AOP 155: Deiodinase 2 inhibition leading to increased mortality via posterior swim bladder inflation - 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 1026: Inhibition, Deiodinase 2 (KE 1002) leads to Decreased, Triiodothyronine (T3) in serum (KE 1003)	**Moderate** Inhibition of DIO2 activity is widely accepted to directly decrease T3 levels, since the conversion of T4 to T3 is inhibited. 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. The importance of DIO2 inhibition in altering serum T3 levels depends on the relative role of different deiodinases in regulating serum versus tissue T3 levels and in negative feedback within the HPT axis, where some uncertainties remain.			
Relationship 1027: Decreased, Triiodothyronine (T3) in serum (KE 1003) leads to Reduced, Posterior swim bladder inflation (KE 1004)	**Moderate** 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 posterior swim bladder chamber is part of the embryonic-to-larval transition in fish, together with structural and functional maturation of the mouth and gastrointestinal tract, and resorption of the yolk sac. Together with empirical evidence, it is plausible to assume that posterior swim bladder inflation is under thyroid hormone regulation but scientific understanding is incomplete.			
Relationship 1028: Reduced, Posterior swim bladder inflation (KE 1004) leads to Reduced, Swimming performance (KE 1005)	**Moderate** The posterior chamber of the swim bladder has a function in regulating the buoyancy of fish. It is highly plausible that impaired inflation impacts swimming performance. There is a lot of evidence of such a relationship but it has been difficult to unambiguously attribute reduced swimming activity to impaired inflation of the posterior chamber, since swimming activity can be altered via different modes of action.			
Relationship 2212: Reduced, Swimming performance (KE 1005) leads to Increaed mortality (KE 351)	**Moderate** Reduced swimming performance is likely to affect essential endpoints such as predator avoidance, feeding behaviour and reproduction. These parameters are biologically plausible to affect survival. Apart from some indirect evidence, it has been difficult to clearly establish this relationship in the laboratory. It may only become apparent in a non-laboratory environment where food is scarce and predators are abundant.			
Relationship 2013: Increased mortality (KE 351) leads to Decrease, Population trajectory (KE 360)	**High** It is widely accepted that mortality increases, the population trajectory will eventually decrease.			
Non-adjacent relationship 1042: Inhibition, Deiodinase 2 (KE 1002) leads to Reduced, Posterior swim bladder inflation (KE 1004)	**Moderate** Inhibition of DIO 2 activity is widely accepted to reduce the conversion of T4 to the more biologically active T3. 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 posterior swim bladder chamber is part of the embryonic-to-larval transition in fish, together with structural and functional maturation of the mouth and gastrointestinal tract, and resorption of the yolk sac. Together with empirical evidence, it is plausible to assume that posterior swim bladder inflation is under thyroid hormone regulation but scientific understanding is incomplete. It follows that disrupted conversion of T4 to T3 is likely to interfere with normal inflation of the posterior swim bladder chamber.			
Non-adjacent relationship 2213: Reduced, Posterior swim bladder inflation (KE 1004) leads to Increased mortality (KE 351)	**High** The posterior chamber of the swim bladder has a function in regulating the buoyancy of fish. Fish rely on the lipid and gas content in their body to regulate their position within the water column. Efficient regulation of buoyancy is energy sparing and allows for fish to expend less energy in maintaining and changing positions in the water column. Because of its roles in energy sparing and swimming performance, it is expected that failure to inflate the swim bladder would create increased oxygen and energy demands leading to decreased growth, which in turn leads to decreased probability of survival. In particular, these impacts would be expected in non-laboratory environments where fish much expend energy to capture food and avoid predators and where available food is limited. Additionally, fish without a functional swim bladder are severely disadvantaged in terms of foraging and avoiding predators, making the likelihood of surviving smaller. There is ample evidence showing that impaired posterior chamber inflation reduces survival.			

