Estrogen Receptor Agonists and Antagonists (OECD 2012).
- Standard Evaluation Procedure (SEP) for estrogen receptor transcriptional activation (Human Cell Line HeLa-9903) assay was developed by the U.S. Environmental Protection Agency (EPA).
- ER-based transactivation assays that have been used to detect ER agonists and antagonist using cell lines include T47D-Kbluc assay (Wehmas et al., 2011), the ERα CALUX assay (Van et al.); MELN assay (Berckmans et al., 2007); and the yeast estrogen screen (YES; (De Boever et al., 2001)). The T47D-Kbluc assay responds to both ERα and ERß agonists but support the assumption that ERα is inducing more reporter expression than ERß. Each of these assays have undergone some level of validation.
- Browne et al. (2015) integrated 18 ER ToxCast high-throughput screening (HTS) assays, measuring ER binding, dimerization, chromatin binding, transcriptional activation and ER-dependent cell proliferation, into the ToxCast ER pathway model. This mathematical model that in vitro assays to predict whether a chemical is an ER agonist or antagonist.

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List of Key Events in the AOP

Event: 307: Increase, Vitellogenin synthesis in liver

Short Name: Increase, Vitellogenin synthesis in liver

Key Event Component

Process	Object	Action
gene expression	vitellogenins	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:29 - Estrogen receptor agonism leading to reproductive dysfunction	KeyEvent

AOP ID and Name	Event Type
Aop:536 - Estrogen receptor agonism leading to reduced survival and population growth due to renal failure	KeyEvent
Aop:537 - Estrogen receptor agonism leads to reduced fecundity via increased vitellogenin in the liver	KeyEvent

Biological Context

Level of Biological Organization

Tissue

Organ term

Organ term

liver

Domain of Applicability

Taxonomic applicability: 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).

Life stage: This KE is applicable to all life stages following the differentiation of the liver. Embryos prior to liver differentiation should not be included.

Sex: This KE is applicable to both sexes.

Key Event Description

Vitellogenin (VTG) is an egg yolk precursor protein synthesized by hepatocytes of oviparous vertebrates(Hara et al., 2016). Transcription of *vtg* is regulated by estrogens and their interaction on ERs.In males expression can be modulated by exogenous compounds. Under high estrogen stimulation the fold increase of *vtg* transcripts increases by orders of magnitude (Brock & Shapiro, 1983).

How it is Measured or Detected

Relative abundance of vitellogenin transcripts or protein can be measured in liver tissue (e.g., Miracle et al., 2006), hepatocytes (e.g., Vaillant et al., 1988), exposed in vitro, or whole-body homogenates from organisms exposed in vivo (Holbech et al., 2001).

mRNA transcripts can be measured using real-time quantitative polymerase chain reaction (qPCR) while protein quantification can be measured using alkali-labile phosphoprotein (e.g., Kramer et al., 1998), or immunochemical methods such as radioimmunoassay (RIA; e.g., Tyler & Sumpter, 1990), enzyme linked immunosorbent assay (ELISA; e.g., Denslow et al., 1999), and Western blotting (e.g., Heppell et al., 1995).

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Event: 220: Increase, Plasma vitellogenin concentrations

Short Name: Increase, Plasma vitellogenin concentrations

Key Event Component

Process	Object	Action
	vitellogenins	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:29 - Estrogen receptor agonism leading to reproductive dysfunction	KeyEvent
Aop:53 - ER agonism leading to reduced survival due to renal failure	KeyEvent
Aop:536 - Estrogen receptor agonism leading to reduced survival and population growth due to renal failure	KeyEvent
Aop:537 - Estrogen receptor agonism leads to reduced fecundity via increased vitellogenin in the liver	KeyEvent

Biological Context

Level of Biological Organization
Organ

Organ term

Organ term
blood plasma

Domain of Applicability

Taxonomic applicability: 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.

Life stage: This KE is applicable to all life stages following the differentiation of the liver. Embryos prior to liver differentiation should not be included.

Sex: This KE is applicable to both sexes.

Key Event Description

Vitellogenins are large serum phospholipoglycoprotein that are encoded by a family of paralog genes whose number varies in the different vertebrate lineages resulting in numerous isoforms (Wahli, 1988). Vtg is synthesized in the liver and is secreted into the blood as ~500 kDa homodimers which circulate to the ovaries for uptake and bind to receptors on the surface of growing oocytes (Wallace, 1985).

How it is Measured or Detected

Vitellogenin concentrations in plasma are typically measured using enzyme linked immunosorbent assay (ELISA; e.g., Denslow et al., 1999; Holbech et al., 2001). Less specific and/or sensitive assays such as determination of alkali-labile phosphoprotein

(e.g., Kramer et al., 1998) and Western blotting (e.g., Heppell et al., 1995) may also be used.

There are also several standardized test guidelines that measure vtg including: Fish Short Term Reproduction Assay (OECD, 2009a), 21-day Fish Assay (OECD, 2009b); Fish Sexual Development Test (OECD, 2011), Medaka Extended One Generation Reproduction Test (OECD, 2015a). Measurement of vtg is also an optional parameter in the Larval Amphibian Growth and Development Assay (OECD, 2015b). The US Environmental Protection Agency (EPA) has similar standardized guidelines (US EPA, 2009, US EPA, 2014) as does the EU as part of the Guidance For The Identification Of Endocrine Disruptors In The Context Of Regulations (EC 2013, EC 2018).

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Event: 252: Increase, Renal pathology due to VTG deposition

Short Name: Increase, Renal pathology due to VTG deposition

Key Event Component

Process	Object	Action
Kidney Diseases		increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:29 - Estrogen receptor agonism leading to reproductive dysfunction	KeyEvent
Aop:536 - Estrogen receptor agonism leading to reduced survival and population growth due to renal failure	KeyEvent

Biological Context

Level of Biological Organization

Organ

Organ term

Organ term

kidney

Domain of Applicability

Taxonomic applicability: All vertebrates with functional kidneys.

Life stage: This KE is applicable to all life stages following the differentiation of the kidney.

Sex: This KE is applicable to both sexes.

Key Event Description

Renal pathology deals with the characterization of the kidneys. The kidneys perform a suite of physiological roles that are critical for organismal homeostasis including waste excretion, osmoregulation, and fluid homeostasis (Preuss, 1993). Each kidney is made up of specialized epithelial cells known as nephrons and while nephron numbers can vary greatly between species their overall function remains conserved in vertebrates (Desgrange & Cereghini, 2015). Nephrons act as filtering units that are composed of glomeruli and tubules which are responsible for removing metabolic waste from the bloodstream, regulating fluids, and balancing electrolytes (Wesselman et al., 2023). Organ tissue damage can occur after exposure to toxins, parasites, or be caused by disease. If pathology is measurable this would be an indication of damage or diseased tissue state and a departure from normal/healthy tissue.

