AOP ID and Title:

SNAPSHOT

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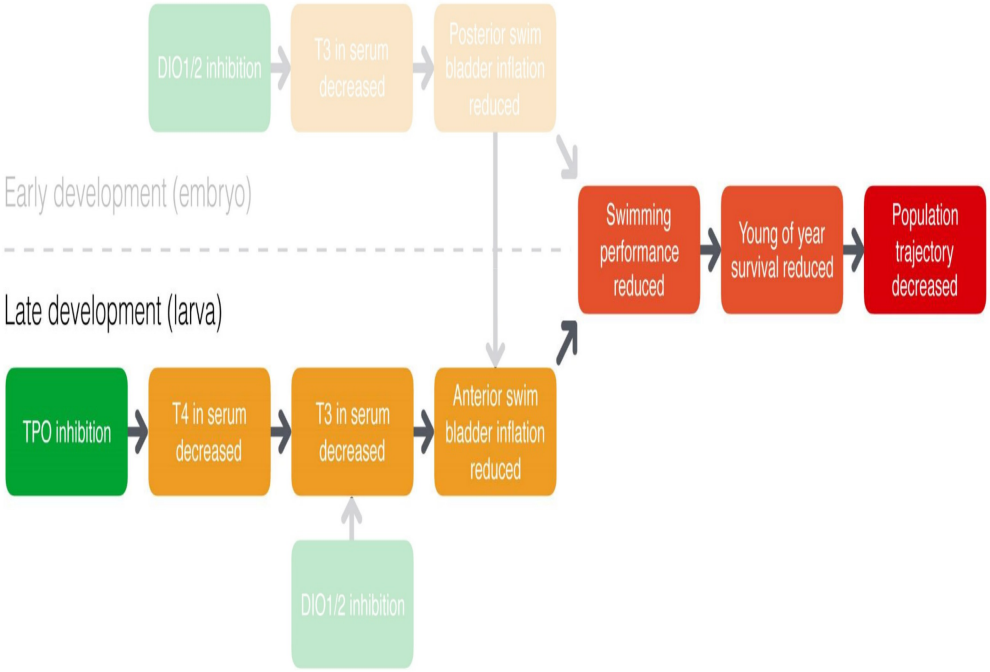
AOP 159: Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation

Short Title: TPOi anterior swim bladder

Graphical Representation



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Status

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Abstract

The AOP describes the effects of inhibition of thyroperoxidase (TPO) on anterior swim bladder inflation leading to reduced young of year survival and population trajectory decline. The inhibition of TPO is the molecular-initiating event (MIE), which results in decreased circulating concentrations of thyroxine (T4) and triiodothyronine (T3) in serum. Disruption of the thyroid hormone (TH) system is increasingly being recognized as an important mode of action that can lead to adverse outcomes, especially during embryonic development. In fish, many different adverse

effects during early development resulting from disruption of the TH endocrine system have been reported (e.g., effects on body and eye size, head-to-trunk angle, heartbeat, otolith formation, pigmentation index, swim bladder inflation, hatching time, somite formation, escape response and photoreceptor development). As in amphibians, the transition in fish between the different developmental phases, including maturation and inflation of the swim bladder, have been shown to be
mediated by THs. Chemicals interfering with the synthesis of T4 have the potential to inhibit anterior chamber inflation which may result in reduced auditory capacity and reduced swimming capacity of the fish, a relevant adverse outcome that can affect feeding behaviour and predator avoidance, resulting in lower survival probability and ultimately population trajectory decline (Czesny et al., 2005; Woolley and Qin, 2010).

Summary of the AOP

Events

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

Sequence	Type	Event ID	Title	Short name
1	MIE	279	Thyroperoxidase, Inhibition (https://aopwiki.org/events/279)	Thyroperoxidase, Inhibition
2	KE	277	Thyroid hormone synthesis, Decreased (https://aopwiki.org/events/277)	TH synthesis, Decreased
3	KE	281	Thyroxine (T4) in serum, Decreased (https://aopwiki.org/events/281)	T4 in serum, Decreased
4	KE	1003	Decreased, Triiodothyronine (T3) in serum (https://aopwiki.org/events/1003)	Decreased, Triiodothyronine (T3) in serum
5	KE	1007	Reduced, Anterior swim bladder inflation (https://aopwiki.org/events/1007)	Reduced, Anterior swim bladder inflation
6	KE	1005	Reduced, Swimming performance (https://aopwiki.org/events/1005)	Reduced, Swimming performance
7	KE	1006	Reduced, Young of year survival (https://aopwiki.org/events/1006)	Reduced, Young of year survival
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
Thyroperoxidase, Inhibition (https://aopwiki.org/relationships/309)	adjacent	Thyroid hormone synthesis, Decreased		
Thyroid hormone synthesis, Decreased (https://aopwiki.org/relationships/305)	adjacent	Thyroxine (T4) in serum, Decreased		
Thyroxine (T4) in serum, Decreased (https://aopwiki.org/relationships/2038)	adjacent	Decreased, Triiodothyronine (T3) in serum		
Decreased, Triiodothyronine (T3) in serum (https://aopwiki.org/relationships/1035)	adjacent	Reduced, Anterior swim bladder inflation		
Reduced, Anterior swim bladder inflation (https://aopwiki.org/relationships/1034)	adjacent	Reduced, Swimming performance		

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Reduced, Swimming performance (https://aopwiki.org/relationships/1029)	adjacent	Reduced, Young of year survival		
Reduced, Young of year survival (https://aopwiki.org/relationships/1030)	adjacent	Decrease, Population trajectory		
Thyroperoxidase, Inhibition (https://aopwiki.org/relationships/366)	non-adjacent	Thyroxine (T4) in serum, Decreased		
Thyroxine (T4) in serum, Decreased (https://aopwiki.org/relationships/1039)	non-adjacent	Reduced, Anterior swim bladder inflation		

Stressors

Name	Evidence
Methimazole	
Mercaptobenzothiazole	
Propylthiouracil	

Overall Assessment of the AOP

Overall, the weight of evidence for the sequence of key events laid out in the AOP is moderate to high. Nonetheless, the exact underlying mechanism of TH disruption leading to impaired swim bladder inflation is not understood. The current domain of applicability is larval life stages of zebrafish and fathead minnow pending future research in other fish species such as medaka.

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
Development	

Taxonomic Applicability

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

Sex Applicability

Sex	Evidence
Unspecific	

The current AOP is only applicable to larval development, which is the period where the anterior swim bladder chamber inflates. The earliest life stages of teleost fish rely on maternally transferred THs to regulate certain developmental processes until embryonic TH synthesis is active (Power et al., 2001). As a result, early developmental processes that are dependent on THs, such as posterior swim bladder chamber inflation, appear to be less sensitive to inhibition of TH synthesis. When maternally derived THs are depleted during late development (larval stage), endogenous TH synthesis becomes more important and inhibition of TPO interferes with proper inflation of the anterior swim bladder chamber (Stinckens et al. submitted; Nelson et al., 2016; Stinckens et al., 2016; Godfrey et al., 2017).

The AOP is currently mainly based on experimental evidence from studies on zebrafish and fathead minnow. A first logical step in expanding the applicability of the AOP network is to assess its relevance to other species that are frequently used in existing fish test guidelines, such as the Japanese rice fish (medaka), three-spined stickleback and rainbow trout.

Sex differences are typically not investigated in tests using early life stages of fish and it is currently unclear whether sex-related differences are important in this AOP. Zebrafish are undifferentiated gonochorists since both sexes initially develop an immature ovary (Maack and Segner, 2003). Immature ovary development progresses until approximately the onset of the third week. Later, in female fish immature ovaries continue to develop further, while male fish undergo transformation of ovaries into testes. Final transformation into testes varies among male individuals, however finishes usually around 6 weeks post fertilization. Since the anterior chamber inflates around 20 days post fertilization, when sex differentiation is still in its early stages, sex differences are expected to play a minor role in the current AOP.

Essentiality of the Key Events

Overall, the confidence in the supporting data for essentiality of KEs within the AOP is high since there is direct evidence from specifically designed experimental studies (knockdown and knockout studies) illustrating that the impact on downstream KEs corresponds to what is predicted by the AOP.

Weight of Evidence Summary

Overall, the weight of evidence for the biological plausibility of the KERs in the AOP is moderate since there is empirical support for an association between the sets of KEs and the KERs are plausible based on analogy to accepted biological relationships, but scientific understanding is not completely established. Especially for some of the upstream KERs biological plausibility is high.

Overall, the empirical support for the KERs in the AOP is moderate since dependent changes in sets of KEs following exposure to a small number of specific stressors has been demonstrated, but there are still some data gaps.

Quantitative Consideration

There is some level of quantitative understanding that can form the basis for development of a quantitative AOP. Quantitative relationships between reduced T4 and reduced T3, and between reduced T3 and reduced anterior chamber inflation were established. The latter is particularly critical for linking impaired swim bladder inflation to TH disruption.

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Appendix 1

List of MIEs in this AOP

Event: 279: Thyroperoxidase, Inhibition (https://aopwiki.org/events/279)

Short Name: Thyroperoxidase, Inhibition

Key Event Component

Process	Object	Action
iodide peroxidase activity	thyroid peroxidase	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:42 - Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	MolecularInitiatingEvent
Aop:119 - Inhibition of thyroid peroxidase leading to follicular cell adenomas and carcinomas (in rat and mouse) (https://aopwiki.org/aops/119)	MolecularInitiatingEvent
Aop:159 - Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	MolecularInitiatingEvent
Aop:175 - Thyroperoxidase inhibition leading to altered amphibian metamorphosis (https://aopwiki.org/aops/175)	MolecularInitiatingEvent
Aop:271 - Inhibition of thyroid peroxidase leading to impaired fertility in fish (https://aopwiki.org/aops/271)	MolecularInitiatingEvent

Stressors

Name
2(3H)-Benzothiazolethione
2-mercaptobenzothiazole
Ethylene thiourea
Mercaptobenzothiazole
Methimazole
Propylthiouracil
Resorcinol
Thiouracil
Ethylenethiourea
Amitrole
131-55-5
2,2',4,4'-Tetrahydroxybenzophenone
Daidzein
Genistein
4-Nonylphenol
4-propoxyphenol
Sulfamethazine

Biological Context

Level of Biological Organization
Molecular

Cell term

Cell term
thyroid follicular cell

Organ term

Organ term
thyroid follicle

Evidence for Perturbation by Stressor

Overview for Molecular Initiating Event

There is a wealth of information on the inhibition of TPO by drugs such as MMI and PTU, as well as environmental xenobiotics. In the landmark paper on thyroid disruption by environmental chemicals, Brucker-Davis (1998) identified environmental chemicals that depressed TH synthesis by inhibiting TPO. Hurley (1998) listed TPO as a major target for thyroid tumor inducing pesticides. More recent work has tested over 1000 chemicals using a high-throughput screening assay (Paul-Friedman et al., 2016).

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)
humans	Homo sapiens	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
pigs	Sus scrofa	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9823)
Xenopus laevis	Xenopus laevis	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8355)
chicken	Gallus gallus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9031)
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)

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Female	High
Male	High

TPO inhibition is a MIE conserved across taxa, with supporting data from experimental models and human clinical testing. This conservation is likely a function of the high degree of protein sequence similarity in the catalytic domain of mammalian peroxidases (Taurog, 1999). Ample data available for human, rat, and porcine TPO inhibition demonstrate qualitative concordance across these species (Schmultzer et al., 2007; Paul et al., 2013; Hornung et al., 2010). A comparison of rat TPO and pig TPO, bovine lactoperoxidase, and human TPO inhibition by genistein demonstrated good qualitative and quantitative (40–66%) inhibition across species, as indicated by quantification of MIT and DIT production (Doerge and Chang, 2002). Ealey et al. (1984) demonstrated peroxidase activity in guinea pig thyroid tissue using 3,3'-diaminobenzidine tetrahydrochloride (DAB) as a substrate that is oxidized by the peroxidase to form a brown insoluble reaction product. Formation of this reaction product was inhibited by 3-amino-1,2,4-triazole and the TPO inhibitor, methimazole (MMI). A comparative analysis of this action of MMI between rat- and human-derived TPO indicates concordance of qualitative response. Data also suggest an increased quantitative sensitivity to MMI in rat compared to human (Vickers et al., 2012). Paul et al. (2013) tested 12 chemicals using the guaiacol assay using both porcine and rat thyroid microsomes. The authors concluded that there was an excellent qualitative concordance between rat and porcine TPO inhibition, as all chemicals that inhibited TPO in porcine thyroid microsomes also inhibited TPO in rat thyroid microsomes when tested within the same concentration range. In addition, these authors noted a qualitative concordance that ranged from 1.5 to 50-fold differences estimated by relative potency. Similary, Takayama et al. (1986) found a very large species difference in potency for sulfamonomethoxine between cynomologus monkeys and rats.