AOP 155: Deiodinase 2 inhibition leading to increased mortality via posterior swim bladder inflation - Weight of evidence evaluation

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 1002 (MIE): Inhibition, deiodinase 2	Bagci et al. (2015) and Heijlen et al. (2013, 2014) reported that knockdown of Dio1+2 in zebrafish resulted in impaired inflation of the posterior swim bladder chamber. Permanent Dio2 knockout also impaired swim bladder inflation and locomotor activity in zebrafish (Houbrechts et al., 2016). Walpita et al. (2009, 2010) reported reduced pigmentation, otic vesicle length and head-trunk angle in the same Dio1+2 and also Dio2 knockdown fish. This confirms that DIO2 is essential for causing downstream effects. These effects were rescued after T3 supplementation but not after T4 supplementation, confirming the importance of T4 to T3 conversion by Dio2 and the essentiality of DIO2 inhibition for causing downstream effects (Walpita et al., 2009, 2010).			
KE 1003: Decreased triiodothyronine (T3) in serum	There is ample evidence confirming the essentiality of decreased T3 levels for the occurrence of reduced posterior chamber inflation (1) from zebrafish knockdown/knockout studies: • Knockdown of deiodinase 1 and 2 (Bagci et al., 2015; Heijlen et al., 2013, 2014), knockdown of TH transporter MCT8 (de Vrieze et al., 2014), knockdown of thryoid hormone receptor alpha or beta (Marelli et al., 2016), and permanent knockout of deiodinase 2 (Houbrechts et al., 2016) in zebrafish resulted in impaired inflation of the posterior swim bladder chamber. Marelli et al. (2016) additionally showed that high T3 doses partially rescued the negative impact in mutants with partially resistant thyroid hormone receptors. • Walpita et al. (2009, 2010) reported reduced pigmentation, otic vesicle length and head-trunk angle in the same Dio1+2 and also Dio2 knockdown fish. These effects were rescued after T3 supplementation, but not after T4 supplementation. While swim bladder inflation was not among the assessed endpoints in this study, this generally confirms the essentiality of decreased T3 in causing downstream effects upon disruption of DIO1 and 2 function (Walpita et al., 2009, 2010). (2) from chemical exposures: • Wang et al. (2020) observed a decrease of whole-body T3 as well as impaired posterior chamber inflation in zebrafish exposed to perfluorooctanoic acid and perfluoropolyether carboxylic acids and exogeneous T3 or T4 supplementation partly rescued this effect. • Maternal injection of T3, resulting in increased T3 concentrations in the eggs of striped bass lead to significant increases in posterior swim bladder inflation (Brown et al., 1988). Similarly, Molla et al. (2019) showed that T3 supplementation increased posterior chamber diameter in zebrafish larvae.			
KE 1004: Reduced, posterior swim bladder inflation	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), confirming the essentiality of posterior swim bladder inflation for the occurrence of the downstream key event 'reduced young of year survival'.			
KE 1005: Reduced, swimming performance	Experimental blocking of this KE is difficult to achieve.			
KE 351: Increased mortality	By definition, increased mortality reduces population size.			
AOP as a whole	**High** Overall, the support for essentiality of the KEs is high since there is direct evidence from specifically designed experimental studies illustrating essentiality for several of the important KEs in the AOP. This includes ample evidence from knockdown studies in zebrafish that use targeted perturbation of key events and show downstream effects, and evidence from both chemical exposure with TH supplementation and knockdown with TH supplementation showing that blocking a KE prevents downstream KEs from occurring.			

AOP 155: Deiodinase 2 inhibition leading to increased mortality via posterior swim bladder inflation - Weight of evidence evaluation

	Defining Questions	High (Strong)	Moderate	Low (Weak)
3. Empirical Support for KERs	Does empirical evidence support that a change in KEup leads to an appropriate change in KEdown? Does KEup occur at lower doses and earlier time points than KE down and is the incidence of KEup > than that for KEdown? 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 1026: Inhibition, Deiodinase 2 (KE 1002) leads to Decreased, Triiodothyronine (T3) in serum (KE 1003)	**Low** Although direct measurements of both KEs in the same organisms are not available in fish, several studies have shown that chemicals able to inhibit DIO2 in vitro, reduce T3 levels. The relative importance of DIO2 versus DIO1 is uncertain, but available evidence suggests that DIO2 is more important.			