How it is Measured or Detected

Histopathology focuses on the changes in tissues and is a technique used for identifying correlations with biochemical markers. Generally renal pathology is measured after either whole organism or specific tissue of interest is fixed, dehydrated, and then embedded in wax, commonly paraffin wax. Sections are then cut to approximately 3–5 µm in thickness and stained before being examined under a microscope (e.g., Folmar et al., 2001; Mihaich et al., 2012; Zha et al., 2007).

- OECD Test No. 123: Guidance document on the diagnosis of endocrine-related histopathology in fish gonads (OECD 2010).
- OECD Test No. 227: Guidance document on medaka histopathology techniques and evaluation for the medaka extended one-generation reproduction test (OECD 2015)
- Crissman et al. (2004) describes best practice guidelines for toxicologic histopathology.
- Fiedler et al. (2023) have written standardized tissue sampling guidelines for histopathological analyses using rainbow trout.

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Event: 351: Increased Mortality

Short Name: Increased Mortality

Key Event Component

Process	Object	Action
mortality		increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:16 - Acetylcholinesterase inhibition leading to acute mortality	AdverseOutcome
Aop:96 - Axonal sodium channel modulation leading to acute mortality	AdverseOutcome
Aop:104 - Altered ion channel activity leading impaired heart function	AdverseOutcome
Aop:113 - Glutamate-gated chloride channel activation leading to acute mortality	AdverseOutcome

AOP ID and Name	Event Type
Aop:160 - Ionotropic gamma-aminobutyric acid receptor activation mediated neurotransmission inhibition leading to mortality	AdverseOutcome
Aop:161 - Glutamate-gated chloride channel activation leading to neurotransmission inhibition associated mortality	AdverseOutcome
Aop:138 - Organic anion transporter (OAT1) inhibition leading to renal failure and mortality	AdverseOutcome
Aop:177 - Cyclooxygenase 1 (COX1) inhibition leading to renal failure and mortality	AdverseOutcome
Aop:186 - unknown MIE leading to renal failure and mortality	AdverseOutcome
Aop:312 - Acetylcholinesterase Inhibition leading to Acute Mortality via Impaired Coordination & Movement	AdverseOutcome
Aop:320 - Binding of SARS-CoV-2 to ACE2 receptor leading to acute respiratory distress associated mortality	AdverseOutcome
Aop:155 - Deiodinase 2 inhibition leading to increased mortality via reduced posterior swim bladder inflation	AdverseOutcome
Aop:156 - Deiodinase 2 inhibition leading to increased mortality via reduced anterior swim bladder inflation	AdverseOutcome
Aop:157 - Deiodinase 1 inhibition leading to increased mortality via reduced posterior swim bladder inflation	AdverseOutcome
Aop:158 - Deiodinase 1 inhibition leading to increased mortality via reduced anterior swim bladder inflation	AdverseOutcome
Aop:159 - Thyroperoxidase inhibition leading to increased mortality via reduced anterior swim bladder inflation	AdverseOutcome
Aop:363 - Thyroperoxidase inhibition leading to altered visual function via altered retinal layer structure	AdverseOutcome
Aop:377 - Dysregulated prolonged Toll Like Receptor 9 (TLR9) activation leading to Multi Organ Failure involving Acute Respiratory Distress Syndrome (ARDS)	AdverseOutcome
Aop:364 - Thyroperoxidase inhibition leading to altered visual function via decreased eye size	AdverseOutcome
Aop:365 - Thyroperoxidase inhibition leading to altered visual function via altered photoreceptor patterning	AdverseOutcome
Aop:399 - Inhibition of Fyna leading to increased mortality via decreased eye size (Microphthalmos)	AdverseOutcome
Aop:413 - Oxidation and antagonism of reduced glutathione leading to mortality via acute renal failure	AdverseOutcome
Aop:410 - GSK3beta inactivation leading to increased mortality via defects in developing inner ear	AdverseOutcome
Aop:450 - Inhibition of AChE and activation of CYP2E1 leading to sensory axonal peripheral neuropathy and mortality	AdverseOutcome
Aop:536 - Estrogen receptor agonism leading to reduced survival and population growth due to renal failure	KeyEvent

Biological Context

Level of Biological Organization

Population

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
all species	all species	High	NCBI

Life Stage Applicability

Life Stage	Evidence

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	Moderate

All living things are susceptible to mortality.

Key Event Description

Increased mortality refers to an increase in the number of individuals dying in an experimental replicate group or in a population over a specific period of time.

How it is Measured or Detected

Mortality of animals is generally observed as cessation of the heart beat, breathing (gill or lung movement) and locomotory movements. Mortality is typically measured by observation. Depending on the size of the organism, instruments such as microscopes may be used. The reported metric is mostly the mortality rate: the number of deaths in a given area or period, or from a particular cause.

Depending on the species and the study setup, mortality can be measured:

- in the lab by recording mortality during exposure experiments
- in dedicated setups simulating a realistic situation such as mesocosms or drainable ponds for aquatic species
- in the field, for example by determining age structure after one capture, or by capture-mark-recapture efforts. The latter is a method commonly used in ecology to estimate an animal population's size where it is impractical to count every individual.

Regulatory Significance of the AO

Increased mortality is one of the most common regulatory assessment endpoints, along with reduced growth and reduced reproduction.