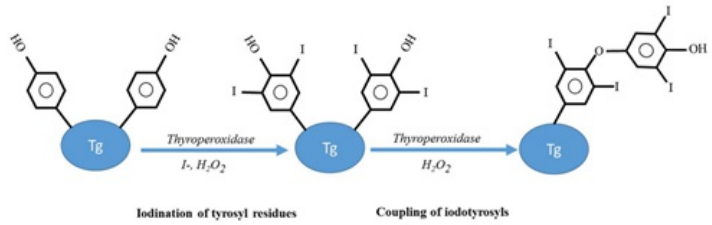
Key Event Description

Thyroperoxidase (TPO) is a heme-containing apical membrane protein within the follicular lumen of thyrocytes that acts as the enzymatic catalyst for thyroid hormone (TH) synthesis. TPO catalyzes several reactions in the thyroid gland, including: the oxidation of iodide; nonspecific iodination of tyrosyl residues of thyroglobulin (Tg); and, the coupling of iodotyrosyls to produce Tg-bound monoiodotyrosine (MIT) and diiodotyrosine (DIT) (Divi et al., 1997; Kessler et al., 2008; Ruf et al., 2006; Taurog et al., 1996). The outcome of TPO inhibition is decreased synthesis of thyroxine (T4) and triiodothyronine (T3), a decrease in release of these hormones from the gland into circulation, and unless compensated, a consequent decrease in systemic concentrations of T4, and possibly T3. The primary product of TPO-catalyzed TH synthesis is T4 (Taurog et al., 1996; Zoeller et al., 2007) that would be peripherally or centrally deiodinated to T3.

It is important to note that TPO is a complex enzyme and that has two catalytic cycles and is capable of iodinating multiple species (Divi et al., 1997). Alterations in all of these events are not covered by some of the commonly used assays that measure “TPO inhibition” (e.g., guicacol and AmplexUltraRed, see below). Therefore, in the context of this AOP we are using TPO inhibiton not in the classical sense, but instead to refer to the empirical data derived from the assays commonly used assays to investigate environmental chemicals.

Figure 1 below illustrates the enzymatic and nonenzymatic reactions mediated by TPO that result in the synthesis of thyroxine (T4) .

Figure 1. Synthesis of thyroxine (T4) by thryoperoxidase showing the iodination of tyrosyl residues and subsequent coupling of iodotyrosyls to form T4.



Inhibition of TPO can be reversible, with transient interaction between the enzyme and the chemical, or irreversible, whereby suicide substrates permanently inactivate the enzyme. Reversible and irreversible TPO inhibition may be determined by the chemical structure, may be concentration dependent, or may be influenced by other conditions, including the availability of iodine (Doerge and Chang, 2002).

The ontogeny of TPO has been determined using both direct and indirect evidence. Available evidence suggests the 11th to 12th fetal week as the beginning of functional TPO in humans. In rodents, TPO function begins late in the second fetal week, with the first evidence of T4 secretion on gestational day 17 (Remy et al., 1980). Thyroid-specific genes appear in the thyroid gland according to a specific temporal pattern; thyroglobulin (*Tg*), TPO (*Tpo*), and TSH receptor (*Tshr*) genes are expressed by gestational day 14 in rats, and the sodium iodide symporter, NIS (*Nis*), is expressed by gestational day 16 in rats. Maturation to adult function is thought to occur within a few weeks after parturition in rats and mice, and within the first few months in neonatal humans (Santisteban and Bernal, 2005). Tg is first detected in human fetuses starting at 5th week of gestation and rises throughout gestation (Thorpe-Beeston et al., 1992), but iodine trapping and T4 production does not occur until around 10-12 weeks. Also, the dimerization of Tg, a characteristic of adult TH storage, is not found until much later in human gestation (Pintar, 2000). In rats, Tg immunoreactivity does not appear until day 15 of gestation (Fukiishi et al., 1982; Brown et al., 2000). The vast majority of research and knowledge on Tg is from mammals, although genomic orthologs are known for a variety of other species (Holzer et al., 2016). It is important to note that prior to the onset of fetal thyroid function, TH are still required by the developing fetus which until that time relies solely on maternal sources. Chemical-induced TPO inhibition can affect synthesis in the maternal gland and in the fetal gland.

How it is Measured or Detected

There are no approved OECD or EPA guideline study protocols for measurement of TPO inhibition.. However, there is an OECD scoping document on identification of chemicals that modulate TH signaling that provides details on a TPO assay (OECD, 2017).

From the early 1960's, microsomal fractions prepared from porcine thyroid glands and isolated porcine follicles were used as a source of TPO for inhibition experiments (Taurog, 2005). Limited information has been published using microsomes from human goiter samples (Vickers et al., 2012) and rat thyroid glands (Paul et al., 2013; 2014; Paul-Friedman et al., 2016).

TPO activity has been measured for decades via indirect assessment by kinetic measurement of the oxidation of guaiacol (Chang & Doerge 2000; Hornung et al., 2010; Schmutzler et al., 2007). This method is a low-throughput assay due to the very rapid kinetics of the guaiacol oxidation reaction. More recently, higher-throughput methods using commercial fluorescent and luminescent substrates with rodent, porcine, and human microsomal TPO have been developed (Vickers et al., 2012; Paul et al., 2013; 2014; Kaczur et al., 1997). This assay substitutes a pre-fluorescent substrate (Amplex UltraRed) for guaiacol, that when incubated with a source of peroxidase and excess hydrogen peroxidase, results in a stable fluorescent product proportional to TPO activity (Vickers et al., 2012). The stability of the fluorescent reaction product allows this assay to be used in a higher throughput format (Paul-Friedman et al., 2016). This approach is appropriate for high-throughput screening but does not elucidate the specific mechanism by which a chemical may inhibit TPO (Paul-Friedman et al., 2016), and as with most in vitro assays, is subject to various sources of assay interference (Thorne et al., 2010).

HPLC has been used to measure of the activity of TPO via formation of the precursors monoiodotyrosine (MIT), diiodotyrosine (DIT), and both T3 and T4, in a reaction mixture containing TPO, or a surrogate enzyme such as lactoperoxidase (Divi & Doerge 1994). The tools and reagents for this method are all available. However, HPLC or other analytical chemistry techniques make this a low throughput assay, depending on the level of automation. A primary advantage of this in vitro method is that it directly informs hypotheses regarding the specific mechanism by which a chemical may impact thyroid hormone synthesis in vitro.

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List of Key Events in the AOP

Event: 277: Thyroid hormone synthesis, Decreased (https://aopwiki.org/events/277)

Short Name: TH synthesis, Decreased

Key Event Component

Process	Object	Action
thyroid hormone generation	thyroid hormone	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:42 - Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	KeyEvent
Aop:65 - XX Inhibition of Sodium Iodide Symporter and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/65)	KeyEvent
Aop:128 - Kidney dysfunction by decreased thyroid hormone (https://aopwiki.org/aops/128)	MolecularInitiatingEvent
Aop:134 - Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	KeyEvent
Aop:54 - Inhibition of Na+/I- symporter (NIS) leads to learning and memory impairment (https://aopwiki.org/aops/54)	KeyEvent
Aop:159 - Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	KeyEvent
Aop:175 - Thyroperoxidase inhibition leading to altered amphibian metamorphosis (https://aopwiki.org/aops/175)	KeyEvent
Aop:176 - Sodium Iodide Symporter (NIS) Inhibition leading to altered amphibian metamorphosis (https://aopwiki.org/aops/176)	KeyEvent
Aop:188 - Iodotyrosine deiodinase (IYD) inhibition leading to altered amphibian metamorphosis (https://aopwiki.org/aops/188)	KeyEvent
Aop:192 - Pendrin inhibition leading to altered amphibian metamorphosis (https://aopwiki.org/aops/192)	KeyEvent
Aop:193 - Dual oxidase (DUOX) inhibition leading to altered amphibian metamorphosis (https://aopwiki.org/aops/193)	KeyEvent
Aop:271 - Inhibition of thyroid peroxidase leading to impaired fertility in fish (https://aopwiki.org/aops/271)	KeyEvent

Stressors

Name
Propylthiouracil
Methimazole

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
thyroid follicular cell

Organ term

Organ term
thyroid gland

Evidence for Perturbation by Stressor

Overview for Molecular Initiating Event

not applicable as this KE is not an MIE

Propylthiouracil

6-n-proylthiouracil is a common positive control

Methimazole

Methimazole is a very common positve control

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)
Pig	Pig	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=0)
Xenopus laevis	Xenopus laevis	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8355)

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

Decreased TH synthesis resulting from TPO or NIS inhibition is conserved across taxa, with *in vivo* evidence from humans, rats, amphibians, some fish specis, and birds, and *in vitro* evidence from rat and porcine microsomes. Indeed, TPO and NIS mutations result in congenital hypothyroidism in humans (Bakker et al., 2000; Spitzweg and Morris, 2010), demonstrating the essentiality of TPO and NIS function toward maintaining euthyroid status. Though decreased serum T4 is used as a surrogate measure to indicate chemical-mediated decreases in TH synthesis, clinical and veterinary management of hyperthyroidism and Grave's disease using propylthiouracil and methimazole, known to decrease TH synthesis, indicates strong medical evidence for chemical inhibition of TPO (Zoeller and Crofton, 2005).

Key Event Description

The thyroid hormones (TH), triiodothyronine (T3) and thyroxine (T4) are thyrosine based hormones. Synthesis of TH is regulated by thyroid-stimulating hormone (TSH) binding to its receptor and thyroidal availability of iodine via the sodium iodide symporter (NIS). Other proteins

contributing to TH production in the thyroid gland, including thyroperoxidase (TPO), dual oxidase enzymes (DUOX), and pendrin are also necessary for iodothyronine production (Zoeller et al., 2007).

The production of THs in the thyroid gland and resulting serum concentrations are controlled by a negatively regulated feedback mechanism. Decreased T4 and T3 serum concentrations activates the hypothalamus-pituitary-thyroid (HPT) axis which upregulates thyroid-stimulating hormone (TSH) that acts to increase production of additional THs (Zoeller and Tan, 2007). This regulatory system includes: 1) the hypothalamic secretion of the thyrotropin-releasing hormone (TRH); 2) the thyroid-stimulating hormone (TSH) secretion from the anterior pituitary; 3) hormonal transport by the plasma binding proteins; 4) cellular uptake mechanisms at the tissue level; 5) intracellular control of TH concentration by deiodinating mechanisms; 6) transcriptional function of the nuclear TH receptor; and 7) in the fetus, the transplacental passage of T4 and T3 (Zoeller et al., 2007).

TRH and the TSH primarily regulate the production of T4, often considered a “pro-hormone,” and to a lesser extent of T3, the transcriptionally active TH. Most of the hormone released from the thyroid gland into circulation is in the form of T4, while peripheral deiodination of T4 is responsible for the majority of circulating T3. Outer ring deiodination of T4 to T3 is catalyzed by the deiodinases 1 and 2 (DIO1 and DIO2), with DIO1 expressed mainly in liver and kidney, and DIO2 expressed in several tissues including the brain (Bianco et al., 2006). Conversion of T4 to T3 takes place mainly in liver and kidney, but also in other target organs such as in the brain, the anterior pituitary, brown adipose tissue, thyroid and skeletal muscle (Gereben et al., 2008; Larsen, 2009).