Relationship 1027: Decreased, Triiodothyronine (T3) in serum (KE 1003) leads to Reduced, Posterior swim bladder inflation (KE 1004)	**Moderate** Many studies showed that chemicals reducing TH synthesis or activation inhibit proper posterior chamber inflation but studies reporting measurements of both endpoints are rare. Uncertainties mainly relate to the mechanism through which altered TH levels result in impaired posterior chamber inflation. Temporal concordance is difficult to establish since swim bladder inflation can only occur at a specific time point.			
Relationship 1028: Reduced, Posterior swim bladder inflation (KE 1004) leads to Reduced, Swimming performance (KE 1005)	**Moderate** There is ample evidence of a link between reduced posterior chamber inflation and reduced swimming performance. This link has been studied both from an aquaculture perspective as well as in chemical exposure experiments. Evidence of dose concordance is limited. Temporal concordance is difficult to establish since swim bladder inflation can only occur at a specific time point.			
Relationship 2212: Reduced, Swimming performance (KE 1005) leads to Increaed mortality (KE 351)	**Low** A direct relationship between reduced swimming performance and increased mortality has been difficult to establish. There is however a lot of indirect evidence linking reduced swim bladder inflation to increased mortality (see non-adjacent KER 2213), which can be plausibly assumed to be related to reduced swimming performance.			
Relationship 2013: Increased mortality (KE 351) leads to Decrease, Population trajectory (KE 360)	**Moderate** 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 population decline.			
Non-adjacent relationship 1042: Inhibition, Deiodinase 2 (KE 1002) leads to Reduced, Posterior swim bladder inflation (KE 1004)	**Moderate** Although direct measurements of both KEs in the same organisms are not available in fish, several studies have shown that chemicals able to inhibit DIO2 in vitro, reduce posterior chamber inflation. The relative importance of DIO1 versus DIO2 is uncertain, but available evidence suggests that DIO2 is more important. The mechanism through which DIO2 inhibition results in impaired posterior chamber inflation is uncertain.			
Non-adjacent relationship 2213: Reduced, Posterior swim bladder inflation (KE 1004) leads to Increased mortality (KE 351)	**Moderate** There is a extensive evidence of a link between reduced posterior chamber inflation and increased mortality based on studies in freshwater and marine fish species. Uncertainties are related to the dependence of the linkage on the circumstances such as food availability and predation.			

							dose and temporal concordance										uncertainties, inconsistencies			