Event: 360: Decrease, Population growth rate

Short Name: Decrease, Population growth rate

Key Event Component

Process	Object	Action
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)	AdverseOutcome
Aop:25 - Aromatase inhibition leading to reproductive dysfunction	AdverseOutcome
Aop:29 - Estrogen receptor agonism leading to reproductive dysfunction	AdverseOutcome
Aop:30 - Estrogen receptor antagonism leading to reproductive dysfunction	AdverseOutcome
Aop:100 - Cyclooxygenase inhibition leading to reproductive dysfunction via inhibition of female spawning behavior	AdverseOutcome
Aop:122 - Prolyl hydroxylase inhibition leading to reproductive dysfunction via increased HIF1 heterodimer formation	AdverseOutcome
Aop:123 - Unknown MIE leading to reproductive dysfunction via increased HIF-1alpha transcription	AdverseOutcome

AOP ID and Name	Event Type
Aop:155 - Deiodinase 2 inhibition leading to increased mortality via reduced posterior swim bladder inflation	AdverseOutcome
Aop:156 - Deiodinase 2 inhibition leading to increased mortality via reduced anterior swim bladder inflation	AdverseOutcome
Aop:157 - Deiodinase 1 inhibition leading to increased mortality via reduced posterior swim bladder inflation	AdverseOutcome
Aop:158 - Deiodinase 1 inhibition leading to increased mortality via reduced anterior swim bladder inflation	AdverseOutcome
Aop:159 - Thyroperoxidase inhibition leading to increased mortality via reduced anterior swim bladder inflation	AdverseOutcome
Aop:101 - Cyclooxygenase inhibition leading to reproductive dysfunction via inhibition of pheromone release	AdverseOutcome
Aop:102 - Cyclooxygenase inhibition leading to reproductive dysfunction via interference with meiotic prophase I /metaphase I transition	AdverseOutcome
Aop:63 - Cyclooxygenase inhibition leading to reproductive dysfunction	AdverseOutcome
Aop:103 - Cyclooxygenase inhibition leading to reproductive dysfunction via interference with spindle assembly checkpoint	AdverseOutcome
Aop:292 - Inhibition of tyrosinase leads to decreased population in fish	AdverseOutcome
Aop:310 - Embryonic Activation of the AHR leading to Reproductive failure, via epigenetic down-regulation of GnRHR	AdverseOutcome
Aop:16 - Acetylcholinesterase inhibition leading to acute mortality	AdverseOutcome
Aop:312 - Acetylcholinesterase Inhibition leading to Acute Mortality via Impaired Coordination & Movement	AdverseOutcome
Aop:334 - Glucocorticoid Receptor Agonism Leading to Impaired Fin Regeneration	AdverseOutcome
Aop:336 - DNA methyltransferase inhibition leading to population decline (1)	AdverseOutcome
Aop:337 - DNA methyltransferase inhibition leading to population decline (2)	AdverseOutcome
Aop:338 - DNA methyltransferase inhibition leading to population decline (3)	AdverseOutcome
Aop:339 - DNA methyltransferase inhibition leading to population decline (4)	AdverseOutcome
Aop:340 - DNA methyltransferase inhibition leading to transgenerational effects (1)	AdverseOutcome
Aop:341 - DNA methyltransferase inhibition leading to transgenerational effects (2)	AdverseOutcome
Aop:289 - Inhibition of 5α-reductase leading to impaired fecundity in female fish	AdverseOutcome
Aop:297 - Inhibition of retinaldehyde dehydrogenase leads to population decline	AdverseOutcome
Aop:346 - Aromatase inhibition leads to male-biased sex ratio via impacts on gonad differentiation	AdverseOutcome
Aop:363 - Thyroperoxidase inhibition leading to altered visual function via altered retinal layer structure	AdverseOutcome
Aop:349 - Inhibition of 11β-hydroxylase leading to decresed population trajectory	AdverseOutcome
Aop:348 - Inhibition of 11β-Hydroxysteroid Dehydrogenase leading to decreased population trajectory	AdverseOutcome
Aop:376 - Androgen receptor agonism leading to male-biased sex ratio	AdverseOutcome
Aop:386 - Deposition of ionizing energy leading to population decline via inhibition of photosynthesis	AdverseOutcome
Aop:387 - Deposition of ionising energy leading to population decline via mitochondrial dysfunction	AdverseOutcome
Aop:388 - Deposition of ionising energy leading to population decline via programmed cell death	AdverseOutcome
Aop:389 - Oxygen-evolving complex damage leading to population decline via inhibition of photosynthesis	AdverseOutcome
Aop:364 - Thyroperoxidase inhibition leading to altered visual function via decreased eye size	AdverseOutcome

AOP ID and Name	Event Type
Aop:365 - Thyroperoxidase inhibition leading to altered visual function via altered photoreceptor patterning	AdverseOutcome
Aop:399 - Inhibition of Fyna leading to increased mortality via decreased eye size (Microphthalmos)	AdverseOutcome
Aop:410 - GSK3beta inactivation leading to increased mortality via defects in developing inner ear	AdverseOutcome
Aop:216 - Deposition of energy leading to population decline via DNA strand breaks and follicular atresia	AdverseOutcome
Aop:238 - Deposition of energy leading to population decline via DNA strand breaks and oocyte apoptosis	AdverseOutcome
Aop:299 - Deposition of energy leading to population decline via DNA oxidation and follicular atresia	AdverseOutcome
Aop:311 - Deposition of energy leading to population decline via DNA oxidation and oocyte apoptosis	AdverseOutcome
Aop:444 - Ionizing radiation leads to reduced reproduction in Eisenia fetida via reduced spermatogenesis and cocoon hatchability	AdverseOutcome
Aop:138 - Organic anion transporter (OAT1) inhibition leading to renal failure and mortality	AdverseOutcome
Aop:177 - Cyclooxygenase 1 (COX1) inhibition leading to renal failure and mortality	AdverseOutcome
Aop:97 - 5-hydroxytryptamine transporter (5-HTT; SERT) inhibition leading to population decline	AdverseOutcome
Aop:203 - 5-hydroxytryptamine transporter inhibition leading to decreased reproductive success and population decline	AdverseOutcome
Aop:218 - Inhibition of CYP7B activity leads to decreased reproductive success via decreased locomotor activity	AdverseOutcome
Aop:219 - Inhibition of CYP7B activity leads to decreased reproductive success via decreased sexual behavior	AdverseOutcome
Aop:323 - PPARalpha Agonism Leading to Decreased Viable Offspring via Decreased 11-Ketotestosterone	AdverseOutcome
Aop:536 - Estrogen receptor agonism leading to reduced survival and population growth due to renal failure	KeyEvent
Aop:540 - Oxidative Stress in the Fish Ovary Leads to Reproductive Impairment via Reduced Vitellogenin Production	AdverseOutcome

Biological Context

Level of Biological Organization

Population

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
all species	all species	High	NCBI

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

A population can be defined as a group of interbreeding organisms, all of the same species, occupying a specific space during a specific time (Vandermeer and Goldberg 2003, Gotelli 2008). As the population is the biological level of organization that is often the focus of ecological risk assessments, population growth rate (and hence population size over time) is important to consider within the context of applied conservation practices.