Most evidence for the ontogeny of TH synthesis comes from measurements of serum hormone concentrations. And, importantly, the impact of xenobiotics on fetal hormones must include the influence of the maternal compartment since a majority of fetal THs are derived from maternal blood early in fetal life, with a transition during mid-late gestation to fetal production of THs that is still supplemented by maternal THs. In humans, THs can be found in the fetus as early as gestational weeks 10-12, and concentations rise continuously until birth. At term, fetal T4 is similar to maternal levels, but T3 remains 2-3 fold lower than maternal levels. In rats, THs can be detected in the fetus as early as the second gestational week, but fetal synthesis does not start until gestational day 17 with birth at gestational day 22-23. Maternal THs continue to supplement fetal production until parturition. (see Howdeshell, 2002; Santisteban and Bernal, 2005 for review). *The ontogeny of TPO inhibition during development by environmental chemicals is a data gap.*

Decreased TH synthesis in the thyroid gland may result from several possible molecular-initiating events (MIEs) including: 1) Disruption of key catalytic enzymes or cofactors needed for TH synthesis, including TPO, NIS, or dietary iodine insufficiency. Theoretically, decreased synthesis of Tg could also affect TH production (Kessler et al., 2008; Yi et al., 1997). Mutations in genes that encode requisite proteins in the thyroid may also lead to impaired TH synthesis, including mutations in pendrin associated with Pendred Syndrome (Dossena et al., 2011), mutations in TPO and Tg (Huang and Jap 2015), and mutations in NIS (Spitzweg and Morris, 2010). 2) Decreased TH synthesis in cases of clinical hypothyroidism may be due to Hashimoto's thyroiditis or other forms of thyroiditis, or physical destruction of the thyroid gland as in radioablation or surgical treatment of thyroid lymphoma. 3) It is possible that TH synthesis may also be reduced subsequent to disruption of the negative feedback mechanism governing TH homeostasis, e.g. pituitary gland dysfunction may result in a decreased TSH signal with concomitant T3 and T4 decreases. 4) More rarely, hypothalamic dysfunction can result in decreased TH synthesis.

Increased fetal thyroid levels are also possible. Maternal Graves disease, which results in fetal thyrotoxicosis (hyperthyroidism and increased serum T4 levels), has been successfully treated by maternal administration of TPO inhibitors (c.f., Sato et al., 2014).

It should be noted that different species and different lifestages store different amounts of TH precursor and iodine within the thyroid gland. Thus, decreased TH synthesis via transient iodine insufficiency or inhibition of TPO may not affect TH release from the thyroid gland until depletion of stored iodinated Tg. Adult humans may store sufficient Tg-DIT residues to serve for several months to a year of TH demand (Greer et al., 2002; Zoeller, 2004). Neonates and infants have a much more limited supply of less than a week.

How it is Measured or Detected

Decreased TH synthesis is often implied by measurement of TPO and NIS inhibition measured clinically and in laboratory models as these enzymes are essential for TH synthesis. Rarely is decreased TH synthesis measured directly, but rather the impact of chemicals on the quantity of T4 produced in the thyroid gland, or the amount of T4 present in serum is used as a marker of decreased T4 release from the thyroid gland (e.g., Romaldini et al., 1988). Methods used to assess TH synthesis include, incorporation of radiolabel tracer compounds, radioimmunoassay, ELISA, and analytical detection.

Recently, amphibian thyroid explant cultures have been used to demonstrate direct effects of chemicals on TH synthesis, as this model contains all necessary synthesis enzymes including TPO and NIS (Hornung et al., 2010). For this work THs was measured by HPLC/ICP-mass spectometry. Decreased TH synthesis and release, using T4 release as the endpoint, has been shown for thiouracil antihyperthyroidism drugs including MMI, PTU, and the NIS inhibitor perchlorate (Hornung et al., 2010).

TIQDT (Thyroxine-immunofluorescence quantitative disruption test) is a method that provides an immunofluorescent based estimate of thyroxine in the gland of zebrafish (Thienpont et al 2011). This method has been used for ~25 xenobiotics (e.g., amitrole, perchlorate, methimazole, PTU, DDT, PCBs). The method detected changes for all chemicals known to directly impact TH synthesis in the thyroid gland (e.g., NIS and TPO inbibitors), but not those that upregulate hepatic catabolism of T4.

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Event: 281: Thyroxine (T4) in serum, Decreased (https://aopwiki.org/events/281)

Short Name: T4 in serum, Decreased

Key Event Component

Process	Object	Action
abnormal circulating thyroxine level	thyroxine	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:42 - Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	KeyEvent
Aop:54 - Inhibition of Na+/I- symporter (NIS) leads to learning and memory impairment (https://aopwiki.org/aops/54)	KeyEvent
Aop:8 - Upregulation of Thyroid Hormone Catabolism via Activation of Hepatic Nuclear Receptors, and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/8)	KeyEvent
Aop:65 - XX Inhibition of Sodium Iodide Symporter and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/65)	KeyEvent
Aop:134 - Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	KeyEvent

AOP ID and Name	Event Type
Aop:152 - Interference with thyroid serum binding protein transthyretin and subsequent adverse human neurodevelopmental toxicity (https://aopwiki.org/aops/152)	KeyEvent
Aop:159 - Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	KeyEvent
Aop:175 - Thyroperoxidase inhibition leading to altered amphibian metamorphosis (https://aopwiki.org/aops/175)	KeyEvent
Aop:176 - Sodium Iodide Symporter (NIS) Inhibition leading to altered amphibian metamorphosis (https://aopwiki.org/aops/176)	KeyEvent
Aop:194 - Hepatic nuclear receptor activation leading to altered amphibian metamorphosis (https://aopwiki.org/aops/194)	KeyEvent

Stressors

Name
Propylthiouracil
Methimazole

Biological Context

Level of Biological Organization
Tissue

Organ term

Organ term
serum

Evidence for Perturbation by Stressor

Propylthiouracil

6-n-propylthouracil is a classic positive control for inhibition of TPO

Perchlorate

Perchlorate ion (ClO_4^-) is a classic positive control for inhibition of NIS

Methimazole

Classic positive control

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
mouse	Mus musculus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
chicken	Gallus gallus	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9031)
Xenopus laevis	Xenopus laevis	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8355)
Pig	Pig	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=0)

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Female	High
Male	High

The overall evidence supporting taxonomic applicability is strong. THs are evolutionarily conserved molecules present in all vertebrate species (Hulbert, 2000; Yen, 2001). Moreover, their crucial role in zebra fish (Thienpont et al., 2011), amphibian and lamprey metamorphoses is well established (Manzon and Youson, 1997; Yaoita and Brown, 1990; Furlow and Neff, 2006). Their existence and importance has also been described in many different animal and plant kingdoms (Eales, 1997; Heyland and Moroz, 2005), while their role as environmental messenger via exogenous routes in echinoderms confirms the hypothesis that these molecules are widely distributed among the living organisms (Heyland and Hodin, 2004). However, the role of TH in the different species depends on the expression and function of specific proteins (e.g receptors or enzymes) under TH control and may vary across species and tissues. As such extrapolation regarding TH action across species should be done with caution.

With few exceptions, vertebrate species have circulating T4 (and T3) that are bound to transport proteins in blood. Clear species differences exist in serum transport proteins (Dohler et al., 1979; Yamauchi and Isihara, 2009). There are three major transport proteins in mammals; thyroid binding globulin (TBG), transthyretin (TTR), and albumin. In adult humans, the percent bound to these proteins is about 75, 15 and 10 percent, respectively (Schussler 2000). In contrast, in adult rats the majority of THs are bound to TTR. Thyroid binding proteins are developmentally regulated in rats. TBG is expressed in rats until approximately postnatal day (PND) 60, with peak expression occurring during weaning (Savu et al., 1989). However, low levels of TBG persist into adult ages in rats and can be experimentally induced by hypothyroidism, malnutrition, or caloric restriction (Rouaze-Romet et al., 1992). While these species differences impact TH half-life (Capen, 1997) and possibly regulatory feedback mechanisms, there is little information on quantitative dose-response relationships of binding proteins and serum hormones during development across different species. Serum THs are still regarded as the most robust measurable key event causally linked to downstream adverse outcomes.

Key Event Description

All iodothyronines are derived from the modification of tyrosine molecules (Taurog, 2000). There are two biologically active thyroid hormones (THs) in serum, triiodothyronine (T3) and T4, and a few inactive iodothyronines (rT3, 3,5-T2). T4 is the predominant TH in circulation, comprising approximately 80% of the TH excreted from the thyroid gland and is the pool from which the majority of T3 in serum is generated (Zoeller et al., 2007). As such, serum T4 changes usually precede changes in other serum THs. Decreased thyroxine (T4) in serum results result from one or more MIEs upstream and is considered a key biomarker of altered TH homeostasis (DeVito et al., 1999).

Serum T4 is used as a biomarker of TH status because the circulatory system serves as the major transport and delivery system for TH delivery to tissues. The majority of THs in the blood are bound to transport proteins (Bartalena and Robbins, 1993). In serum, it is the unbound, or ‘free’ form of the hormone that is thought to be available for transport into tissues. Free hormones are approximately 0.03 and 0.3 percent for T4 and T3, respectively. There are major species differences in the predominant binding proteins and their affinities for THs (see below). However, there is broad agreement that changes in serum concentrations of THs is diagnostic of thyroid disease or chemical-induced disruption of thyroid homeostasis (DeVito et al., 1999; Miller et al., 2009; Zoeller et al., 2007).

Normal serum T4 reference ranges can be species and lifestage specific. In rodents, serum THs are low in the fetal circulation, increasing as the fetal thyroid gland becomes functional on gestational day 17, just a few days prior to birth. After birth serum hormones increase steadily, peaking

at two weeks, and falling slightly to adult levels by postnatal day 21 (Walker et al., 1980; Harris et al., 1978; Goldey et al., 1995; Lau et al., 2003). Similarly, in humans, adult reference ranges for THs do not reflect the normal ranges for children at different developmental stages, with TH concentrations highest in infants, still increased in childhood, prior to a decline to adult levels coincident with pubertal development (Corcoran et al. 1977; Kapelari et al., 2008). In some frog species, there is an analogous peak in thyroid hormones in tadpoles that starts around embryonic NF stage 56, peaks at Stage 62 and the declines to lower levels by Stage 56 (Sternberg et al., 2011; Leloup and Buscaglia, 1977).

How it is Measured or Detected

Serum T3 and T4 can be measured as free (unbound) or total (bound + unbound). Free hormone concentrations are clinically considered more direct indicators of T4 and T3 activities in the body, but in animal studies, total T3 and T4 are typically measured. Historically, the most widely used method in toxicology is the radioimmunoassay (RIA). The method is routinely used in rodent endocrine and toxicity studies. The ELISA method is a commonly used as a human clinical test method. Analytical determination of iodothyronines (T3, T4, rT3, T2) and their conjugates, though methods employing HPLC, liquid chromatography, immuno luminescence, and mass spectrometry are less common, but are becoming increasingly available (Hornung et al., 2015; DeVito et al., 1999; Baret and Fert, 1989; Spencer, 2013; Samanidou V.F et al., 2000; Rathmann D. et al., 2015). It is important to note that thyroid hormones concentrations can be influenced by a number of intrinsic and extrinsic factors (e.g., circadian rhythms, stress, food intake, housing, noise) (see for example, Döhler et al., 1979).

Any of these measurements should be evaluated for the relationship to the actual endpoint of interest, repeatability, reproducibility, and lower limits of quantification using a fit-for-purpose approach (i.e., different regulatory needs will require different levels of confidence in the AOP). This is of particular significance when assessing the very low levels of TH present in fetal serum. Detection limits of the assay must be compatible with the levels in the biological sample. All three of the methods summarized above would be fit-for-purpose, depending on the number of samples to be evaluated and the associated costs of each method. Both RIA and ELISA measure THs by an indirect methodology, whereas analytical determination is the most direct measurement available. All these methods, particularly RIA, are repeatable and reproducible.

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Event: 1003: Decreased, Triiodothyronine (T3) in serum (https://aopwiki.org/events/1003)

Short Name: Decreased, Triiodothyronine (T3) in serum

Key Event Component

Process	Object	Action
abnormal circulating hormone level		decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:155 - Deiodinase 2 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/155)	KeyEvent
Aop:156 - Deiodinase 2 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/156)	KeyEvent
Aop:157 - Deiodinase 1 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/157)	KeyEvent
Aop:158 - Deiodinase 1 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/158)	KeyEvent
Aop:189 - Type I iodothyronine deiodinase (DIO1) inhibition leading to altered amphibian metamorphosis (https://aopwiki.org/aops/189)	KeyEvent
Aop:159 - Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	KeyEvent

Biological Context

Level of Biological Organization
Tissue

Organ term

Organ term
serum

Domain of Applicability

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	

Sex Applicability

Sex	Evidence
Unspecific	

The overall evidence supporting taxonomic applicability is strong. With few exceptions vertebrate species have circulating T3 and T4 that are bound to transport proteins in blood. Clear species differences exist in transport proteins (Yamauchi and Isihara, 2009). Specifically, the majority of supporting data for TH decreases in serum come from rat studies, and the predominant iodothyronine binding protein in rat serum is transthyretin (TT4). TT4 demonstrates a reduced binding affinity for T4 when compared with thyroxine binding globulin (TBG), the predominant serum binding protein for T4 in humans. This difference in serum binding protein affinity for THs is thought to modulate serum half-life for T4; the half-life of T4 in rats is 12-24 hr, wherease the half-life in humans is 5-9 days (Capen, 1997). While these species differences impact hormone half-life, possibly regulatory feedback mechanisms, and quantitative dose-response relationships, measurement of serum THs is still regarded as a measurable key event causatively linked to downstream adverse outcomes.

