AOP 363: Thyroperoxidase inhibition leading to altered visual function via altered retinal layer structure - Weight of evidence evaluation

	Defining Question	**High (Strong)**	**Moderate**	**Low (Weak)**
1. Support for Biological Plausibility of KERs	Is there a mechanistic relationship between KE_{up} and KE_{down} consistent with established biological knowledge?	Extensive understanding of the KER based on extensive previous documentation and broad acceptance.	KER is plausible based on analogy to accepted biological relationships, but scientific understanding is incomplete	Empirical support for association between KEs, but the structural or functional relationship between them is not understood.
Relationship: 309 Thyroperoxidase, inhibition (KE 279) leads to TH synthesis, decreased (KE 277)	**High (reviewed and endorsed previously)** The role and importance of thyroperoxidase (TPO) in thyroid hormone synthesis across vertebrates is well established. TPO is the only enzyme capable of de novo synthesis of TH. Therefore, inhibition of TPO activity is widely accepted to directly impact TH synthesis.			
Relationship: 305 TH synthesis, decreased (KE 277) leads to T4 in serum, decreased (KE 281)	**High (reviewed and endorsed previously)** It is commonly accepted that decreased thyroid hormone synthesis leads to decreased serum T4 levels.			
Non-adjacent relationship: 366 Thyroperoxidase, inhibition (KE 279) leads to T4 in serum, decreased (KE 281)	**High (reviewed and endorsed previously)** The role of thyroperoxidase in the synthesis of thyroid hormones that are then released to the blood is well established.			
Relationship 2038: T4 in serum, decreased (KE 281) leads to decreased, triiodothyronine (T3) (KE 1003)	**Moderate (reviewed and endorsed previously)** When serum thyroxine (T4) levels are decreased, less T4 is available for conversion to the more biologically active triiodothyronine (T3). Since in fish early life stages THs are typically measured on a whole-body level, it is currently uncertain whether T3 level 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. While there is empirical support for the association between decreased serum T4 and decreased T3 levels in fish, the 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. The role of taxonomic differences in this relationship is currently unclear.			
Relationship 2373: Decreased, triiodothyronine (T3) (KE 1003) leads to altered, retinal layer structure (KE 1877)	**Moderate** THs are generally accepted to be important for eye and retinal development across vertebrates. There is ample knowledge of this relationship in different vertebrate taxa. Although there is also some mechanistic knowledge on the importance of certain signaling pathways for retinal development, the exact mechanisms responsible for altered retinal layer structure in hypothyroid animals are not entirely understood.			
Relationship 2374: Altered, retinal layer structure (KE 1877) leads to altered, visual function (KE 1643)	**High** The retina is the key structure mediating visual perception and it is generally accepted that an improperly formed retina does not allow for normal visual function.			
Relationship 2375: Altered, visual	**High**			

function (KE 1643) leads to increased mortality (KE 351)	Since vision is essential to locate prey and avoid predators, it is generally accepted that reduced visual function reduces survival. It should be noted that this relationship is not always evident in a laboratory context and may only become apparent in a realistic environment where food is scarce and predators are present.
Relationship 2013: Increased mortality (KE 351) leads to decrease, population growth rate (KE 360)	**High** It is widely accepted that mortality increases, the population growth rate will eventually decrease.