If N is the size of the population and t is time, then the population growth rate (dN/dt) is proportional to the instantaneous rate of increase, r, which measures the per capita rate of population increase over a short time interval. Therefore, r, is a difference between the instantaneous birth rate (number of births per individual per unit of time; b) and the instantaneous death rate (number of deaths per individual per unit of time; d) [Equation 1]. Because r is an instantaneous rate, its units can be changed via division. For example, as there are 24 hours in a day, an r of 24 individuals/(individual x day) is equal to an r of 1 individual/(individual/hour) (Caswell 2001, Vandermeer and Goldberg 2003, Gotelli 2008, Murray and Sandercock 2020).

Equation 1: $r = b - d$

This key event refers to scenarios where $r < 0$ (instantaneous death rate exceeds instantaneous birth rate).

Examining r in the context of population growth rate:

- A population will decrease to extinction when the instantaneous death rate exceeds the instantaneous birth rate ($r < 0$).
- The smaller the value of r below 1, the faster the population will decrease to zero.
- A population will increase when resources are available and the instantaneous birth rate exceeds the instantaneous death rate ($r > 0$)
- The larger the value that r exceeds 1, the faster the population can increase over time
- A population will neither increase or decrease when the population growth rate equals 0 (either due to $N = 0$, or if the per capita birth and death rates are exactly balanced). For example, the per capita birth and death rates could become exactly balanced due to density dependence and/or to the effect of a stressor that reduces survival and/or reproduction (Caswell 2001, Vandermeer and Goldberg 2003, Gotelli 2008, Murray and Sandercock 2020).

Effects incurred on a population from a chemical or non-chemical stressor could have an impact directly upon birth rate (reproduction) and/or death rate (survival), thereby causing a decline in population growth rate.

- Example of direct effect on r: Exposure to 17b-trenbolone reduced reproduction (i.e., reduced b) in the fathead minnow over 21 days at water concentrations ranging from 0.0015 to about 41 mg/L (Ankley et al. 2001; Miller and Ankley 2004).

Alternatively, a stressor could indirectly impact survival and/or reproduction.

- Example of indirect effect on r: Exposure of non-sexually differentiated early life stage fathead minnow to the fungicide prochloraz has been shown to produce male-biased sex ratios based on gonad differentiation, and resulted in projected change in population growth rate (decrease in reproduction due to a decrease in females and thus recruitment) using a population model. (Holbech et al., 2012; Miller et al. 2022)

Density dependence can be an important consideration:

- The effect of density dependence depends upon the quantity of resources present within a landscape. A change in available resources could increase or decrease the effect of density dependence and therefore cause a change in population growth rate via indirectly impacting survival and/or reproduction.
- This concept could be thought of in terms of community level interactions whereby one species is not impacted but a competitor species is impacted by a chemical stressor resulting in a greater availability of resources for the unimpacted species. In this scenario, the impacted species would experience a decline in population growth rate. The unimpacted species would experience an increase in population growth rate (due to a smaller density dependent effect upon population growth rate for that species).

Closed versus open systems:

- The above discussion relates to closed systems (there is no movement of individuals between population sites) and thus a declining population growth rate cannot be augmented by immigration.
- When individuals depart (emigrate out of a population) the loss will diminish population growth rate.

Population growth rate applies to all organisms, both sexes, and all life stages.

How it is Measured or Detected

Population growth rate (instantaneous growth rate) can be measured by sampling a population over an interval of time (i.e. from time t = 0 to time t = 1). The interval of time should be selected to correspond to the life history of the species of interest (i.e. will be different for rapidly growing versus slow growing populations). The population growth rate, r, can be determined by taking the difference (subtracting) between the initial population size, $N_{t=0}$ (population size at time t=0), and the population size at the end of the interval, $N_{t=1}$ (population size at time t = 1), and then subsequently dividing by the initial population size.

Equation 2: $r = (N_{t=1} - N_{t=0}) / N_{t=0}$

The diversity of forms, sizes, and life histories among species has led to the development of a vast number of field techniques for estimation of population size and thus population growth over time (Bookhout 1994, McComb et al. 2021).

- For stationary species an observational strategy may involve dividing a habitat into units. After setting up the units, samples are performed throughout the habitat at a select number of units (determined using a statistical sampling design) over a time interval (at time t = 0 and again at time t = 1), and the total number of organisms within each unit are counted. The numbers recorded are assumed to be representative for the habitat overall, and can be used to estimate the population growth rate within the entire habitat over the time interval.

- For species that are mobile throughout a large range, a strategy such as using a mark-recapture method may be employed (i.e. tags, bands, transmitters) to determine a count over a time interval (at time = 0 and again at time =1).

Population growth rate can also be estimated using mathematical model constructs (for example, ranging from simple differential equations to complex age or stage structured matrix projection models and individual based modeling approaches), and may assume a linear or nonlinear population increase over time (Caswell 2001, Vandermeer and Goldberg 2003, Gotelli 2008, Murray and Sandercock 2020). The AOP framework can be used to support the translation of pathway-specific mechanistic data into responses relevant to population models and output from the population models, such as changing (declining) population growth rate, can be used to assess and manage risks of chemicals (Kramer et al. 2011). As such, this translational capability can increase the capacity and efficiency of safety assessments both for single chemicals and chemical mixtures (Kramer et al. 2011).

Some examples of modeling constructs used to investigate population growth rate:

- A modeling construct could be based upon laboratory toxicity tests to determine effect(s) that are then linked to the population model and used to estimate decline in population growth rate. Miller et al. (2007) used concentration–response data from short term reproductive assays with fathead minnow (*Pimephales promelas*) exposed to endocrine disrupting chemicals in combination with a population model to examine projected alterations in population growth rate.

- A model construct could be based upon a combination of effects-based monitoring at field sites (informed by an AOP) and a population model. Miller et al. (2015) applied a population model informed by an AOP to project declines in population growth rate for white suckers (Catostomus commersoni) using observed changes in sex steroid synthesis in fish exposed to a complex pulp and paper mill effluent in Jackfish Bay, Ontario, Canada. Furthermore, a model construct could be comprised of a series of quantitative models using KERs that culminates in the estimation of change (decline) in population growth rate.