THs are evolutionarily conserved molecules present in all vertebrate species (Hulbert, 2000; Yen, 2001). Moreover, their crucial role in amphibian and larbean metamorphoses is well established (Manzon and Youson, 1997; Yaoita and Brown, 1990). Their existence and importance has been also described in many differrent animal and plant kingdoms (Eales, 1997; Heyland and Moroz, 2005), while their role as environmental messenger via exogenous routes in echinoderms confirms the hypothesis that these molecules are widely distributed among the living organisms (Heyland and Hodin, 2004). However, the role of TH in the different species may differ depending on the expression or function of specific proteins (e.g receptors or enzymes) that are related to TH function, and therefore extrapolation between species should be done with cautious.

Key Event Description

There are two biological active thyroid hormones (THs), triiodothyronine (T3) and thyroxine (T4), and a few inactive iodothyronines (rT3, 3,5-T2), which are all derived from the modification of tyrosine molecules (Hulbert, 2000). However, the plasma concentrations of the other iodothyronines are significantly lower than those of T3 and T4. The different iodothyronines are formed by the sequential outer or inner ring monodeiodination of T4 by the deiodinating enzymes, Dio1, Dio2, and Dio3 (Gereben et al., 2008). Deiodinase structure is considered to be unique, as THs are the only molecules in the body that incorporate iodide.

The circulatory system serves as the major transport and delivery system for THs from synthesis in the gland to delivery to tissues. The majority of THs in the blood are bound to transport proteins (Bartalena and Robbins, 1993). In humans, the major transport proteins are TBG (thyroxine binding globulin), TTR (transthyretin) and albumin. The percent bound to these proteins in adult humans is about 75, 15 and 10 percent, respectively (Schussler 2000). Unbound (free) hormones are approximately 0.03 and 0.3 percent for T4 and T3, respectively. In serum, it is the free form of the hormone that is active.

There are major species differences in the predominant binding proteins and their affinities for THs (see section below on Taxonomic applicability). However, there is broad agreement that changes in serum concentrations of THs is diagnostic of thyroid disease or chemical-induced disruption of thyroid homeostasis (Zoeller et al., 2007).

It is notable that the changes measured in the TH concentration reflect mainly the changes in the serum transport proteins rather than changes in the thyroid status. These thyroid-binding proteins serve as hormonal store which ensure their even and constant distribution in the different tissues, while they protect the most sensitive ones in the case of severe changes in thyroid availability, like in thyroidectomies (Obregon et al., 1981). Until recently, it was believed that all of the effects of TH were mediated by the binding of T3 to the thyroid nuclear receptors (TRa and TRb), a notion which is now questionable due to the increasing evidence that support the non-genomic action of TH (Davis et al., 2010, Moeller et al., 2006). Many non-nuclear TH binding sites have been identified to date and they usually lead to rapid cellular response in TH-effects (Bassett et al., 2003), but the specific pathways that are activated in this regard need to be elucidated.

The production of THs in the thyroid gland and the circulation levels in the bloodstream are self-controlled by an efficiently regulated feedback mechanism across the Hypothalamus-Pituitary-Thyroid (HPT) axis. One of the most unique characteristics of TH is their ability to regulate their own concentration, not only in the plasma level, but also in the individual cell level, to maintain their homeostasis. This is succeed by the efficient regulatory mechanism of the thyroid hormone axis which consists of the following: (1) the hypothalamic secretion of the thyrotropin-releasing hormone (TRH), (2) the thyroid-stimulating hormone (TSH) secretion from the anterior pituitary, (3) hormonal transport by the plasma binding proteins, (4) cellular uptake mechanisms in the cell level, (5) intracellular control of TH concentration by the deiodinating mechanism (6) transcriptional function of the nuclear thyroid hormone receptor and (7) in the fetus, the transplacental passage of T4 and T3 (Cheng et al., 2010).

In regards to the brain, the TH concentration involves also an additional level of regulation, namely the hormonal transport through the Blood Brain Barrier (BBB) (Williams, 2008). The TRH and the TSH are actually regulating the production of pro-hormone T4 and in a lesser extent of T3, which is the biologically active TH. The rest of the required amount of T3 is produced by outer ring deiodination of T4 by the deiodinating enzymes D1 and D2 (Bianco et al., 2006), a process which takes place mainly in liver and kidneys but also in other target organs such as in the brain, the anterior pituitary, brown adipose tissue, thyroid and skeletal muscle (Gereben et al., 2008; Larsen, 2009). Both hormones exert their action in almost all tissues of mammals and they are acting intracellularly, and thus the uptake of T3 and T4 by the target cells is a crucial step of the overall pathway. The trans-membrane transport of TH is performed mainly through transporters that differ depending on the cell type (Hennemann et al., 2001; Friesema et al., 2005; Visser et al., 2008). Many transporter proteins have been identified up to date but the monocarboxylate transporters (Mct8, Mct10) and the anion-transporting polypeptide (OATP1c1) show the highest degree of affinity towards TH (Jansen et al., 2005).

T3 and T4 have significant effects on normal development, neural differentiation, growth rate and metabolism (Yen, 2001; Brent, 2012; Williams, 2008), with the most prominent ones to occur during the fetal development and early childhood. The clinical features of hypothyroidism and hyperthyroidism emphasize the pleiotropic effects of these hormones on many different pathways and target organs. The thyroidal actions though are not only restricted to mammals, as their high significance has been identified also for other vertebrates, with the most well-studied to be the amphibian metamorphosis (Furlow and Neff, 2006). The importance of the thyroid-regulated pathways becomes more apparent in iodine deficient areas of the world, where a higher rate of cretinism and growth retardation has been observed and linked to decreased TH levels (Gilbert et al., 2012). Another very common cause of severe hypothyroidism in human is the congenital hypothyroidism, but the manifestation of these effects is only detectable in the lack of adequate treatment and is mainly related to neurological impairment and growth retardation (Glinoer, 2001), emphasizing the role of TH in neurodevelopment in all above cases. In adults, the thyroid-related effects are mainly linked to metabolic activities, such as deficiencies in oxygen consumption, and in the metabolism of the vitamin, proteins, lipids and carbohydrates, but these defects are subtle and reversible (Oetting and Yen, 2007). Blood tests to detect the amount of thyroid hormone (T4) and thyroid stimulating hormone (TSH) are routinely done for newborn babies for the diagnosis of congenital hypothyroidism at the earliest stage possible.

How it is Measured or Detected

T3 and T4 can be measured as free (unbound) or total (bound + unbound). Free hormone are considered more direct indicators of T4 and T3 activities in the body. The majority of T3 and T4 measurements are made using either RIA or ELISA kits. In animal studies, total T3 and T4 are typically measured as the concentrations of free hormone are very low and difficult to detect. Historically, the most widely used method in toxicology is RIA. The method is routinely used in rodent endocrine and toxicity studies. The ELISA method has become more routine in rodent studies. The ELISA method is a commonly used as a human clinical test method. Least common is analytical determination of iodothyronines (T3, T4, rT3, T2) and their conjugates, though methods employing HLPC and mass spectrometry (DeVito et al., 1999; Miller et al., 2009).

Any of these measurements should be evaluated for fit-for-purpose, relationship to the actual endpoint of interest, repeatability, and reproducibility. All three of the methods summarized above would be fit-for-purpose, depending on the number of samples to be evaluated and the associated costs of each method. Both RIA and ELISA measure THs by a an indirect methodology, whereas analytical determination is the most direct measurement available. All of these methods, particularly RIA, are repeatable and reproducible.

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Event: 1007: Reduced, Anterior swim bladder inflation (https://aopwiki.org/events/1007)

Short Name: Reduced, Anterior swim bladder inflation

Key Event Component

Process	Object	Action
swim bladder inflation	anterior chamber swim bladder	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:155 - Deiodinase 2 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/155)	KeyEvent
Aop:156 - Deiodinase 2 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/156)	KeyEvent
Aop:157 - Deiodinase 1 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/157)	KeyEvent
Aop:158 - Deiodinase 1 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/158)	KeyEvent
Aop:159 - Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	KeyEvent

Biological Context

Level of Biological Organization
Organ

Organ term

Organ term
swim bladder

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=7955)

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

The evidence for impaired inflation of the anterior chamber of the swim bladder currently comes from work on zebrafish and fathead minnow.

Key Event Description

The swim bladder of bony fish is evolutionary homologous to the lung (Zheng et al., 2011). The teleost swim bladder is a gas-filled structure that consists of two chambers, the posterior and anterior chamber. In zebrafish, the posterior chamber inflates around 96 h post fertilization (hpf) which is 2 days post hatch, and the anterior chamber inflates around 21 dpf. In fathead minnow, the posterior and anterior chamber inflate around 6 and 14 dpf respectively. Inflation of the anterior swim bladder chamber is part of the larval-to-juvenile transition in fish, together with the development of adult fins and fin rays, ossification of the axial skeleton, formation of an adult pigmentation pattern, scale formation, maturation and remodeling of organs including the lateral line, nervous system, gut and kidneys (McMenamin and Parichy, 2013).

The anterior chamber is formed by evagination from the cranial end of the posterior chamber (Robertson et al., 2007). Dumbarton et al. (2010) showed that the anterior chamber of zebrafish has particularly closely packed and highly organized bundles of muscle fibres, suggesting that contraction of these muscles would reduce swim bladder volume. While it had previously been suggested that the posterior chamber had a more important role as a hydrostatic organ, this implies high importance of the anterior chamber for buoyancy. The anterior chamber has an additional role in hearing (Bang et al., 2002). Weberian ossicles (the Weberian apparatus) connect the anterior chamber to the inner ear resulting in an amplification of sound waves. Reduced inflation of the anterior chamber may manifest itself as either a complete failure to inflate the chamber or reduced size of the chamber. Reduced size is often associated with a deviating morphology.

How it is Measured or Detected

In several fish species, inflation of the anterior chamber can be observed using a stereomicroscope because the larvae are still transparent during the larval stage. This is for example true for zebrafish and fathead minnow. Anterior chamber size can then be measured based on photographs with a calibrator.

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Event: 1005: Reduced, Swimming performance (https://aopwiki.org/events/1005)

Short Name: Reduced, Swimming performance

Key Event Component

Process	Object	Action
aquatic locomotion		decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:155 - Deiodinase 2 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/155)	KeyEvent
Aop:156 - Deiodinase 2 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/156)	KeyEvent

AOP ID and Name	Event Type
Aop:157 - Deiodinase 1 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/157)	KeyEvent
Aop:158 - Deiodinase 1 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/158)	KeyEvent
Aop:159 - Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	KeyEvent
Aop:242 - Inhibition of lysyl oxidase leading to enhanced chronic fish toxicity (https://aopwiki.org/aops/242)	KeyEvent
Aop:334 - Glucocorticoid Receptor Agonism Leading to Impaired Fin Regeneration (https://aopwiki.org/aops/334)	KeyEvent

Biological Context

Level of Biological Organization
Individual

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=7955)
teleost fish	teleost fish		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=70862)

Life Stage Applicability

Life Stage	Evidence
All life stages	

Sex Applicability

Sex	Evidence
Mixed	

Importance of swimming performance for natural behaviour is generally applicable to fish.

Key Event Description

Adequate swimming performance in fish is essential for behaviour such as foraging, predator avoidance and reproduction.

How it is Measured or Detected

For fish larvae, automated observation and tracking systems are commercially available and increasingly used for measuring swimming performance including distance travelled, duration of movements, swimming speed, etc. This kind of measurements is often included in publications describing effects of chemicals in zebrafish larvae (Hagenaars et al., 2014; Stinckens et al., 2016; Vergauwen et al., 2015).