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2. Essentiality of KEs	**Defining question**	**High (Strong)**	**Moderate**	**Low (Weak)**
	Are downstream KEs and/or the AO prevented if an upstream KE is blocked?	Direct evidence from specifically designed experimental studies illustrating essentiality for at least one of the important KEs	Indirect evidence that sufficient modification of an expected modulating factor attenuates or augments a KE	No or contradictory experimental evidence of the essentiality of any of the KEs.
KE 279 (MIE): Thyroperoxidase, inhibition	● There is evidence of recovery of serum T4 levels after cessation of exposure to a TPO inhibitor in different species such as rat, human, mice and pigs (Vickers et al 2012, Taurog, 1999; Paul et al., 2013; Cooper et al., 1982; 1983; AOP 42) but TPO inhibition is not directly measured in the test subjects. ● Baumann et al (2019) showed that treatment with the TPO inhibitor PTU led to effects on transcript levels of genes in the gene ontology (GO)-class "sensory perception" specifically in the eyes of zebrafish. They showed over 90% down-regulation in PTU-exposed fish. Pathways involved in repair mechanisms were significantly upregulated in the recovery treatment, which indicates activation of regeneration processes in the eyes after stopping the exposure to the TPO inhibitor. When blocking a KE prevents downstream KEs from happening, this supports essentiality of the former KE. In this case, cessation of exposure (i.e., blocking the MIE) resulted in reversal of downstream transcriptional changes which are implicitly part of the downstream pathway.			
KE 277: Thyroid hormone synthesis, decreased	● There is evidence of recovery of serum T4 levels in athyroid mice following grafting of *in vitro*-derived follicles (Antonica et al., 2012; AOP 42). ● Stop/recovery experiments demonstrate recovery of serum thyroxine concentrations due to cessation of developmental exposure to chemical stressors that inhibit TH synthesis (e.g., Crofton et al., 2000), with similar findings in adult rats (Cooper et al.,1984). Studies of Hill et al. (1998) on adult rats show a similar recovery after cessation of dosing.			
KE 281: Thyroxine (T4) in serum, decreased	● There is ample evidence of recovery of phenotypes after cessation of exposure to TPO inhibitors and subsequent T4 recovery in mammals (Cooke et al., 1993; Goldey et al., 1995; Axelstad et al., 2008; Shibutani et al., 2009; Lasley and Gilbert, 2011; AOP 42) but specific effects on retinal structure and visual function were not included.			
KE 1003: Decreased triiodothyronine (T3)	● Houbrechts et al. (2016a) used a knockdown of deiodinase 1 and 2 in zebrafish embryos to decrease T3 levels. At 3 dpf, the ganglion cell layer of the knockdown embryos was wider and less dense compared to controls. Knockdown using morpholinos is known to cause a transient blocking of translation. By 7 dpf, the ganglion cell layer had recovered. This was likely due to recovered deiodinase expression and T3 levels, thus blocking this KE. TH levels were not measured in the study of Houbrechts et al. (2016a), but Houbrechts et al. (2016b) showed that permanent knockout of DIO2 resulted in decreased T3 levels with unaltered T4 levels. Together, this confirms that reduced T3 levels are most likely essential for causing the downstream effects on the retina. ● Bhumika et al. (2014): Adult zebrafish were exposed to 10 µM of iopanoic acid (IOP), which lowered intracellular 3,5,3′-triiodothyronine (T3) availability, or to 7 µM of the thyroid hormone receptor β antagonist methylsulfonylnitrobenzoate (C1). Both treatments accelerated optic tectum (OT) reinnervation. At 7 days post injury (7 dpi) there was a clear increase in the biocytin labeled area in the OT following anterograde tracing as well as an increased immunostaining of Gap43, a protein expressed in outgrowing axons. This effect was attenuated by T3 supplementation to IOP-treated fish. This shows that reduced T3 signaling is essential for the effects on optic tectum reinnervation. ON crush induced limited cell death and proliferation at the level of the retina in control, IOP- and C1-treated fish. The authors stated the absence of toxic effects of the drug treatment and indicated that the observed effect is not due to activities on cell survival or proliferation. Lowering T3 Levels had no influence on mitosis in the zebrafish retina.			

	• Marelli et al. (2016) showed that resistance to thyroid hormones (by creating morphant heterozygous, dominant-negative (DN) THRA (RTHa) or THRB (RTHb) mutations) results in severe developmental abnormalities, such as abnormal eyes and otoliths. The co-expression of wild-type, but not mutant human TRs can rescue the phenotype in both morphants. High doses of T3 can partially reverse the dominant negative effect of mutant TRs in the morphant fish. • Duval and Allison et al. (2018) and Suzuki et al. (2013) showed that knocking out thrb leads to an almost complete absence of red cones and an increase in UV cones (by about 35%). Red cones appear to have a critical time window for induction by thrb shortly after the onset of crx expression, since in thrb morphants red cones can be rescued by thrb expression driven by the crx promoter (i.e. as early as 19 hpf), as described by Suzuki et al. In the same article, induction of thrb after the last cell division resulted in cones co-expressing red cone opsin with another cone opsin, a kind of imperfect "rescue". • Roberts et al (2006) demonstrated that both T3 and T4 are present in the developing retina of mice and that T3 is required for normal development of both S and M cones. They showed that exogenous T3 inhibits S-opsin when experimentally elevated at the time of S-opsin onset, and activates M-opsin when animals are treated at the time of M-opsin onset. Analysis of a mouse with a mutation in the ligand binding domain of thyroid receptor beta indicates that binding of endogenous TH to the thyroid receptor beta is required to inhibit S-opsin and to activate M-opsin in vivo. These results show a requirement for Thyroid hormone in the developmental regulation of cone opsins.
KE 1877: Altered, Retinal layer structure	• Houbrechts et al. (2016) showed that the ganglion cell layer was wider and less dense in 3 dpf zebrafish embryos after combined knockdown of deiodinase 1 and 2, together with a reduced response to light (normal response is increase of swimming activity) at 4 dpf. By 7 dpf, the ganglion cell layer had recovered, effectively blocking this KE, probably due to the transient nature of the morpholino knockdown. The authors observed a corresponding recovery of the response to light. In a similar study, investigating D1D2MO zebrafish larvae, hatching occurred at a normal rate, heart rate was normal, growth was not significantly reduced, there was no pericardial edema, but the frequency of swim bladder inflation was decreased, and the swimming activity was reduced (Bagci et al., 2015). This does not suggest that the observed effects on retinal development are the consequence of secondary effects or systemic toxicity.
KE 1643: Altered, Visual function	• While it is plausible to assume that the change in visual function as a consequence of altered retinal development is essential for downstream changes on survival, no studies rescuing visual function and showing that this leads to reduced mortality have been found.
KE 351: Increased mortality	• Increased mortality is generally assumed to be essential for reduced population size.
AOP as a whole	**High** Evidence for essentiality in this AOP can be classified as high. Direct evidence from specifically designed experimental studies illustrating essentiality is available for several KEs in the AOP. Especially the evidence of essentiality of decreased T3 levels for effects on the eyes is very important and strongly supports this AOP.