- A quantitative adverse outcome pathway (qAOP) has been defined as a mathematical construct that models the dose–response or response–response relationships of all KERs described in an AOP (Conolly et al. 2017, Perkins et al. 2019). Conolly et al. (2017) developed a qAOP using data generated with the aromatase inhibitor fadrozole as a stressor and then used it to predict potential population-level impacts (including decline in population growth rate). The qAOP modeled aromatase inhibition (the molecular initiating event) leading to reproductive dysfunction in fathead minnow (Pimephales promelas) using 3 computational models: a hypothalamus–pituitary–gonadal axis model (based on ordinary differential equations) of aromatase inhibition leading to decreased vitellogenin production (Cheng et al. 2016), a stochastic model of oocyte growth dynamics relating vitellogenin levels to clutch size and spawning intervals (Watanabe et al. 2016), and a population model (Miller et al. 2007).

- Dynamic energy budget (DEB) models offer a methodology that reverse engineers stressor effects on growth, reproduction, and/or survival into modular characterizations related to the acquisition and processing of energy resources (Nisbet et al. 2000, Nisbet et al. 2011). Murphy et al. (2018) developed a conceptual model to link DEB and AOP models by interpreting AOP key events as measures of damage-inducing processes affecting DEB variables and rates.

- Endogenous Lifecycle Models (ELMs), capture the endogenous lifecycle processes of growth, development, survival, and reproduction and integrate these to estimate and predict expected fitness (Etterson and Ankley, 2021). AOPs can be used to inform ELMs of effects of chemical stressors on the vital rates that determine fitness, and to decide what hierarchical models of endogenous systems should be included within an ELM (Etterson and Ankley, 2021).

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.

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Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

Relationship: 128: Agonism, Estrogen receptor leads to Increase, Vitellogenin synthesis in

liver

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Estrogen receptor agonism leading to reproductive dysfunction	adjacent	High	
Estrogen receptor agonism leading to reduced survival and population growth due to renal failure	adjacent	High	Low
Estrogen receptor agonism leads to reduced fecundity via increased vitellogenin in the liver	adjacent		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio	High	NCBI
fathead minnow	Pimephales promelas	High	NCBI

Taxonomic applicability: Oviparous vertebrates.

Life stage: This KER is applicable to all life stages following the differentiation of the liver. Larvae prior to liver differentiation should not be included.

Sex: This KER is applicable to both sexes.

Evidence Supporting this KER

Biological Plausibility

Original text - unknown contributor

High degree of plausibility in fathead minnow, zebrafish and other cyprinid species.

Added by C. Baettig June 24, 2024

In egg laying vertebrates such as fish vitellogenin (VTG) synthesis occurs in the female liver after activation of estrogen receptors (ERs), including ERα and ERβ isoforms, by endogenous steroids and a variety of exogenous chemicals that bind to ERs (e.g., Brock & Shapiro, 1983; Denslow et al., 1999; Miracle et al., 2006). In mature female fish VTG is incorporated into growing oocytes by the ovary and is converted into yolk protein. However, neither adult male fish nor juvenile fish normally produce VTG, but the hepatic ER is present in males, as are the genes that encode for *vtg* expression and can therefore be induced by exogenous compounds (Heppell et al., 1995).

Agonism of the ER is expected to increase *vtg* transcription and translation and under high estrogen stimulation the fold increase of *vtg* transcripts increases by orders of magnitude (Brock & Shapiro, 1983). As such, induction of VTG levels in male fish has been used extensively as a biomarker of estrogen exposure (Wheeler et al., 2005).

Empirical Evidence

Original text - unknown contributor

A wide range of studies using adult fish show that induction of plasma vitellogenin (VTG) occurs within 21 days in vivo aquatic exposure to estrogen receptor agonists (eg 17beta-estradiol and 4-tert pentylphenol) as shown during the successful validation of the OECD Test Guideline 229 and related protocols. A smaller number of experiment studies with fish have shown that within the OECD Test Guideline 2010, larval fish can also show induction of whole body VTG levels within 21 days aquatic exposure to estrogen receptor agonists.

Added by C. Baettig June 24, 2024

There are numerous publications supporting this relationship including multiple review articles (e.g., Matozzo et al., 2008; Palmer & Selcer, 1996; Verderame & Scudiero, 2017). A few specific examples are listed below.

- Estradiol and diarylpropionitrile (DPN), an ERβ selective agonist, induced a dose-dependent increase in VTG synthesis in rainbow trout hepatocytes (Leaños-Castañeda & Van Der Kraak, 2007).
- DPN has also been shown to increase ERα and *vtg* expression and synthesis post-injection in Mozambique tilapia in vivo (Davis et al., 2010).
- A study focusing on benzophenone derivatives found that BP1 (2,4-dihydroxybenzophenone), BP2 (2,2′,4,4′-tetrahydroxybenzophenone), and THB (2,4,4′-trihydroxybenzophenone) were human ERα (hERα) and hERβ and rainbow trout ERα (rtERα) and rtERβ agonists. To investigate ER activation profiles of the derivatives in vitro tests, i.e., competitive binding, reporter gene based assays, vitellogenin (Vtg) induction in isolated rainbow trout hepatocytes, and proliferation based assays were completed. hERβ was more strongly activated, which is an inverse finding to natural ligand 17β-estradiol (E2) where hERα is more strongly activated. BPs were more active in rtERα than in hERα assays. Significant VTG induction was detected in hERα, hERβ, rtERα, and rtERβ cultures (Molina-Molina et al., 2008).
- Tollefsen et al. (2003) looked at multiple endogenous (e.g., estrone (E1), estradiol (E2), and estriol (E3)) and exogenous estrogens (e.g., ethynyloestradiol (EE2), diethylstilbestrol (DES), genistein, zearalenone, bisphenol A) and found they induced dose-dependent VTG synthesis in Atlantic salmon hepatocytes.
- Shen et al. (2021) used in silico methods to screen 1056 pesticides for potential agonistic activity. They found 72 pesticides to be potential ER agonists, 14 of which have been previously reported as ER agonists. To test whether these pesticides were ER agonists, 10 were selected from the list, three that were previously reported as ER agonists and seven previously unreported as ER agonists. They found all 10 pesticides exhibited ERα agonistic activity in human or zebrafish cells and of the 10, seven also induced *vtg1* and *vtg2* mRNA in zebrafish.
- Xu et al (2020) also showed increase in plasma VTG following exposure to aryloxy-phenoxypropionate (APP) herbicides, after measuring the binding patterns of quizalofop-P-ethyl (QPE), clodinafop-propargyl (CP) and haloxyfop-P (HP) with ERα.
- In male fathead minnows exposed to E2 and 1H,1H,10H,10H-perfluorodecane-1,10-diol (FC-10 diol) for 21 days expression of hepatic *esr1* and *vtg* were both significantly increased (Ankley et al. in prep).
- In male fathead minnows exposed to methoxychlor, a weak estrogen agonist, there was a clear induction of VTG (Ankley et al. 2001). In the same study exposure to methyltestosterone, a synthetic androgen, caused a significant induction of VTG in both male and female fathead minnows. This level of induction in female fathead minnows resulted in a dose-dependent increase in VTG, to concentrations approximately 10-fold higher than those observed in control fish. These funding were likely due to the conversion of methyltestosterone to methylestradiol (Hornung et al., 2004).