For juvenile and adult fish, measurements of swim performance vary. However, in some circumstances, a swim tunnel has been used to measure various data (Fu et al., 2013).

References

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Hagenaars, A., Stinckens, E., Vergauwen, L., Bervoets, L., Knapen, D., 2014. PFOSaffects posterior swim bladder chamber inflation and swimming performanceof zebrafish larvae. Aquat. Toxicol. 157, 225–235.

Stinckens, E., Vergauwen, L., Schroeder, A.L., Maho, W., Blackwell, B., Witter, H.,Blust, R., Ankley, G.T., Covaci, A., Villenueve, D.L., Knapen, D., 2016. Disruption of thyroid hormone balance after 2-mercaptobenzothiazole exposure causes swim bladder inflation impairment—part II: zebrafish. Aquat. Toxicol. 173:204-17.

Vergauwen, Lucia; Nørgaard Schmidt, Stine; Maho, Walid; Stickens, Evelyn; Hagenaars, An; Blust, Ronny; Mayer, Philipp; Covaci, Adrian; Knapen, Dries. 2014. A high throughput passive dosing format for the Fish Embryo Acute Toxicity test. Chemosphere. 139: 9-17.

Event: 1006: Reduced, Young of year survival (https://aopwiki.org/events/1006)

Short Name: Reduced, Young of year survival

Key Event Component

Process	Object	Action
survival		decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:155 - Deiodinase 2 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/155)	KeyEvent
Aop:156 - Deiodinase 2 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/156)	KeyEvent
Aop:157 - Deiodinase 1 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/157)	KeyEvent
Aop:158 - Deiodinase 1 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/158)	KeyEvent
Aop:159 - Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	KeyEvent

Biological Context

Level of Biological Organization
Individual

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=7955)

Survival is important for all species.

Key Event Description

Young of year refers to young animals (usually fish) produced in one reproductive year, which have not yet reached one year of age. Small fish, hatched from eggs spawned in the current year, are considered young of year.

Young of year survival directly impacts population structure, growth and fitness. Maintenance of sustainable fish and wildlife populations is an accepted regulatory goal upon which risk assessments and risk management decisions are based.

How it is Measured or Detected

Young of year survival can be measured:

- in the lab by recording survival during prolonged exposure experiments
- in dedicated mesocosms, or in drainable ponds
- in the field, for example by determining age structure after one capture, or by capture-tag-recapture efforts

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

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: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 ID and Name	Event Type
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 fertility 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

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: 309: Thyroperoxidase, Inhibition leads to TH synthesis, Decreased (https://aopwiki.org/relationships/309)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	**adjacent**	**High**	**Low**
Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	**adjacent**		
Inhibition of thyroid peroxidase leading to impaired fertility in fish (https://aopwiki.org/aops/271)	**adjacent**		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

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

Term	Scientific Term	Evidence	Links
Xenopus laevis	Xenopus laevis	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8355)

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

Inhibition of TPO activity is widely accepted to directly impact TH synthesis. This is true for both rats and humans, as well as some fishes, frogs and birds. Most of the data supporting a causative relationship between TPO inhibition and altered TH synthesis is derived from animal studies, in vitro thyroid microsomes from rats or pigs, and a limited number of human ex vivo (Nagasaka and Hidaka, 1976; Vickers et al., 2012) and clinical studies. There are data to support that gene mutations in TPO result in congenital hypothyroidism, underscoring the essential role of TPO in human thyroid hormone synthesis.

Key Event Relationship Description

Thyroperoxidase (TPO) is a heme-containing apical membrane protein within the follicular lumen of thyrocytes that acts as the enzymatic catalyst for thyroid hormone (TH) synthesis (Taurog, 2005). Two commonly used reference chemicals, propylthiouracil (PTU) and methimazole (MMI), are drugs that inhibit the ability of TPO to: a) activate iodine and transfer it to thyroglobulin (Tg) (Davidson et al., 1978); and, b) couple thyroglobulin (Tg)-bound iodotyrosyls to produce Tg-bound thyroxine (T4) and triiodothyronine (T3) (Taurog, 2005).

Evidence Supporting this KER

The weight of evidence supporting a direct linkage between the MIE, TPO inhibition, and the KE of decreased TH synthesis, is strong and supported by more than three decades of research in animals, including humans (Cooper et al., 1982; Cooper et al.,1983; Divi and Doerge, 1994).

Biological Plausibility

The biological plausibility for this KER is rated Strong. TPO is the only enzyme capable of de novo systhesis of TH. TPO catalyzes several reactions, including the oxidation of iodide, nonspecific iodination of tyrosyl residues of thyroglobulin (Tg) to form monoiodotyrosyl (MIT) or diiodotyrosyl (DIT) residues, and the coupling of these Tg-bound iodotyrosyls to produce Tg-bound T3 and T4 (Divi and Doerge, 1994; Kessler et al., 2008; Ruf et al., 2006; Taurog et al., 1996, 2005). Therefore, inhibition of TPO activity is widely accepted to directly impact TH synthesis.

Empirical Evidence

Empirical support for this KER is strong. There are several papers that have measured alterations in TPO and subsequent effects on TH synthesisTaurog et al. (1996) showed decreased guicaol activity, decreased bound I^{125}, and subsequent decreases in newly formed T3 and T4 per molecule of Tg, following exposure to PTU, MMI and some antibiotics. Following in vivo exposure to PTU in rats (Cooper et al., 1982; 1983), there are concentration and time-dependent decreases in thyroid protein bound iodine and serum T4 and T3 that recovered one month after cessation of PTU exposure. In addition, measures of thyroidal iodine content were highly correlated with intra-thyroidal PTU concentration. Vickers et al. (2012) demonstrated dose- and time- dependent inhibition of TPO activity in both human and rat thyroid homogenates exposed to MMI. Tietge et al (2010) recently showed decreases in thyroidal T4 following MMI exposure in Xenopus. Doerge et al (1998) showed that a tryphenylmethane dye, malachite green, inhibited TPO and lowered thyroxine production. A recent paper used a series of benzothiazoles and showed TPO inhibition (guicaol assay) and inhibition of TSH stimulated thyroxine release from Xenopus thyroid gland explant cultures (Hornung et al., 2015).

> *Temporal Evidence:* The temporal nature of this KER is applicable to all life stages, including development (Seed et al., 2005). The impact of decreased TPO activity on thyroidal hormone synthesis is similar across all ages. Good evidence for the temporal relationship of the KER comes from thyroid system modeling (e.g., Degon et al., 2008; Fisher et al., 2013) using data using data from studies of iodine deficiency and chemicals that inhibit NIS. In addition, there is ample evidence of the temporal impacts of TPO inhibition on TH synthesis, using ex vivo and in vitro measures that demonstrate the time course of inhibition following chemical exposures, including some data from human thyroid microsomes and ex vivo thyroid slices (Vickers et al., 2012). Future work is needed that measures both TPO inhibition and TH production during development.
>
> *Dose-Response Evidence:* Dose-response data is available from a number of studies that correlate TPO inhibition with decreased TH production measured using a variety of endpoints including iodine organification (e.g., Taurog et al., 1996), inhibition of guicaol oxidation in thyroid microsomes (e.g., Doerge and Chang, 2002), and direct measure of thyroid gland T4 concentrations (e.g., Hornung et al., 2015). However, there is a lack of dose-response data from developmental studies showing direct linkages from TPO inhibition to thyroidal TH synthesis.

Uncertainties and Inconsistencies

While it is clear that TPO inhibition will lead to altered hormone synthesis, there is a need for data that will inform quantitative modeling of the relationship between TPO inhibition and the magnitude of effects on thyroid hormone synthesis.

It is important to note that data from studies on genistein highlight this uncertainty. Doerge and colleagues have demonstrated that for this compound up to 80% TPO inhibition did not result in decreased serum T4 in rats (Doerge and Chang, 2002). This is not consistent with other prototypical TPO inhibitors (e.g., PTU, MMI). It remains to be determined, if for some presently unknown reason, that genistein is an outlier or not. This again points to the need for quantitative modeling of the relationship between TPO inhibtion and downstream KEs.

Quantitative Understanding of the Linkage

Response-response relationship

There are only a limited number of studies where both TPO inhibition and iodine organification have been measured in vivo, and there are not enough data available to make any definitive quantitative correlations. One in vivo study in rats exposed to the TPO inhibitor genistein found no in vivo impact on serum thyroid hormone concentrations, even when TPO was inhibited up to 80% (Chang and Doerge, 2000).

References

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Tietge JE, Butterworth BC, Haselman JT, Holcombe GW, Hornung MW, Korte JJ, Kosian PA, Wolfe M, Degitz SJ. Early temporal effects of three thyroid hormone synthesis inhibitors in Xenopus laevis. Aquat Toxicol. 2010 98(1):44-50

Vickers AE, Heale J, Sinclair JR, Morris S, Rowe JM, Fisher RL. Thyroid organotypic rat and human cultures used to investigate drug effects on thyroid function, hormone synthesis and release pathways. Toxicol Appl Pharmacol. 2012 260(1):81-8.

Relationship: 305: TH synthesis, Decreased leads to T4 in serum, Decreased (https://aopwiki.org/relationships/305)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	**adjacent**	**High**	**Moderate**
XX Inhibition of Sodium Iodide Symporter and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/65)	**adjacent**	**High**	**Moderate**
Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	**adjacent**	**High**	**High**

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of Na+/I- symporter (NIS) leads to learning and memory impairment (https://aopwiki.org/aops/54)	**adjacent**	**High**	**Moderate**
Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	**adjacent**		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
rat	Rattus norvegicus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
mouse	Mus musculus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
Xenopus laevis	Xenopus laevis	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8355)

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

While a majority of the empirical evidence comes from work with laboratory rodents, there is a large amount of supporting data from humans (with anti-hyperthyroidism drugs including propylthiouracil and methimazole), some amphibian species (e.g., frog), and some avian species (e.g, chicken). The following are samples from a large literature that supports this concept: Cooper et al. (1982; 1983); Hornung et al. (2010); Van Herck et al. (2013); Paul et al. (2013); Alexander et al. (2017).

Key Event Relationship Description

Thyroid hormones (THs), thyroxine (T4) and triiodothyronine (T3) are synthesized by NIS and TPO in the thyroid gland as iodinated thyroglobulin (Tg) and stored in the colloid of thyroid follicles. Secretion from the follicle into serum is a multi-step process. The first involves thyroid stimulating hormone (TSH) stimulation of the separation of the peptide linkage between Tg and TH. The next steps involve endocytosis of colloid, fusion of the endosome with the basolateral membrane of the thyrocyte, and finally release of TH into blood. More detailed descriptions of this process can be found in reviews by Braverman and Utiger (2012) and Zoeller et al. (2007).

Evidence Supporting this KER

The weight of evidence linking these two KEs of decreased TH synthesis and decreased T4 in serum is strong. It is commonly accepted dogma that decreased synthesis in the thyroid gland will result in decreased circulating TH (serum T4).

Biological Plausibility
The biological relationship between two KEs in this KER is well understood and documented fact within the scientific community.

Empirical Evidence
It is widely accepted that TPO inhibition leads to declines in serum T4 levels in adult mammals. This is due to the fact that the sole source for circulating T4 derives from hormone synthesis in the thyroid gland. Indeed, it has been known for decades that insufficient dietary iodine will lead

to decreased serum TH concentrations due to inadequate synthesis. Strong qualitative and quantitative relationships exist between reduced TH synthesis and reduced serum T4 (Ekerot et al., 2013; Degon et al., 2008; Cooper et al., 1982; 1983; Leonard et al., 2016; Zoeller and Tan, 2007). There is more limited evidence supporting the relationship between decreased TH synthesis and lowered circulating hormone levels during development. Lu and Anderson (1994) followed the time course of TH synthesis, measured as thyroxine secretion rate, in non-treated pregnant rats and correlated it with serum T4 levels More recently, modeling of TH in the rat fetus demonstrates the quantitative relationship between TH synthesis and serum T4 concentrations (Hassan et al., 2017). Furthermore, a wide variety of drugs and chemicals that inhibit TPO are known to result in decreased release of TH from the thyroid gland, as well as decreased circulating TH concentrations. This is evidenced by a very large number of studies that employed a wide variety of techniques, including thyroid gland explant cultures, tracing organification of 131-I and in vivo treatment of a variety of animal species with known TPO inhibitors (King and May,1984; Atterwill et al., 1990; Brown et al., 1986; Brucker-Davis, 1998; Hornung et al., 2010; Hurley et al., 1998; Kohrle, 2008).

