

AOP 156: Deiodinase 2 inhibition leading to increased mortality via reduced anterior 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 1035: Decreased, Triiodothyronine (T3) in serum (KE 1003) leads to Reduced, Anterior swim bladder inflation (KE 1007)	Moderate Thyroid hormones, especially the more biologically active T3, 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. Together with empirical evidence, it is plausible to assume that anterior inflation is under thyroid hormone regulation but scientific understanding is incomplete.			
Relationship 1034: Reduced, Anterior swim bladder inflation (KE 1007) leads to Reduced, Swimming performance (KE 1005)	Moderate Next to a role in hearing, the anterior chamber of the swim bladder has a function in regulating the buoyancy of fish. Stoyek et al. (2011) showed that the anterior chamber 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 posterior chamber volume remains largely invariable. Therefore, it is plausible to assume that functionality of the swim bladder is affected when anterior chamber inflation is incomplete, even when the posterior chamber appears to fully compensate the gas volume of the swim bladder.			
Relationship 2212: Reduced, Swimming performance (KE 1005) leads to Increased 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.			

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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 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	<p>There is ample evidence confirming the essentiality of decreased T3 levels for the occurrence of reduced posterior chamber inflation, confirming a direct link between T3 levels and the swim bladder system in general.</p> <p>(1) from zebrafish knockdown/knockout studies:</p> <ul style="list-style-type: none"> 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 thyroid 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). <p>(2) from chemical exposures:</p> <ul style="list-style-type: none"> Wang et al. (2020) observed a decrease of whole-body T3 as well as impaired posterior chamber inflation in zebrafish exposed to perfluoroctanoic acid and perfluoropolyether carboxylic acids and exogenous 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. <p>Less information is available about the essentiality of reduced T3 levels for reduced anterior chamber inflation in particular.</p> <ul style="list-style-type: none"> Chopra et al. (2019) provided indirect evidence showing that knockdown of dual oxidase - expected to lead to reduced T4 and T3 levels since dual oxidase is important for thyroid hormone synthesis - reduced anterior swim bladder inflation. <p>Proving essentiality of reduced T3 levels for reduced anterior chamber inflation is further complicated by the complexity of the swim bladder system and the difficulty of distinguishing effects resulting from altered anterior chamber inflation from those resulting from altered posterior chamber inflation.</p>			
KE 1007: Reduced, anterior swim bladder inflation	Stinckens et al. (2020) showed that at the time point where control zebrafish inflate the anterior chamber, larvae exposed to PTU have a lower frequency of inflated anterior chambers together with reduced swimming distance. Later during the exposure the frequency of non-inflated anterior chambers decreased and the effect on swimming distance disappeared confirming the essentiality of reduced anterior chamber inflation for the downstream effect on swimming performance.			
KE 1005: Reduced, swimming performance	Experimental blocking of this KE is difficult to achieve.			
KE 351: Increased mortality	By definition, increased mortality is essential for reduced population size.			
AOP as a whole	<p>Moderate</p> <p>Overall, the confidence in the supporting data for essentiality of KEs within the AOP is moderate. There is evidence from deiodinase knockdowns showing the link with reduced posterior chamber inflation and the essentiality for downstream effects, but anterior chamber inflation was not studied. There is additional indirect evidence that reduced thyroid hormone synthesis causes reduced anterior swim bladder inflation: Chopra et al. (2019) showed that knockdown of dual oxidase, important for thyroid hormone synthesis, reduced anterior swim bladder inflation. It should be noted that dual oxidase also plays a role in oxidative stress. There is also evidence that alleviation of the effect on anterior chamber inflation reduces the effect on swimming performance.</p>			

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	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 1035: Decreased, Triiodothyronine (T3) in serum (KE 1003) leads to Reduced, Anterior swim bladder inflation (KE 1007)	Moderate Several studies showed both T3 decreases and reduced inflation of the anterior chamber with some evidence of dose concordance. 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 1034: Reduced, Anterior swim bladder inflation (KE 1007) leads to Reduced, Swimming performance (KE 1005)	Moderate There is extensive evidence of a link between reduced anterior chamber inflation and reduced swimming performance including some evidence of dose concordance. Temporal concordance is specifically supported by the study of Stinckens et al. (2020): First, after 21 d of exposure to 111 mg/L propylthiouracil around 30% of anterior chambers were not inflated and swimming distance was reduced, while by 32 days post fertilization all larvae had inflated their anterior chamber (although chamber surface was still smaller) and the effect on swimming distance had disappeared.			