Uncertainties and Inconsistencies

Original text - unknown contributor

There are generally few inconsistencies for experimental studies using model fish species dervied from pathogen-free laboratory cultures. However, there can some uncertainties where wild fish have been used for experimental purposes.

Added by C. Baettig June 24, 2024

- Some uncertainty remains regarding which ER subtypes regulate *vtg* gene expression in the liver of fish. In general, the literature suggests a close interplay between ER subtypes, primarily ERα and Erβ, in the regulation of vitellogenesis. Consequently, at present, the key event relationship is generalized to impacts on all ER subtypes, even though it remains possible that impacts on a particular sub-type may drive the effect on vitellogenin transcription and translation.
- Using selective agonists and antagonists for ERα and ERβ, it was concluded that ERβ was primarily responsible for inducing vitellogenin production in rainbow trout and that compounds exhibiting ERα selectivity would not be detected using a vitellogenin ELISA bioassay (Leaños-Castañeda & Van Der Kraak, 2007). However, a subsequent study conducted in tilapia concluded that agonistic and antagonistic characteristics of mammalian, isoform-specific ER agonists and antagonists, cannot be reliably extrapolated to piscine ERs (Davis et al., 2010).
- Based on RNA interference knock-down experiments Nelson and Habibi (2010) proposed a model in which all ER subtypes are involved in E2-mediated vitellogenesis, with ERβ isoforms stimulating expression of both vitellogenin and ERα gene expression, and ERα helping to drive vitellogenesis, particularly as it becomes more abundant following sensitization.

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Relationship: 336: Increase, Vitellogenin synthesis in liver leads to Increase, Plasma vitellogenin concentrations

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Estrogen receptor agonism leading to reproductive dysfunction	adjacent	High	
Estrogen receptor agonism leading to reduced survival and population growth due to renal failure	adjacent	High	Moderate
Estrogen receptor agonism leads to reduced fecundity via increased vitellogenin in the liver	adjacent		

Evidence Supporting Applicability of this Relationship

Taxonomic applicability: 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.

Life stage: This KER is applicable to all life stages following the differentiation of the liver. Embryos prior to liver differentiation should not be included.

Sex: This KER is applicable to both sexes. However, as males do not have the ability to clear plasma VTG via uptake into the oocytes the outcome is more likely to be problematic in males. Therefore, this KER has more relevance in males both in the context of monitoring for exogenous estrogens and potential biological consequences of elevated VTG.

Evidence Supporting this KER

Biological Plausibility

Original text - unknown contributor

High level of physiological plausibility in fish.

Added by C. Baettig on June 24, 2024

The liver is the primary source of VTG synthesis and production and after it is synthesized it is secreted into the blood (Wallace, 1985). Vitellogenin transcription and translation results in protein production, although there is a delay between expression of *vtg* and actual production/detection of VTG (e.g., Korte et al. 2000).

Empirical Evidence

- In male tilapia, 48 hours after 17β-estradiol (E2) treatment, *vtg* hepatic mRNA expression was elevated as was plasma VTG (Davis et al., 2008).
- In time course studies an increase in *vtg* mRNA synthesis precedes increases in plasma VTG concentration. For example, a study using male fathead minnows injected with E2, vtg mRNA was detected in the liver within 4 hours, reached a maximum around 48 hours, and returned to normal levels after 6 days. Plasma VTG was detectable within 16 hours of treatment, reached maximum levels at about 72 hours, and did not return to normal levels for at least 18 days (Korte et al., 2000).
- Similar results were observed in a flow-through experiment using sheepshead minnows exposed to E2 and p-nonylphenol. A dose dependent increase in hepatic vtg mRNA initially occurred followed by plasma VTG increase. Their results further supported that hepatic *vtg* mRNA rapidly diminishes after termination of estrogenic exposure, but plasma VTG clearance is concentration and time dependent (Hemmer et al., 2002).
- Bowman et al. (2000) also found a time lag between *vtg*, which was elevated after 4 hours while induction of plasma VTG wasn't detected until 24 hours in male sheepshead minnows injected with E2.
- During waterborne exposures to 17α-ethinylestradiol (EE2), male fathead minnows showed a strong increase of *vtg* mRNA within 3 days (first sampling time point in the study), which remained elevated for the entirety of the 35-day exposure. Although plasma VTG was first detectable on day 3 it did not significantly increase until day 14 further illustrating the lag between *vtg* mRNA and plasma increase (Schmid et al., 2002).
- In male fathead minnows exposed to E2 and FC-10 diol for 21 days, expression of hepatic *vtg* was significantly increased as was the plasma VTG (Ankley et al. in prep).

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.

Quantitative Understanding of the Linkage

Response-response relationship

Models and statistical relationships that define quantitative relationships between circulating E2 concentrations and circulating VTG concentrations have been developed (Ankley et al., 2008; Li et al., 2011; Murphy et al., 2009; Murphy et al., 2005). However, much of this work has focused on decreased VTG as a function of decreased E2, rather than induction.

Time-scale

Due to the timeline between induction of mRNA transcription, translation, and the appearance of protein in plasma, as well as variable rates of uptake of VTG from plasma into oocytes, a precise quantitative relationship describing all steps of vitellogenesis transcription/translation has not been described.

However, studies in fish suggest that that the temporal lag between mRNA transcription and increased plasma concentrations takes place within 24 hours. For example, in fish injected with E2 there is generally an increase of *vtg* mRNA beginning around 4 hours whereas plasma VTG isn't measurable until 16-24 hours (Bowman et al., 2000; Korte et al., 2000). Additionally, in waterborne exposure of estrone (E1) in juvenile rainbow trout, elevated vtg mRNA occurred on day 4 of exposure while plasma VTG was elevated on day 5 (Osachoff et al., 2016).

Known Feedforward/Feedback loops influencing this KER

There is no known feedback as plasma VTG does not appear to regulate expression levels in the liver.