Temporal Evidence: The temporal nature of this KER is applicable to all life stages, including development (Seed et al., 2005). There are currently no studies that measured both TPO synthesis and TH production during development. However, the impact of decreased TH synthesis on serum hormones is similar across all ages. Good evidence for the temporal relationship comes from thyroid system modeling of the impacts of iodine deficiency and NIS inhibition (e.g., Degon et al., 2008; Fisher et al., 2013). In addition, recovery experiments have demonstrated that serum thyroid hormones recovered in athyroid mice following grafting of in-vitro derived follicles (Antonica et al., 2012).

Dose-response Evidence: Dose-response data is lacking from studies that include concurrent measures of both TH synthesis and serum TH concentrations. However, data is available demonstrating correlations between thyroidal TH and serum TH concentrations during gestation and lactation during development (Gilbert et al., 2013). This data was used to develop a rat quantitative biologically-based dose-response model for iodine deficiency (Fisher et al., 2013).

Uncertainties and Inconsistencies

There are no inconsistencies in this KER, but there are some uncertainties. The first uncertainty stems from the paucity of data for quantitative modeling of the relationship between the degree of synthesis decrease and resulting changes in circulating T4 concentrations. In addition, most of the data supporting this KER comes from inhibition of TPO, and there are a number of other processes (e.g., endocytosis, lysosomal fusion, basolateral fusion and release) that are not as well studied.

Quantitative Understanding of the Linkage

Response-response relationship

Fisher et al. (2013) published a quantitative biologically-based dose-response model for iodine deficiency in the rat. This model provides quantitative relationships for thyroidal T4 synthesis (iodine organification) and predictions of serum T4 concentrations in developing rats. There are other computational models that include thyroid hormone synthesis. Ekerot et al. (2012) modeled TPO, T3, T4 and TSH in dogs and humans based on exposure to myeloperoxidase inhibitors that also inhibit TPO. This model was recently adapted for rat (Leonard et al., 2016) and Hassan et al (2017) have extended it to include the pregnant rat dam in response to TPO inhibition induced by PTU. While the original model predicted serum TH and TSH levels as a function of oral dose, it was not used to explicitly predict the relationship between serum hormones and TPO inhibition, or thyroidal hormone synthesis. Leonard et al. (2016) recently incorporated TPO inhibition into the model. Degon et al (2008) developed a human thyroid model that includes TPO, but does not make quantitative prediction of organification changes due to inhibition of the TPO enzyme.

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Relationship: 2038: T4 in serum, Decreased leads to Decreased, Triiodothyronine (T3) in serum (https://aopwiki.org/relationships/2038)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	**adjacent**		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=7955)
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
Juvenile	Moderate

Sex Applicability

Sex	Evidence
Unspecific	High

This key event relationship is applicable to late larvae and juveniles rather than to embryos, because of the presence of maternal TH in embryos.

Embryo stage:

- A decrease in T4 was observed in fathead minnows exposed to 1 mg/L 2-mercaptobenzothiazole (MBT), a thyroperoxidase inhibitor, through 6 dpf (Nelson et al., 2016). In contrast, there was no observed effect on T3 in fathead minnows exposed to MBT through 6 dpf. Comparably, zebrafish exposed to 0.4 or 0.7 mg/L MBT through 120 hpf showed decreased T4 but not T3 (Stinckens et al., 2016). During this early larval life stage, T3 may have been derived from maternal T4. In addition, it could be produced from further depletion of any T4 still produced by the thyroid gland (as thyroperoxidase may not have been fully inhibited at the tested exposure concentrations).
- Since exposure to PFAS did result in decreased whole-body T4 and T3 in 5 day old zebrafish, the life-stage specificity possibly depends on the mechanism that lies at the basis of the TH changes (Wang et al., 2020). The exact mechanisms by which PFAS disrupt the thyroid hormone system remain uncertain. Compounds that directly reduce T3 levels (e.g., deiodinase inhibitors) in addition to reducing T4 levels via another mechanism can be expected to result in decreased T4 and T3 levels.

The evidence for a relationship between circulating T4 and T3 levels currently comes from work on zebrafish and fathead minnow.

This KER is probably not sex-dependent since both females and males have the same mechanisms of TH synthesis and conversion. Additionally, zebrafish are undifferentiated gonochorists, and gonad differentiation starts only around 23-25 dpf (Uchida et al., 2002), and the current data is based on larvae younger than or around that age.

Key Event Relationship Description

When serum thyroxine (T4) levels are decreased, less T4 is available for conversion to the more biologically active triiodothyronine (T3). While some thyroid hormone (TH) disrupting mechanisms can immediately affect T3 levels, including deiodinase inhibition, other mechanisms reduce T4 levels, for example through inhibition of TH synthesis, leading to decreased T3 levels.

Since in fish early life stages TH are typically measured on a whole body level, it is currently uncertain whether TH levels changes occur at the serum and/or tissue level. Pending more dedicated studies, whole body TH levels are considered a proxy for serum TH levels.

This key event relationship is not always evident. This could be due to feedback/compensatory mechanisms that in some cases seem to be able to maintain T3 levels even though T4 levels are reduced, for example through increased conversion of T4 to T3 by deiodinases.

Evidence Supporting this KER

Biological Plausibility
When serum thyroxine (T4) levels are decreased, less T4 is available for conversion to the more biologically active triiodothyronine (T3). It is plausible to assume that while some thyroid hormone (TH) disrupting mechanisms can immediately affect T3 levels, including deiodinase inhibition, other mechanisms reduce T4 levels, for example through inhibition of TH synthesis, leading to decreased T3 levels.

Empirical Evidence

- A decrease in whole-body T4 and T3 was observed in zebrafish exposed to methimazole from fertilization until the age of 21 and 32 days and to propylthiouracil until the age of 14, 21 and 32 days (Stinckens et al., submitted). Both compounds are thyroperoxidase inhibitors expected to inhibit thyroid hormone synthesis.
- A dose-dependent decrease in whole-body T4 and T3 was observed in zebrafish exposed to perfluorooctanoic acid and perfluoropolyether carboxylic acids from fertilization until the age of 5 days (Wang et al., 2020). The exact mechanisms by which PFAS disrupt the thyroid hormone system remain uncertain.
- While T4 measurements could not be acquired in fathead minnows exposed to 1 mg/L 2-mercaptobenzothiazole, a thyroperoxidase inhibitor, for 14 days, a significant decrease in T3 was observed (Nelson et al., 2016). The decreased T3 levels were likely the result of reduced T4 synthesis.

Uncertainties and Inconsistencies

- Since in fish early life stages THs are typically measured on a whole body level, it is currently uncertain whether TH levels changes occur at the serum and/or tissue level. Pending more dedicated studies, whole body TH levels are considered a proxy for serum TH levels.
- This key event relationship is not always evident. This could be due to feedback/compensatory mechanisms that in some cases seem to be able to maintain T3 levels even though T4 levels are reduced, for example through increased conversion of T4 to T3 by deiodinases. Examples of studies showing reduced T4 levels in the absence of reduced T3 levels:
    - Zebrafish exposed to 0.35 mg/L 2-mercaptobenzothiazole, a thyroperoxidase inhibitor, through 32 dpf showed decreased whole-body T4, but T3 levels showed particularly large variation and overall were not significantly decreased (Stinckens et al., 2016).
    - Although T4 content of 28 dpf larval fathead minnows exposed to 32 or 100 µg/l methimazole, a thyroperoxidase inhibitor, was reduced, these fish showed no change in whole body T3 content (Crane et al., 2006). Significantly higher T3/T4 ratios in fish held in 100 µg/l methimazole suggest an increased conversion of T4 to T3 or reduced degradation and conjugation during continued exposure to methimazole.

Quantitative Understanding of the Linkage

Known Feedforward/Feedback loops influencing this KER
This key event relationship is not always evident. This could be due to feedback/compensatory mechanisms that in some cases seem to be able to maintain T3 levels even though T4 levels are reduced, for example through increased conversion of T4 to T3 by deiodinases. Examples of studies showing reduced T4 levels in the absence of reduced T3 levels:

- Zebrafish exposed to 0.35 mg/L 2-mercaptobenzothiazole, a thyroperoxidase inhibitor, through 32 dpf showed decreased whole-body T4, but T3 levels showed particularly large variation and overall were not significantly decreased (Stinckens et al., 2016).
- Although T4 content of 28 dpf larval fathead minnows exposed to 32 or 100 µg/l methimazole, a thyroperoxidase inhibitor, was reduced, these fish showed no change in whole body T3 content (Crane et al., 2006). Significantly higher T3/T4 ratios in fish held in 100 µg/l methimazole suggest an increased conversion of T4 to T3 or reduced degradation and conjugation during continued exposure to methimazole.

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Nelson, K., Schroeder, A., Ankley, G., Blackwell, B., Blanksma, C., Degitz, S., Flynn, K., Jensen, K., Johnson, R., Kahl, M., Knapen, D., Kosian, P., Milsk, R., Randolph, E., Saari, T., Stinckens, E., Vergauwen, L., Villeneuve, D., 2016. Impaired anterior swim bladder inflation following exposure to the thyroid peroxidase inhibitor 2-mercaptobenzothiazole part I: Fathead minnow. Aquatic Toxicology 173, 192-203.

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Relationship: 1035: Decreased, Triiodothyronine (T3) in serum leads to Reduced, Anterior swim bladder inflation (https://aopwiki.org/relationships/1035)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deiodinase 2 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/156)	**adjacent**		
Deiodinase 1 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/158)	**adjacent**		
Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	**adjacent**		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
Juvenile	High

Sex Applicability

Sex	Evidence
Unspecific	High

This KER is probably not sex-dependent since both females and males rely on THs for regulation of vital processes. Additionally, zebrafish are undifferentiated gonochorists, and gonad differentiation starts only around 23-25 dpf (Uchida et al., 2002), after the time point of anterior chamber inflation (around 21 dpf).

Key Event Relationship Description

Thyroid hormones are known to be involved in development, especially in metamorphosis in amphibians and in embryonic-to-larval transition and larval-to-juvenile transition in fish. Inflation of the anterior swim bladder chamber is part of the larval-to-juvenile transition in fish, together with the development of adult fins and fin rays, ossification of the axial skeleton, formation of an adult pigmentation pattern, scale formation, maturation and remodeling of organs including the lateral line, nervous system, gut and kidneys.

Evidence Supporting this KER

Biological Plausibility

Thyroid hormones are known to be involved in development, especially in metamorphosis in amphibians and in embryonic-to-larval transition (Liu and Chan, 2002) and larval-to-juvenile transition (Brown et al., 1997) in fish. Inflation of the anterior swim bladder chamber is part of the larval-to-juvenile transition in fish, together with the development of adult fins and fin rays, ossification of the axial skeleton, formation of an adult pigmentation pattern, scale formation, maturation and remodeling of organs including the lateral line, nervous system, gut and kidneys (Brown, 1997; Liu and Chan, 2002; McMenamin and Parichy, 2013).

Empirical Evidence

- In a study in which embryo-larval fathead minnows (Pimephales promelas) were exposed to the thyroid peroxidase inhibitor 2-mercaptobenzothiazole (MBT), T3 concentrations measured at 14dpf were reduced at the same concentration (1 mg/L) that significantly reduced anterior swim bladder inflation at the same time-point (Nelson et al. 2016).
- Maternal injection of T3, resulting in increased T3 concentrations in the eggs of striped bass (Morone saxatilis) lead to significant increases in both swim bladder inflation and survival (Brown et al., 1988).
- In the study of Cavallin et al. (2017) fathead minnow larvae were exposed to IOP, a model iodothyronine deiodinase inhibitor that is assumed to inhibit all three deiodinase enzymes (DIO1,2,3). The authors observed pronounced decreases of whole body T3 concentrations and increases of whole body T4 concentrations, together with impaired inflation of the anterior swim bladder chamber. More specifically, inflation was delayed and the size of the swim bladder chamber was reduced until the end of the exposure experiment. Since exposure was started after inflation of the posterior chamber, this study shows that DIO inhibition can directly affect anterior chamber inflation.
- In the study of Stinckens et al. (submitted) a strong correlation between reduced T3 levels and reduced anterior chamber inflation was observed in zebrafish exposed to iopanoic acid, a deiodinase inhibitor, as well as methimazole and propylthiouracil, both thyroperoxidase inhibitors, from fertilization until the age of 32 days. Anterior chamber inflation was delayed and a number of larvae did not manage to inflate the anterior chamber by the end of the 32 day exposure period. Additionally, exposed fish that had inflated the swim bladder had reduced anterior chamber sizes.