reference	species	chemical	expected MIE	exposure period	time point	concentrations tested	TPO inhibition	DIO1 inhibition	DIO2 inhibition	TH synthesis decreased	T4 in serum decreased	T3 in serum decreased	posterior swim bladder chamber inflation reduced	anterior swim bladder chamber inflation reduced	swimming performance reduced	increased mortality	decreased tpo mRNA	decreased dio1 mRNA	serum T4 increased	serum T3 increased
Cavallin et al. (2017)	fathead minnow	iopanoic acid	DIO1 and 2 inhibition	0-6dpf	4 dpf	0.6, 1.9, 6.0 mg/L	n/a	n/a	n/a	n/a	n/a	-£	n/a	n/a	n/a	-			0.6, 1.9, 6.0 mg/L£	6 mg/L£
Cavallin et al. (2017)	fathead minnow	iopanoic acid	DIO1 and 2 inhibition	0-6dpf	6 dpf	0.6, 1.9, 6.0 mg/L	n/a	-*	-*	n/a	n/a	-£	6 mg/L	n/a	n/a	-			-£	1.9, 6.0 mg/L£
Cavallin et al. (2017)	fathead minnow	iopanoic acid	DIO1 and 2 inhibition	6-21 dpf	10 dpf	0.6, 1.9, 6.0 mg/L	n/a	-*	-*	n/a	n/a	0.6, 1.9, 6.0 mg/L£	n/a	n/a	n/a	-			0.6, 1.9, 6.0 mg/L£	-£
Cavallin et al. (2017)	fathead minnow	iopanoic acid	DIO1 and 2 inhibition	6-21 dpf	14 dpf	0.6, 1.9, 6.0 mg/L	n/a	-*	0.6, 1.9, 6.0 mg/L*	n/a	n/a	0.6, 1.9, 6.0 mg/L£	n/a	0.6, 1.9, 6.0 mg/L	n/a	-			1.9, 6.0 mg/L£	-£
Cavallin et al. (2017)	fathead minnow	iopanoic acid	DIO1 and 2 inhibition	6-21 dpf	18 dpf	0.6, 1.9, 6.0 mg/L	n/a	-*	0.6, 1.9, 6.0 mg/L*	n/a	n/a	0.6, 1.9, 6.0 mg/L£	n/a	0.6, 1.9, 6.0 mg/L	n/a	-			0.6, 1.9, 6.0 mg/L£	-£
Cavallin et al. (2017)	fathead minnow	iopanoic acid	DIO1 and 2 inhibition	6-21 dpf	21 dpf	0.6, 1.9, 6.0 mg/L	n/a	-*	0.6, 1.9, 6.0 mg/L*	n/a	n/a	0.6, 1.9, 6.0 mg/L£	n/a	0.6, 1.9, 6.0 mg/L	n/a	6 mg/L			0.6, 1.9, 6.0 mg/L£	-£
Stinckens et al. (2016)	zebrafish	2-mercaptobenzothiazole	TPO inhibition	0-168 hpf	120 hpf	0.1, 0.35, 0.56, 0.7, 0.88, 1.75, 3.5, 7 mg/L	n/a	n/a	n/a	n/a	0.35, 0.7 mg/L£ (0.1 mg/L no	-	-	n/a	0.35, 0.56, 0.7, 0.88, 1.75, 3.	3.5, 7 mg/L			-£	-£
Stinckens et al. (2016)	zebrafish	2-mercaptobenzothiazole	TPO inhibition	0-32 dpf	20 dpf	0.1, 0.35 mg/L	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0.35 mg/L	n/a	-			-£	-£
Stinckens et al. (2016)	zebrafish	2-mercaptobenzothiazole	TPO inhibition	0-32 dpf	21 dpf	0.1, 0.35 mg/L	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0.35 mg/L	n/a	-			-£	-£
Stinckens et al. (2016)	zebrafish	2-mercaptobenzothiazole	TPO inhibition	0-32 dpf	22 dpf	0.1, 0.35 mg/L	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0.35 mg/L	n/a	-			-£	-£
Stinckens et al. (2016)	zebrafish	2-mercaptobenzothiazole	TPO inhibition	0-32 dpf	23 dpf	0.1, 0.35 mg/L	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0.35 mg/L	n/a	-			-£	-£
Stinckens et al. (2016)	zebrafish	2-mercaptobenzothiazole	TPO inhibition	0-32 dpf	24 dpf	0.1, 0.35 mg/L	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0.35 mg/L	n/a	-			-£	-£