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	Defining Questions	**High (Strong)**	**Moderate**	**Low (Weak)**
3. Empirical Support for KERs	Does empirical evidence support that a change in KE_{up} leads to an appropriate change in KE_{down}? Does KE_{up} occur at lower doses and earlier time points than KE down and is the incidence of KE_{up} > than that for KE_{down}? Inconsistencies ?	if there is dependent change in both events following exposure to a wide range of specific stressors (extensive evidence for temporal, dose-response and incidence concordance) and no or few data gaps or conflicting data	if there is demonstrated dependent change in both events following exposure to a small number of specific stressors and some evidence inconsistent with the expected pattern that can be explained by factors such as experimental design, technical considerations, differences among laboratories, etc.	if there are limited or no studies reporting dependent change in both events following exposure to a specific stressor (i.e., endpoints never measured in the same study or not at all), and/or lacking evidence of temporal or dose-response concordance, or identification of significant inconsistencies in empirical support across taxa and species that don't align with the expected pattern for the hypothesised AOP
Relationship: 309 Thyroperoxidase, Inhibition (KE 279) leads to TH synthesis, Decreased (KE 277)	**Low (reviewed and endorsed previously)** Direct measurements of both KEs in the same study are not available in fish, but studies have shown that known TPO inhibitors reduce TH synthesis in the thyroid follicles and alter thyroid follicle histology in fish.			
Relationship: 305 TH synthesis, Decreased (KE 277) leads to T4 in serum, Decreased (KE 281)	**Low (reviewed and endorsed previously)** Direct measurements of both KEs in the same study are not available in fish, but separate studies have shown that known TPO inhibitors reduce TH synthesis in the thyroid follicles, alter thyroid follicle histology and reduce T4 in fish.			
Non-adjacent relationship: 366 Thyroperoxidase, Inhibition (KE 279) leads to T4 in serum, Decreased (KE 281)	**Moderate (reviewed and endorsed previously)** Although direct measurements of both KEs in the same organisms are not available in fish, several studies have shown that chemicals able to inhibit TPO in vitro, reduce T4 levels. In rare cases, increased T4 levels have been observed after longer exposures to TPO inhibitors, which is probably due to compensatory feedback mechanisms.			
Relationship 2038: T4 in serum, Decreased (KE 281) leads to Decreased, Triiodothyronine (T3) (KE 1003)	**Moderate (reviewed and endorsed previously)** Several studies have shown both T4 and T3 decreases upon exposure to chemicals that inhibit TH synthesis including a strong correlation between T4 and T3 levels and evidence of time and dose concordance. In some cases, T4 and T3 levels do not change in the same direction. This can mostly be explained by feedback mechanisms. This relationship depends on the MIE that is causing the decrease in T3. For example, deiodinase inhibition results in reduced activation of T4 to T3 and thus in reduced T3 levels; increased T4 levels have been observed, probably as a compensatory mechanism in response to the lower T3 levels. Additionally, thyroid binding proteins in plasma including transthyretin, thyroxin-binding globulin and albumin determine the fraction of T4 that is available for conversion to T3. This may buffer the impact of reduced T4 levels on downstream T3 levels.			
Relationship 2373:	**Moderate**			

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Decreased, Triiodothyronine (T3) (KE 1003) leads to Altered, Retinal layer structure (KE 1877)	Convincing evidence of a link between decreased T3 levels and altered retinal layer structure has been generated using different methods to reduce T3 levels, mostly in zebrafish, but also in other fish species: genetic knockdown, thyroidectomy and exposure to chemicals inhibiting thyroid hormone synthesis as well as other thyroid hormone system disruptors, each leading to decreased T3 levels, have been shown to result in altered retinal layer structure.
Relationship 2374: Altered, Retinal layer structure (KE 1877) leads to Altered, Visual function (KE 1643)	**Moderate** Convincing evidence of a link between altered retinal layer structure and altered visual function has been generated using a variety of stressors and methods for measuring visual function, including clear evidence of time concordance.
Relationship 2375: Altered, Visual function (KE 1643) leads to Increased mortality (KE 351)	**Moderate** There is convincing evidence of a link between visual responsiveness and ability to avoid a predator but only a limited number of studies directly measured both KEs in an exposure context. Therefore evidence of time and dose concordance is scarce. Studies often report poor performance in vision-related behaviors in a laboratory environment but mostly do not directly address the impact on survival in a realistic scenario.
Relationship 2013: Increased mortality (KE 351) leads to Decrease, Population growth rate (KE 360)	**Moderate (reviewed and endorsed previously)** Survival rate is an obvious determinant of population size and is therefore included in population modeling. The extent to which increased mortality may impact population sizes in a realistic, environmental exposure scenario depends on the circumstances. Under some conditions, reduced larval survival may be compensated by reduced predation and increased food availability, and therefore not result in decreased population growth rate.