Uncertainties and Inconsistencies

- The mechanism underlying the link between reduced T3 and reduced anterior chamber inflation remains unclear, but several hypotheses exist (Stinckens et al., submitted). For example, altered gas distribution between chambers could be the result of impaired development of smooth muscle fibers, delayed and/or impaired evagination of the anterior chamber, impaired anterior budding through altered Wnt and hedgehog signalling, etc.
- Increased T3 levels also seem to result in reduced swim bladder inflation. For example, Li et al. (2011) reported impairment of swim bladder inflation in Chinese rare minnows (Gobiocypris rarus) exposed to exogenous T3.

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Relationship: 1034: Reduced, Anterior swim bladder inflation leads to Reduced, Swimming performance (https://aopwiki.org/relationships/1034)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deiodinase 2 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/155)	**adjacent**		

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deiodinase 2 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/156)	**adjacent**		
Deiodinase 1 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/157)	**adjacent**		
Deiodinase 1 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/158)	**adjacent**		
Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	**adjacent**		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

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

Importance of swimming performance for natural behaviour is generally applicable to fish.

Key Event Relationship Description

Effects on swim bladder inflation can alter swimming performance and buoyancy of fish, which is essential for predator avoidance, energy sparing, migration, reproduction and feeding behaviour, resulting in lower young-of-year survival.

Evidence Supporting this KER

The weight of evidence supporting a direct linkage between these two KEs, i.e. reduced anterior swim bladder inflation and reduced swimming performance, is weak.

Biological Plausibility

The anterior chamber of the swim bladder has a function in regulating the buoyancy of fish, by altering the volume of the swim bladder (Roberston et al., 2007). Fish rely on the lipid and gas content in their body to regulate their position within the water column, with the latter being more efficient at increasing body buoyancy. Therefore, fish with functional swim bladders have no problem supporting their body (Brix 2002), while it is highly likely that impaired inflation severely impacts swimming performance. Fish with no functional swim bladder can survive, but are severely disadvantaged., making the likelihood of surviving smaller.

Several studies in zebrafish and fathead minnow showed that a smaller AC was associated with a larger posterior chamber (Nelson et al., 2016; Stinckens et al., 2016; Cavallin et al., 2017, Stinckens et al., submitted) suggesting a possible compensatory mechanism. As shown by Stoyek et al. (2011) however, the AC volume is highly dynamic under normal conditions due to a series of regular corrugations running along the chamber wall, and is in fact the main driver for adjusting buoyancy while the basic PC volume remains largely invariable. Therefore, it is plausible to assume that functionality of the swim bladder is affected when AC inflation is incomplete, even when the PC appears to fully compensate the gas volume of the swim bladder.

Empirical Evidence

- After exposure to 2-mercaptobenzothiazole, a TPO inhibitor, from 0 to 32 days post fertilization (dpf) in zebrafish, the swimming activity of fish was impacted starting at 26 dpf if the inflation of the anterior chamber of the swim bladder was impaired or had no normal structure/size (Stinckens et al., 2016).
- Methimazole (MMI) and propylthiouracil (PTU), two thyroperoxidase inhibitors, and iopanoic acid (IOP), a deiodinase inhibitor, each reduced both anterior chamber (AC) inflation and swimming distance in zebrafish exposed from fertlization until the age of 32 days (Stinckens et al., submitted). The current dataset provides evidence for a specific, direct link between AC inflation and reduced swimming performance. First, after 21 d of exposure to 111 mg/L PTU around 30% of ACs were not inflated and swimming distance was reduced, while by 32 dpf all larvae had inflated ACs and the effect on swimming distance had disappeared. The most direct way to assess the role of AC inflation in swimming performance, however, is to compare larvae with and without inflated AC at the same time point and within the same experimental treatment. Both in the PTU exposure at 21 dpf and in the IOP exposure at 21 and 32 dpf, swimming distance was clearly reduced in larvae lacking an inflated AC, while the swimming distance of larvae with inflated AC was equal to that of controls.
-
- It has also been reported that larvae that fail to inflate their swim bladder use additional energy to maintain buoyancy (Lindsey et al., 2010, Goodsell et al. 1996), possibly contributing to reduced swimming activity. Furthermore, Chatain (1994) associated larvae with non-inflated swim bladders with numerous complications, such as spinal deformities and lordosis and reduced growth rates, adding to the impact on

swimming behaviour.
- An increasing incidence of swim bladder non-inflation has also been reported in Atlantic salmon (Poppe et al. 1997). Affected fish had severely altered balance and buoyancy, observed through a specific swimming behaviour, as the affected fish were swimming upside down in an almost vertical position (Poppe et al. 1997).

Uncertainties and Inconsistencies
After exposure to 100 mg/L MMI, 95% of the zebrafish larvae failed to inflate their AC at 32 dpf and swimming distance was reduced (Stinckens et al., submitted). On the other hand, there was no effect of impaired AC inflation on swimming distance in the MMI exposure of 50 mg/L. Also, inflated but smaller ACs did not result in a decreased swimming performance in the present study. A similar result, where non-inflated ACs did not consistently lead to reduced swimming performance, was previously found after exposure to MBT (Stinckens et al., 2016). In summary, the precise relationship between these two KEs is not easy to determine and may be different for different chemicals. Swimming capacity can be affected via other processes which may or may not depend on the HPT axis, such as decreased cardiorespiratory function, energy metabolism and growth.

As Robertson et al., (2007) reported, the swim bladder only starts regulating buoyancy actively from 32 dpf onward in zebrafish, possibly explaining the lack of effect on swimming capacity in some cases.

The anterior chamber is also important for producing and transducing sound through the Weberian Apparatus (Popper, 1974; Lechner and Ladich, 2008). It is highly plausible that impaired inflation or size of the anterior swim bladder could lead to a reduction in young-of-year survival as hearing loss would affect their ability to respond to their surrounding environment, thus impacting ecological relevant endpoints such as predator avoidance or prey seeking (Wisenden et al., 2008; Fay, 2009).

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Relationship: 1029: Reduced, Swimming performance leads to Reduced, Young of year survival (https://aopwiki.org/relationships/1029)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deiodinase 2 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/155)	**adjacent**		
Deiodinase 2 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/156)	**adjacent**		
Deiodinase 1 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/157)	**adjacent**		
Deiodinase 1 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/158)	**adjacent**		
Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	**adjacent**		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	

Sex Applicability

Sex	Evidence
Unspecific	

Importance of swimming performance on young of year survival is generally applicable to fish.

Evidence Supporting this KER

Biological Plausibility

Reduced swimming performance is likely to affect essential endpoints such as predator avoidance, feeding behaviour and reproduction. These parameters are biologicaly plausible to affect young-of-year survival, especially in a non-laboratory environment where food is scarce and predators are abundant.

Empirical Evidence

All zebrafish larvae that failed to inflate the posterior chamber after exposure to 2 mg/L iopanoic acid (IOP), died by the age of 9 dpf (Stinckens et al., unpublished). Since larvae from the same group that were able to inflate the posterior chamber survived, it is plausible to assume that the cause of death was the inability to swim and find food due to the failure to inflate the posterior swim bladder chamber.

Quantitative Understanding of the Linkage

Time-scale

Reduced swimming performance is not expected to immediately lead to mortality. Depending on the extent of the reduction in swimming performance and depending on the cause of death (e.g., starvation due to the inability to find food, being caught by a predator) the lag time may vary.

As an example, Stinckens et al. (unpublished) found that zebrafish larvae that failed to inflate the swim bladder at 5 dpf and did not manage to inflate it during the days afterwards died by the age of 9 dpf. Since zebrafish initiate exogenous feeding around day 5 when the yolk is almost completely depleted, there was a lag period of around 4 days after which reduced feeding resulted in mortality. Obviously, in a laboratory setup there is no increased risk of being caught by a predator.

All zebrafish larvae that failed to inflate the posterior chamber after exposure to 2 mg/L iopanoic acid (IOP), died by the age of 9 dpf (Stinckens et al., unpublished). Since larvae from the same group that were able to inflate the posterior chamber survived, it is plausible to assume that the cause of death was the inability to swim and find food due to the failure to inflate the posterior swim bladder chamber.

Relationship: 1030: Reduced, Young of year survival leads to Decrease, Population trajectory (https://aopwiki.org/relationships/1030)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deiodinase 2 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/155)	**adjacent**		
Deiodinase 2 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/156)	**adjacent**		
Deiodinase 1 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/157)	**adjacent**		
Deiodinase 1 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/158)	**adjacent**		
Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	**adjacent**		

Evidence Supporting Applicability of this Relationship

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)

Key Event Relationship Description

If young of year survival is reduced, ultimately the population trajectory will decrease.

Evidence Supporting this KER

Biological Plausibility

It is widely accepted that if young of year survival is reduced, the population trajectory will eventually decrease.

List of Non Adjacent Key Event Relationships

Relationship: 366: Thyroperoxidase, Inhibition leads to T4 in serum, Decreased (https://aopwiki.org/relationships/366)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	**non-adjacent**	**High**	**Moderate**
Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	**non-adjacent**		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Xenopus laevis	Xenopus laevis	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8355)
rat	Rattus norvegicus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
chicken	Gallus gallus	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9031)
human	Homo sapiens	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

Use of TPO inhibitors as anti-hyperthyroidism drugs, in humans and pets (Emiliano et al., 2010; Trepanier, 2006), in amphibian and avian species (Coady et al., 2010; Grommen et al., 2011; Rosebrough et al., 2006; Tietge et al., 2012), demonstrate decreased serum TH concentrations in vivo in rats (US EPA, 2005) and strongly supports a causative linkage between inhibition of TPO and decreased serum T4 across species.

Key Event Relationship Description

Thyroperoxidase (TPO) is the enzyme that catalyzes iodine organification of thyroglobulin to produce thyroglobulin (Tg)-bound T3 and T4 in the lumen of thyroid follicles. Tg-bound THs are endocytosed across the apical lumen-follicular cell membrane, undergo thyroglobulin proteolysis, followed by hormone section into the blood stream (see Taurog, 2005 for review). This indirect KER describes the relationship of TPO inhibition to reduced circulating levels of thyroid hormone (TH) in the serum.

Evidence Supporting this KER

The weight of evidence linking thyroperoxidase inhibition to reductions in circulating serum TH is strong. Many studies support this basic linkage. There is no inconsistent data.

Biological Plausibility
It is a well-accepted fact that inhibition of the only enzyme capable of synthesizing THs, TPO, results in subsequent decrease in serum TH concentrations. A large amount of evidence from clinical and animal studies clearly support the commonly accepted dogma that inhibition of TPO leads to decreased serum THs.

Empirical Evidence
The majority of research in support of this KER involve exposure to known TPO inhibitors and measurement of serum hormones. There are many in vivo studies that link decreases in serum TH concentrations with exposure to xenobiotics that inhibit thyroperoxidase (TPO) (Brucker-Davis, 1998; Hurley, 1998; Boas et al., 2006; Crofton, 2008; Kohrle, 2008; Pearce and Braverman, 2009; Murk et al., 2013).

While these studies support the connection between exposure to a known TPO inhibitor and decreased TH, many of these studies do not empirically measure TPO inhibition or decreased TH synthesis. Thus, many studies support the indirect linkage between TPO inhibition (for chemicals identified as TPO inhibitors in in vivo or ex vivo studies) and decreased TH, with the well accepted theory that these proceed via decreased TH synthesis. That exposure to TPO inhibitors leads to decreased serum TH concentrations, via decreased TH synthesis is strongly supported by decades of mechanistic research in a variety of species.

This indirect relationship is also evidenced by the use of clinically-relevant anti-hyperthyroidism drugs, MMI and PTU (Laurberg & Anderson, 2014; Sundaresh et al., 2013). These drugs are both recognized TPO inhibitors and are part of a standard drug-based regimen of care for clinically hyperthyroid patients including those with Grave's disease. Serum THs are measured as the bioindicator of successful treatment with anti-hyperthyroidism drugs; the actual decrease in TH synthesis in the thyroid gland is implied in the efficacious use of these drugs (Trepanier, 2006).