Stinckens et al. (2016)	zebrafish	2-mercaptobenzothiazole	TPO inhibition	0-32 dpf	25 dpf	0.1, 0.35 mg/L	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0.35 mg/L	n/a	-			-£	-£
Stinckens et al. (2016)	zebrafish	2-mercaptobenzothiazole	TPO inhibition	0-32 dpf	26 dpf	0.1, 0.35 mg/L	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0.35 mg/L	0.35 mg/L	-			-£	-£
Stinckens et al. (2016)	zebrafish	2-mercaptobenzothiazole	TPO inhibition	0-32 dpf	27 dpf	0.1, 0.35 mg/L	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0.35 mg/L	n/a	-			-£	-£
Stinckens et al. (2016)	zebrafish	2-mercaptobenzothiazole	TPO inhibition	0-32 dpf	28 dpf	0.1, 0.35 mg/L	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0.35 mg/L	n/a	-			-£	-£
Stinckens et al. (2016)	zebrafish	2-mercaptobenzothiazole	TPO inhibition	0-32 dpf	29 dpf	0.1, 0.35 mg/L	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0.35 mg/L	0.35 mg/L	-			-£	-£
Stinckens et al. (2016)	zebrafish	2-mercaptobenzothiazole	TPO inhibition	0-32 dpf	30 dpf	0.1, 0.35 mg/L	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0.35 mg/L	0.35 mg/L	-			-£	-£
Stinckens et al. (2016)	zebrafish	2-mercaptobenzothiazole	TPO inhibition	0-32 dpf	31 dpf	0.1, 0.35 mg/L	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0.35 mg/L	n/a	-			-£	-£
Stinckens et al. (2016)	zebrafish	2-mercaptobenzothiazole	TPO inhibition	0-32 dpf	32 dpf	0.1, 0.35 mg/L	n/a	n/a	n/a	n/a	0.35 mg/L£	-£	n/a	0.35 mg/L	n/a	-			-£	-£
Nelson et al. (2016)	fathead minnow	2-mercaptobenzothiazole	TPO inhibition	0-21 dpf	6 dpf	0.25, 0.5, 1 mg/L	-	n/a	n/a	n/a	1 mg/L£	-£	-	n/a	n/a	-			-£	-£
Nelson et al. (2016)	fathead minnow	2-mercaptobenzothiazole	TPO inhibition	0-21 dpf	14 dpf	0.25, 0.5, 1 mg/L	0.5, 1 mg/L*	n/a	n/a	0.5, 1 mg/L$	n/a	1 mg/L£	n/a	0.5, 1 mg/L	n/a	-			n/a	-£
Nelson et al. (2016)	fathead minnow	2-mercaptobenzothiazole	TPO inhibition	0-21 dpf	21 dpf	0.25, 0.5, 1 mg/L	1 mg/L*	n/a	n/a	0.5, 1 mg/L$	-£	-£	n/a	0.5, 1 mg/L	n/a	-			0.25, 0.5, 1 mg/L£	-£
Wei et al. (2018)	zebrafish	bisphenol S	unknown	adults	F1 96 hpf	1, 10, 100 µg/L	n/a	n/a	n/a	n/a	1, 10, 100 µg/L£		1, 10, 100 µg/L	n/a	1, 10, 100 µg/L				-	1, 10, 100 µg/L£
Crane et al. (2005)	fathead minnow	ammonium perchlorate	NIS inhibition	0-28 dpf	28 dpf	1, 10, 100 mg/L	n/a	n/a	n/a	1, 10, 100 mg/L$	-£	-£	n/a	n/a	n/a	-			100 mg/L	-
Crane et al. (2006)	fathead minnow	methimazole	TPO inhibition	0-84 dpf	28 dpf	32, 100, 320 µg/L	n/a	n/a	n/a	n/a	32, 100 µg/L£	320 µg/L£	n/a	n/a	n/a	32, 100 µg/L			-£	-£
Crane et al. (2006)	fathead minnow	methimazole	TPO inhibition	0-84 dpf	56 dpf	32, 100, 320 µg/L	n/a	n/a	n/a	n/a	-£	100 µg/L£	n/a	n/a	n/a	32, 100 µg/L			320 µg/L£	-£
Crane et al. (2006)	fathead minnow	methimazole	TPO inhibition	0-84 dpf	84 dpf	32, 100, 320 µg/L	n/a	n/a	n/a	n/a	-	-	n/a	n/a	n/a	32, 100 µg/L			-	-
Stinckens et al. (2020)	zebrafish	methimazole	TPO inhibition	0-32 dpf	21 dpf	50, 100 mg/L	n/a	n/a	n/a	n/a	50, 100 mg/L£	50, 100 mg/L£	-	50, 100 mg/L	n/a					