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Relationship: 254: Increase, Plasma vitellogenin concentrations leads to Increase, Renal pathology due to VTG deposition

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Estrogen receptor agonism leading to reproductive dysfunction	adjacent	High	
Estrogen receptor agonism leading to reduced survival and population growth due to renal failure	adjacent	Moderate	Low

Evidence Supporting Applicability of this Relationship

Original text - unknown contribution

Publish studies specifically relate to fish, although it is plausible that the same response may occur in the aquatic life-stages of amphibians.

Added by C. Baettig on June 24, 2024

Taxonomic applicability: Oviparous vertebrates that synthesize yolk precursor proteins and have functional kidneys.

Life stage: This KER is applicable to all life stages following the differentiation of the liver and kidney.

Sex: This KER is applicable to both sexes.

Evidence Supporting this KER

Biological Plausibility

Original text - unknown contribution

High level of biological plausibility in fish.

Added by C. Baettig on June 24, 2024

When large quantities of VTG are circulating hyalin material can accumulate in the kidneys which can cause significant pathology (Folmar et al., 2001; Herman & Kincaid, 1988; Palace et al., 2002). Additionally, eosinophilic material is known to accumulate in kidney tubules and has been proposed to be due to high circulating VTG (Hahlbeck et al., 2004). Similarly, cilia proliferation observed in renal tubules is assumed to be related to increased absorption of circulating vitellogenin (Zha et al., 2008).

Empirical Evidence

Original text - unknown contribution

Laboratory in vivo aquatic exposures of fish (fathead minnow) to 17alpha-ethinylestradiol led to renal pathology within 16 weeks, concomitant with macroscopic evidence of osmoregulatory dysfunction and morbidity (Laenge et al., 2001).

Added by C. Baettig on June 24, 2024

- Male summer flounder injected with 17β-estradiol (E2) had increased levels of circulating VTG. The accumulation of VTG resulted in obstruction or rupture of renal glomeruli (Folmar et al., 2001).
- Male rare minnow exposed to 17α-ethinylestradiol (EE2) and 4-nonylphenol (NP) had significantly increased plasma VTG concentrations, as did females after EE2 exposure. This resulted in hemorrhages in male kidney tubules, hypertrophy of tubular epithelia, and accumulated eosinophilic material in renal tissue (Zha et al., 2007).
- Elevated levels of VTG and kidney hypertrophy in juvenile three-spined sticklebacks was observed after exposure to E2 and EE2 (Hahlbeck et al., 2004).
- Male fathead minnows experimentally exposed to EE2 within a whole lake experiment showed 9000-fold higher VTG concentrations than fish captured from the same lake prior to the EE2 additions. Edema in the interstitium between kidney tubules and eosinophilic deposits in the kidney tubule lumen were also observed in the EE2-exposed male fatheads (Palace et al., 2002).
- After exposure to bisphenol A VTG levels increased in fathead minnows resulting in glomerular epithelial cell hyperplasia, hyaline droplets in glomeruli, glomerular mesangial membrane thickening, intravascular proteinaceous fluid, tubular dilation, and dilation of Bowman’s spaces (Mihaich et al., 2012).
- Fathead minnow embryos exposed to EE2 exhibited increased whole body VTG levels and tubular degeneration and dilation and glomerulonephritis/glomerulosclerosis was observable after 16 weeks (Länge et al., 2001).
- In male fathead minnows exposed to E2 and an estrogenic PFAS, FC-10 diol, for 21 days plasma VTG was significantly increased. Neuropathy in the kidneys of diol-exposed fish was observed, specifically tubule dilation, tubule protein, enlarged glomeruli, glomerular protein, and thickened basement membranes. Additionally, interstitial and intravascular proteinaceous fluid was significantly elevated (Ankley et al. in prep).

Uncertainties and Inconsistencies

Original text - unknown contribution

None that the author of this entry is aware of.

Added by C. Baettig on June 24, 2024

Although the accumulation of hyalin material/lipoprotein within the kidneys has been confirmed to be partially caused by accumulated VTG, some of the accumulated proteins do not respond to VTG antibody (e.g., Folmar et al., 2001). Because male fish will also express other estrogen inducible proteins such as vitelline envelope and zona radiata some renal pathology could be caused by these related proteins rather than VTG (Johan Hyllner et al., 1994; Oppen-Berntsen et al., 1994).

Proliferative kidney disease (PKD) in fish caused by the parasite *Tetracapsuloides bryosalmonae* results in significant kidney pathology. However, when PKD infection took place under simultaneous exposure to EE2, kidney pathology was less pronounced despite the fact that hepatic *vtg* was elevated in fish exposed to the estrogen (Bailey et al., 2019; Rehberger et al., 2020).

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Relationship: 3258: Increase, Renal pathology due to VTG deposition leads to Increased Mortality

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Estrogen receptor agonism leading to reduced survival and population growth due to renal failure	adjacent	Moderate	Low

Evidence Supporting Applicability of this Relationship

Taxonomic applicability: All vertebrates with functional kidneys.

Life stage: This KER is applicable to all life stages following the differentiation of the kidney.

Sex: This KER is applicable to both sexes.

Evidence Supporting this KER

Biological Plausibility

The kidneys perform a suite of physiological roles that are critical for organismal homeostasis including waste excretion, osmoregulation, and fluid homeostasis (Preuss, 1993). The renal system can incur damage from a variety of sources which can lead to a loss of renal functions such as decreased glomerular filtration rate or impaired clearance of waste products which can lead to death (McKee & Wingert, 2015).

For example, in fish exposed to estrogenic compounds there is evidence that excessive production of vitellogenin (VTG), which leads to renal failure, increases mortality in fish (Herman & Kincaid, 1988). Generally, the molecular mass of proteins in glomerular filtrate are lower than albumin but when proteins like VTG are deposited in the kidneys they cannot be resorbed and the excess protein can lead to glomerular rupturing or hemorrhaging (Tojo & Kinugasa, 2012). Ultimately these pathologies can cause acute renal failure resulting in mortality.