Relationship 2212: Reduced, Swimming performance (KE 1005) leads to Increased 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.			

reference	species	chemical	expected MIE	exposure period	time point	concentrations tested	dose and temporal concordance										uncertainties, inconsistencies				
							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	
Cavallini et al. (2017)	fathead minnow	ipanoic 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	n/a	-	0.6, 1.9, 6.0 mg/L	6 mg/L			
Cavallini et al. (2017)	fathead minnow	ipanoic acid	DIO1 and 2 inhibition	0-6dpf	6 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	19, 60 mg/L			
Cavallini et al. (2017)	fathead minnow	ipanoic 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	19, 60 mg/L			
Cavallini et al. (2017)	fathead minnow	ipanoic 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	n/a	n/a	-	0.6, 1.9, 6.0 mg/L	19, 60 mg/L			
Cavallini et al. (2017)	fathead minnow	ipanoic 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	n/a	n/a	-	0.6, 1.9, 6.0 mg/L	19, 60 mg/L			
Cavallini et al. (2017)	fathead minnow	ipanoic 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	n/a	n/a	6 mg/L	0.6, 1.9, 6.0 mg/L	19, 60 mg/L			
Stinckens et al. (2016)	zebrafish	2-mercaptopbenzothiazole	TPO inhibition	0-168 hpf	120 hpf	0.88, 1.75, 3.3, 7 mg/L	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-mercaptopbenzothiazole	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	0.35 mg/L	n/a	-	-.*	-.*	-.*			
Stinckens et al. (2016)	zebrafish	2-mercaptopbenzothiazole	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	0.35 mg/L	n/a	-	-.*	-.*	-.*			
Stinckens et al. (2016)	zebrafish	2-mercaptopbenzothiazole	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	0.35 mg/L	n/a	-	-.*	-.*	-.*			
Stinckens et al. (2016)	zebrafish	2-mercaptopbenzothiazole	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	0.35 mg/L	n/a	-	-.*	-.*	-.*			
Stinckens et al. (2016)	zebrafish	2-mercaptopbenzothiazole	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	0.35 mg/L	n/a	-	-.*	-.*	-.*			
Stinckens et al. (2016)	zebrafish	2-mercaptopbenzothiazole	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	0.35 mg/L	n/a	-	-.*	-.*	-.*			
Stinckens et al. (2016)	zebrafish	2-mercaptopbenzothiazole	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	0.35 mg/L	0.35 mg/L	-	-.*	-.*	-.*			
Stinckens et al. (2016)	zebrafish	2-mercaptopbenzothiazole	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	0.35 mg/L	n/a	-	-.*	-.*	-.*			
Stinckens et al. (2016)	zebrafish	2-mercaptopbenzothiazole	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	0.35 mg/L	n/a	-	-.*	-.*	-.*			
Stinckens et al. (2016)	zebrafish	2-mercaptopbenzothiazole	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	0.35 mg/L	n/a	-	-.*	-.*	-.*			