In rats, MMI and PTU are often used as control chemicals to decrease serum THs to study biological phenomena related to disruption of TH homeostasis (many examples, including Zoeller and Crofton, 2005; Morreale de Escobar et al, 2004; Schwartz et al., 1997; Herwig et al., 2014; Wu et al., 2013; Pathak et al., 2011). Further, MMI is recommended as a positive control for use in the Amphibian Metamorphosis (Frog) Assay within Tier 1 of the U.S. EPA Endocrine Disruptor Screening Program (US EPA, 2009; Coady et al., 2010), an assay used to evaluate the

potential for chemicals to disrupt TH homeostasis. PTU has been suggested a positive control chemical in the guidance for the Comparative Developmental Thyroid Assay (US EPA, 2005), a non-guideline assay used to evaluate the potential for chemicals to disrupt TH homeostasis during gestation and early neonatal development.

Additionally, evidence is available from studies investigating responses to environmentally relevant TPO inhibitors. Stinckens et al. (2016) showed that exposure to 2-mercaptobenzothiazole (MBT), a potent TPO inhibitor, decreased whole body T4 levels in continuously exposed 5 and 32 day old zebrafish larvae. A high concentration of MBT also decreased whole body T4 levels in 6 day old fathead minnows, but recovery was observed at the age of 21 days (Nelson et al., 2016).

Thus, an indirect key event relationship between TPO inhibition and decreased serum THs is strongly supported by a large database of clinical medicine and investigative research with whole animals (with a great deal of supporting evidence in rats and frogs).

> *Temporal Evidence:* The temporal nature of this KER is applicable to all life stages, including development (Seed et al., 2005). The qualitative impact of thyroperoxidase inhibition on serum hormones is similar across all ages. The temporal nature of the impact on serum THs by TPO inhibitors in developmental exposure studies is evidenced by the duration of exposure and developmental age (Goldey et al., 1995; Ahmed et al., 2010; Tietge et al., 2010), as well as recovery after cessation of exposure (Cooke et al., 1993; Goldey et al., 1995; Sawin et al., 1998; Axelstad et al., 2008; Shibutani et al., 2009; Lasley and Gilbert, 2011). The temporal relationship between TPO inhibitor exposure duration and serum hormone decreases in adult organisms has been widely demonstrated (e.g., Hood et al., 1999; Mannisto et al., 1979). In addition, MMI and PTU induced decreases in serum T4 are alleviated by thyroid hormone replacement in both fetal and postnatal age rats (Calvo et al., 1990; Sack et al., 1995; Goldey and Crofton, 1998). Computational modeling of the thyroid also provides evidence for the indirect temporal relationship between these two KEs (e.g., Degon et al., 2008; Fisher et a., 2013).
>
> *Dose-Response Evidence:* Empirical data is available from enough studies in animals treated with TPO inhibitors during development to make it readily accepted dogma that a dose-response relationship exists between TPO inhibition and serum TH concentrations. Again, these studies do not empirically measure TPO inhibition or decreased TH synthesis, but rely on the strong support of decades of mechanistic research in a variety of species of the causative relationship between these KEs. Examples of dose-responsive changes in TH concentrations following developmental exposure to TPO inhibitors include studies a variety of species, including: rodents (Blake and Henning, 1985; Goldey et al., 1995; Sawin et al., 1998); frogs (Tietge et al., 2013); fish tissue levels (Elsalini and Rohr, 2003.); and, chickens (Wishe et al., 1979). Computational modeling of the thyroid also provides evidence for the indirect dose-response relationship between these two KEs (e.g., Leonard et al., 2016; Fisher et a., 2013).

Uncertainties and Inconsistencies

There are no inconsistencies in this KER, but there are some uncertainties. The predominant uncertainty regarding the indirect key event relationship between inhibition of TPO activity and decreased serum T4 is the quantitative nature of this relationship, i.e., to what degree must TPO be inhibited in order to decrease serum T4 by a certain magnitude. Many animal (rat) studies typically employ relatively high exposures of TPO-inhibiting chemicals that result in hypothyroidism (severe decrements in T4 and T3). Thus, a dose-response relationship between TPO inhibition and decreased serum T4 is not typically defined. However, there are numerous publications demonstrating clear dose- and duration-dependent relationships between TPO inhibitors dose and reduced serum T3 and T4 in rodent models (see for example: Cooper et al., 1983; Hood et al., 1999; Goldey et al., 2005; Gilbert, 2011). The relationship between maternal and fetal levels of hormone following chemically-induced TPO inhibiton has not been well characterized and may differ based on kinetics. Reductions in serum TH in the fetus, in rat and human is derived a chemical's effect on the maternal thyroid gland as well as the fetal thyroid gland.

Quantitative Understanding of the Linkage

Response-response relationship

The indirect linkage between exposure to known TPO inhibitors and decreased serum TH has not been defined quantitatively. The two key event relationships that mediate this relationship (TPO inhibition leading to decreased TH synthesis, and decreased TH synthesis leading to decreased serum TH) have been incorporated into some quantitative models. A quantitative biologically-based dose-response model for iodine deficiency in the rat includes relationships between thyroidal T4 synthesis and serum T4 concentrations in developing rats Fisher et al. (2013). Ekerot et al. (2012) modeled TPO, T3, T4 and TSH in dogs and humans based on exposure to myeloperoxidase inhibitors that also inhibit TPO and was has recently adapted for rat (Leonard et al., 2016). While the original model predicted serum TH and TSH levels as a function of oral dose, it was not used to explicitly predict the relationship between serum hormones and TPO inhibition, or thyroidal hormone synthesis. Leonard et al. (2016) recently incorporated TPO inhibition into the model. Degon et al (2008) developed a human thyroid model that includes TPO but does not make quantitative prediction of organification changes due to inhibition of the TPO enzyme.

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Relationship: 1039: T4 in serum, Decreased leads to Reduced, Anterior swim bladder inflation (https://aopwiki.org/relationships/1039)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	**non-adjacent**		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links

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

Sex Applicability

Sex	Evidence
Unspecific	High

The evidence for a relationship between circulating T4 levels and inflation of the anterior chamber of the swim bladder currently comes from work on zebrafish and fathead minnow.

This KER is probably not sex-dependent since both females and males rely on synthesis of THs for regulation of vital processes. Additionally, zebrafish are undifferentiated gonochorists, and gonad differentiation starts only around 23-25 dpf (Uchida et al., 2002), after the time point of anterior chamber inflation (around 21 dpf).

Key Event Relationship Description

Reduced T4 levels in serum prohibit local production of active T3 hormone by deiodinases expressed in the target tissues. There is evidence suggesting that anterior swim bladder inflation relies on increased thyroid hormone levels at this specific developmental time point.

While evidence supports a link between reduced serum T4 levels and reduced anterior swim bladder inflation, experimental evidence suggests that inhibition of T4 synthesis does not result in reduced posterior swim bladder inflation. The absence of effects on posterior chamber inflation is possibly due to maternal transfer of T4 into the eggs. These maternally derived THs are depleted at 21 dpf and cannot offset TPO inhibition, resulting in impaired anterior chamber inflation. This has been previously suggested by Reider and Connaughton (2014) with specific reference to eye development.

Maternal thyroid hormone levels in embryos have been demonstrated in zebrafish, fathead minnow, brown trout, striped bass, tilapia, rabbitfish, conger eel, sea bream and different species of salmon (Ruuskanen and Hsu, 2018; Walpita et al., 2007; Chang et al., 2012; Hsu et al., 2014; Power et al., 2001; Brown et al., 1987, 1988). Campinho et al. (2014) confirmed that maternal thyroid hormones are essential for normal brain development in zebrafish by knocking down MCT8, responsable for transporting thyroid hormones into the cells. Alt et al. (2006) found a first differentiated thyroid follicle in zebrafish at 55 hours post fertilization. Elsalini et al. (2003) used immunohistochemistry to show the development of the first thyroid follicles producing thyroid hormone at 72 hours post fertilization in zebrafish. During further larval development, the number of follicles increases. Therefore early developmental processes (before thyroid activation) that are dependent on T4, such as posterior swim bladder inflation, might not be affected by chemicals reducing T4 synthesis. Nelson et al. (2016) and Stinckens et al. (2016) indeed found that MBT (a thyroperoxidase inhibitor) decreased T4 levels in both zebrafish (5 days post fertilization) and fathead minnow (6 days post fertilization), which is after activation of the thyroid gland for both species, while it did not affect posterior inflation. Godfrey et al. (2017) also reported normal posterior chamber inflation after exposure of zebrafish embryos to methimazole, a TPO inhibitor.

Evidence Supporting this KER

Biological Plausibility

Thyroid hormones are known to be involved in development, especially in metamorphosis in amphibians and in embryonic-to-larval transition (Liu and Chan, 2002) and larval-to-juvenile transition (Brown et al., 1997) in fish. The formation of the anterior chamber coincides with the second transition phase (Winata et al., 2009) and with a peak in T4 synthesis (Chang et al., 2012) suggesting that anterior inflation is under thyroid hormone regulation.

Empirical Evidence

- Chang et al. (2012) observed an increase of whole body T4 concentrations in zebrafish larvae at 21 days post fertilization, corresponding to the timing of anterior swim bladder inflation.
- Nelson et al. (2016) showed reduced whole body T4 levels at 6 days post fertilization and delayed anterior inflation (lower proportion of inflation at 14 dpf compared to controls) after exposure of fathead minnow embryos to 2-mercaptobenzothiazole. All anterior chambers eventually inflated but their size was reduced and morphology deviated.
- Stinckens et al. (2016) showed reduced whole body T4 levels both at 5 (before anterior inflation) and 32 days post fertilization (after anterior inflation) when zebrafish were exposed to 2-mercaptobenzothiazole (a thyroperoxidase inhibitor) from 0 to 32 days post fertilization. A large percentage of MBT-exposed fish had an uninflated anterior swim bladder, although some recovery was observed over time.
- Stinckens et al. (2016) further showed a significant correlation between whole body T4 levels and anterior chamber volume, with reduced T4 levels leading to smaller anterior chambers.
- Methimazole, a TPO inhibitor expected to result in decreased T4 levels, impaired anterior chamber inflation in zebrafish (Godfrey et al., 2017; Stinckens et al., unpublished data). Stinckens et al. continued the follow-up until 32 dpf and observed no recovery.
- Chopra et al. (2019) found that a nonsense mutation of the duox gene, coding for the enzyme dual oxidase involved in thyroid hormone synthesis, resulted in decreased intrafollicular T4 levels and impaired anterior chamber inflation until at least 54 dpf in zebrafish.

Uncertainties and Inconsistencies

The mechanism through which reduced T4 hormone concentrations in serum result in anterior chamber inflation impairment is not yet understood. The anterior chamber is formed by evagination from the cranial end of the posterior chamber (Robertson et al., 2007, Winata et al., 2009). Several hypotheses could explain effects on anterior chamber inflation due to reduced T4 levels:

- Evagination from the posterior chamber could be impaired. Villeneuve et al. (unpublished results) showed that although the anterior bud was present after exposure to a deiodinase 2 inhibitor, the anterior chamber did not inflate.
- The formation of the tissue layers of the anterior swim bladder could be affected, although Villeneuve et al. (unpublished results) observed intact tissue layers of the anterior swim bladder after exposure to a deiodinase 2 inhibitor.
- The anterior chamber is inflated with gas from the posterior chamber through the communicating duct. Impaired gas exchange between the two chambers could be at the basis of impaired anterior inflation. Both Nelson et al. (2016) and Stinckens et al. (2016) found that posterior chambers were larger when anterior chambers were smaller or not inflated at all. The sum of the areas of the posterior and anterior chambers remained constant independent of inflation of the anterior chamber (Stinkens et al., 2016). These results suggest retention of the gas in the posterior chamber.
- Since gas exchange relies on a functional communicating duct between the posterior and anterior chamber, and the communicating duct is known to progressively narrow and eventually close during development, a dysfunctional communicating duct or a closure prior to anterior inflation could inhibit inflation. However, Villeneuve et al. (unpublished results) showed that the communicating duct was anatomically intact and open after exposure to iopanoic acid (a deiodinase 2 inhibitor), still leading to impaired anterior inflation.
- Lactic acid production which is essential for producing gas to fill the swim bladder could be affected, although the observation that the total amount of gas in both chambers is not affected when anterior inflation is impaired seems to contradict this (Stinckens et al., 2016).
- Possibly there is an effect on the production of surfactant, which is crucial to maintain the surface tension necessary for swim bladder inflation.

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