Empirical Evidence

- Male summer flounder injected with 17β-estradiol (E2) had increased levels of circulating VTG. The accumulation of VTG resulted in obstruction or rupture of renal glomeruli. Glomerular injury including immunoreactive hyalin material within the glomerular capsule, increased drainage into Bowman's space and renal tubules. Mortality observed after E2 treatment likely resulted from acute renal failure associated with excessive VTG accumulation in the kidney (Folmar et al., 2001).
- High mortality was observed in rainbow trout fed E2. The accumulation of circulating VTG most likely resulted in hypertrophy of the kidneys (Herman & Kincaid, 1988).
- Abdel-Tawwab et al. (2020) found that in European sea bass fed dietary zearalenone combined with exposure to a

pathogen, *Vibrio alginolyticus,* increased mortality. A depletion of serum total protein, albumin, and globulin was observed in in zearalenone fed fish which resulted in kidney dysfunction and ultimately increased mortality.
- Exposure to microcystin-LR (MC-LR) resulted in kidney lesions consisting of coagulative tubular necrosis with a dilation of Bowman's space and caused mortality in rainbow trout (Kotak et al., 1996). Mortality is most likely due to MC-LR resulting in significantly dysregulating proteins related to ionic regulation (Shahmohamadloo et al., 2022).
- Laboratory in vivo aquatic exposures of fathead minnow to EE2 led to renal pathology within 16 weeks, concomitant with macroscopic evidence of osmoregulatory dysfunction and morbidity (Länge et al., 2001).
- Proliferative kidney disease caused by *Tetracapsuloides bryosalmonae* in salmonid fish result in significant kidney lesions and often resulted in mortality (e.g., Bettge et al., 2009; Schmidt-Posthaus et al., 2015; Sterud et al., 2007).
- In male fathead minnows exposed to the estrogenic PFAS FC-10 diol for 21 days neuropathy in the kidneys was observed, specifically tubule dilation, tubule protein, enlarged glomeruli, glomerular protein, and thickened basement membranes. Additionally, interstitial and intravascular proteinaceous fluid was significantly elevated. Elevated mortality in males was also observed (Ankley et al. in prep).

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Relationship: 2013: Increased Mortality leads to Decrease, Population growth rate

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Acetylcholinesterase Inhibition leading to Acute Mortality via Impaired Coordination & Movement	adjacent		
Acetylcholinesterase inhibition leading to acute mortality	adjacent	Moderate	Moderate
Deiodinase 2 inhibition leading to increased mortality via reduced posterior swim bladder inflation	adjacent	Moderate	Moderate
Deiodinase 2 inhibition leading to increased mortality via reduced anterior swim bladder inflation	adjacent	Moderate	Moderate
Deiodinase 1 inhibition leading to increased mortality via reduced posterior swim bladder inflation	adjacent	Moderate	Moderate
Deiodinase 1 inhibition leading to increased mortality via reduced anterior swim bladder inflation	adjacent	Moderate	Moderate
Thyroperoxidase inhibition leading to increased mortality via reduced anterior swim bladder inflation	adjacent	Moderate	Moderate

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Thyroperoxidase inhibition leading to altered visual function via altered retinal layer structure	adjacent	Moderate	Moderate
Thyroperoxidase inhibition leading to altered visual function via decreased eye size	adjacent		
Thyroperoxidase inhibition leading to altered visual function via altered photoreceptor patterning	adjacent		
Inhibition of Fyna leading to increased mortality via decreased eye size (Microphthalmos)	adjacent	High	High
GSK3beta inactivation leading to increased mortality via defects in developing inner ear	adjacent	High	High
Estrogen receptor agonism leading to reduced survival and population growth due to renal failure	adjacent	Moderate	Moderate

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio	High	NCBI
fathead minnow	Pimephales promelas	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	Moderate

Taxonomic: All organisms must survive to reproductive age in order to reproduce and sustain populations. The additional considerations related to survival made above are applicable to other fish species in addition to zebrafish and fathead minnows with the same reproductive strategy (r-strategist as described in the theory of MaxArthur and Wilson (1967). The impact of reduced survival on population size is even greater for k-strategists that invest more energy in a lower number of offspring.

Life stage: Density dependent effects start to play a role in the larval stage of fish when free-feeding starts (Hazlerigg et al., 2014).

Sex: This linkage is independent of sex.

Key Event Relationship Description

Increased mortality in the reproductive population may lead to a declining population. This depends on the excess mortality due to the applied stressor and the environmental parameters such as food availability and predation rate. Most fish species are r-strategist, meaning they produce a lot of offspring instead of investing in parental care. This results in natural high larval mortality causing only a small percentage of the larvae to survive to maturity. If the excess larval mortality due to a stressor is small, the population dynamics might result in constant population size. Should the larval excess be more significant, or last on the long-term, this will affect the population. To calculate the long-term persistence of the population, population dynamic models should be used.

Evidence Supporting this KER

Survival rate is an obvious determinant of population size and is therefore included in population modeling (e.g., Miller et al., 2020).

Biological Plausibility

- Survival to reproductive maturity is a parameter of demographic significance. Assuming resource availability (i.e., food, habitat, etc.) is not limiting to the extant population, sufficient mortality in the reproductive

population may ultimately lead to declining population trajectories.
- Under some conditions, reduced larval survival may be compensated by reduced predation and increased food availability, and therefore not result in population decline (Stige et al., 2019).

Empirical Evidence

- According to empirical data, combined with population dynamic models, feeding larvae are the crucial life stage in zebrafish (and other r-strategists) for the regulation of the population. (Schäfers et al., 1993)
- In fathead minnow, natural survival of early life stages has been found to be highly variable and influential on population growth (Miller and Ankley, 2004)
- Rearick et al. (2018) used data from behavioural assays linked to survival trials and applied a modelling approach to quantify changes in antipredator escape performance of larval fathead minnows in order to predict changes in population abundance. This work was done in the context of exposure to an environmental oestrogen. Exposed fish had delayed response times and slower escape speeds, and were more susceptible to predation. Population modelling showed that this can result in population decline.
- In the context of fishing and fisheries, ample evidence of a link between increased mortality and a decrease of population size has been given. Important insights can result from the investigation of optimum modes of fishing that allow for maintaining a population (Alekseeva and Rudenko, 2018). Jacobsen and Essington (2018) showed the impact of varying predation mortality on forage fish populations.
- Boreman (1997) reviewed methods for comparing the population-level effects of mortality in fish populations induced by pollution or fishing.

Uncertainties and Inconsistencies

- The extent to which larval mortality affects population size could depend on the fraction of surplus mortality compared to a natural situation.
- There are scenarios in which individual mortality may not lead to declining population size. These include instances where populations are limited by the availability of habitat and food resources, which can be replenished through immigration. Effects of mortality in the larvae can be compensated by reduced competition for resources (Stige et al., 2019).
- The direct impact of pesticides on migration behavior can be difficult to track in the field, and documentation of mortality during migration is likely underestimated (Eng 2017).

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