Stinckens et al. (2020)	zebrafish	methimazole	TPO inhibition	0-32 dpf	32 dpf	50, 100 mg/L	n/a	n/a	n/a	n/a	50, 100 mg/L£	50, 100 mg/L£	-	50, 100 mg/L	100 mg/L					
Stinckens et al. (2020)	zebrafish	propylthiouracil	TPO inhibition	0-32 dpf	14 dpf	37, 111 mg/L	n/a	n/a	n/a	n/a	37, 111 mg/L£	111 mg/L£	-	n/a	111 mg/L					
Stinckens et al. (2020)	zebrafish	propylthiouracil	TPO inhibition	0-32 dpf	21 dpf	37, 111 mg/L	n/a	n/a	n/a	n/a	37, 111 mg/L£	111 mg/L£	-	37, 111 mg/L	111 mg/L					
Stinckens et al. (2020)	zebrafish	propylthiouracil	TPO inhibition	0-32 dpf	32 dpf	37, 111 mg/L	n/a	n/a	n/a	n/a	37, 111 mg/L£	37, 111 mg/L£	-	37, 111 mg/L	-					
Stinckens et al. (2020)	zebrafish	iopanoic acid	DIO1 and 2 inhibition	0-32 dpf	9 dpf	2 mg/L	n/a	n/a	n/a	n/a	n/a	n/a	2 mg/L	n/a	n/a	2 mg/L				
Stinckens et al. (2020)	zebrafish	iopanoic acid	DIO1 and 2 inhibition	0-32 dpf	14 dpf	0.35, 1 mg/L	n/a	n/a	n/a	n/a	-£	-£	-	n/a	1, 2 mg/L					
Stinckens et al. (2020)	zebrafish	iopanoic acid	DIO1 and 2 inhibition	0-32 dpf	21 dpf	0.35, 1 mg/L	n/a	n/a	n/a	n/a	-£	0.35, 1 mg/L£	-	0.35, 1, 2 mg/L	0.35, 1, 2 mg/L					
Stinckens et al. (2020)	zebrafish	iopanoic acid	DIO1 and 2 inhibition	0-32 dpf	32 dpf	0.35, 1, 2 mg/L	n/a	n/a	n/a	n/a	-£	0.35, 1, 2 mg/L£	-	0.35, 1, 2 mg/L	0.35, 1, 2 mg/L					
Wang et al. (2020)	zebrafish	perfluorooctanoic acid (PFOA)	DIO1 and 2 inhibition	0-5 dpf	5 dpf	0, 50, 100, 150, 200, 2502, 300, 350, 400, 450, 500 mg/L	-*	-	125, 250, 500 mg/L*	-	250, 500 mg/L£	250, 500 mg/L£	200, 250, 300, 350, 400, 45(	n/a	n/a	300, 400, 450, 500 mg/L	-	500 mg/L	-£	-£
Wang et al. (2020)	zebrafish	PFO3OA	unknown	0-5 dpf	5 dpf	0, 400, 600, 800, 1000, 1200, 1400, 1600, 1800, 2000, 2200, 2400 mg/L	1200, 2200 mg/L*	-*	600, 1200, 2200 mg/L*	-	600, 1200, 2200 mg/L£	1200, 2200 mg/L£	800, 1000, 1200, 1400, 160(	n/a	n/a	-	-	-	-£	-£
Wang et al. (2020)	zebrafish	PFO4DA	unknown	0-5 dpf	5 dpf	0, 30, 45, 60, 90, 120, 150, 180, 210, 240 mg/L	-*	240 mg/L*	-*	-	60, 120, 240 mg/L£ (lower cc	60, 120, 240 mg/L£ (lower c	45, 60, 90, 120, 150, 180, 21	n/a	n/a	-	-	-	-£	-£
Wang et al. (2020)	zebrafish	PFO5DoDA	unknown	0-5 dpf	5 dpf	0, 5, 10, 15, 20, 25, 30, 35, 40 mg/L	-*	-*	10, 20, 40 mg/L*	-	10, 20, 40 mg/L£	10, 20, 40 mg/L£	20, 25, 30, 35, 40 mg/L§	n/a	n/a	-	10 mg/L	-	-£	-£
Rehberger et al. (2018)	zebrafish	propylthiouracil	TPO inhibition	0-5 dpf	5 dpf	0, 2.5, 10, 25, 50 mg/L	n/a	n/a	n/a	10, 25, 50 mg/L	n/a	n/a	n/a	n/a	n/a	n/a				

Legend
n/a: not measured
* based on increased mRNA levels of the target as indirect measurement of MIE
$ based on thyroid histopathology
£ based on whole body measurement
§ based on visual evaluation of graphs because no statistics have been reported

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