Stinckens et al. (2016)	zebrafish	2-mercaptopbenzothiazole	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	0.35 mg/L	0.35 mg/L	-	-.*	-.*	-.*			
Stinckens et al. (2016)	zebrafish	2-mercaptopbenzothiazole	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	0.35 mg/L	n/a	-	-.*	-.*	-.*			
Stinckens et al. (2016)	zebrafish	2-mercaptopbenzothiazole	TPO inhibition	0-32 dpf	32 dpf	0.1, 0.35 mg/L	n/a	n/a	n/a	n/a	n/a	0.35 mg/L	0.35 mg/L	n/a	-	-.*	-.*	-.*			
Nelson et al. (2016)	fathead minnow	2-mercaptopbenzothiazole	TPO inhibition	0-21 dpf	6 dpf	0.25, 0.5, 1 mg/L	-	n/a	n/a	1 mg/L ^c	-.*	-	n/a	n/a	-	-.*	-.*	-.*			
Nelson et al. (2016)	fathead minnow	2-mercaptopbenzothiazole	TPO inhibition	0-21 dpf	14 dpf	0.25, 0.5, 1 mg/L	0.5, 1 mg/L ^c	n/a	n/a	0.5, 1 mg/L ^c	n/a	1 mg/L ^c	n/a	0.5, 1 mg/L	n/a	-	n/a	0.25, 0.5, 1 mg/L ^c	-.*		
Nelson et al. (2016)	fathead minnow	2-mercaptopbenzothiazole	TPO inhibition	0-21 dpf	21 dpf	0.25, 0.5, 1 mg/L	1 mg/L ^c	n/a	n/a	0.5, 1 mg/L ^c	-.*	-.*	n/a	0.5, 1 mg/L	n/a	-	1, 10, 100 µg/L	1, 10, 100 µg/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	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	1, 10, 100 mg/L ^c	-.*	-.*	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	32, 100 µg/L ^c	320 µg/L ^c	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	100 µg/L ^c	n/a	n/a	n/a	n/a	32, 100 µg/L	320 µg/L ^c	-.*	-.*			
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	50, 100 mg/L ^c	50, 100 mg/L ^c	n/a	50, 100 mg/L	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	50, 100 mg/L ^c	50, 100 mg/L ^c	n/a	50, 100 mg/L	n/a	-	100 mg/L	-	-			
Stinckens et al. (2020)	zebrafish	methimazole	TPO inhibition	0-32 dpf	32 dpf	50, 100 mg/L	n/a	n/a	n/a	50, 100 mg/L ^c	50, 100 mg/L ^c	n/a	50, 100 mg/L	100 mg/L	-	100 mg/L	-	-			
Stinckens et al. (2020)	zebrafish	propylthiouracil	TPO inhibition	0-32 dpf	14 dpf	37, 111 µg/L	n/a	n/a	n/a	37, 111 µg/L ^c	111 µg/L ^c	n/a	n/a	111 µg/L	-	111 µg/L	-	-			
Stinckens et al. (2020)	zebrafish	propylthiouracil	TPO inhibition	0-32 dpf	21 dpf	37, 111 µg/L	n/a	n/a	n/a	37, 111 µg/L ^c	111 µg/L ^c	n/a	37, 111 µg/L	111 µg/L	-	37, 111 µg/L	-	-			
Stinckens et al. (2020)	zebrafish	propylthiouracil	TPO inhibition	0-32 dpf	32 dpf	37, 111 µg/L	n/a	n/a	n/a	37, 111 µg/L ^c	37, 111 µg/L ^c	n/a	37, 111 µg/L	-	-	37, 111 µg/L	-	-			
Stinckens et al. (2020)	zebrafish	ipanoic acid	DIO1 and 2 inhibition	0-32 dpf	9 dpf	2 mg/L	n/a	n/a	n/a	n/a	n/a	2 mg/L	n/a	n/a	2 mg/L	-	2 mg/L	-	-		
Stinckens et al. (2020)	zebrafish	ipanoic acid	DIO1 and 2 inhibition	0-32 dpf	14 dpf	0.35, 1 mg/L	n/a	n/a	n/a	0.35, 1 mg/L ^c	-.*	-	n/a	1, 2 mg/L	-	-	1, 2 mg/L	-	-		
Stinckens et al. (2020)	zebrafish	ipanoic acid	DIO1 and 2 inhibition	0-32 dpf	21 dpf	0.35, 1 mg/L	n/a	n/a	n/a	0.35, 1 mg/L ^c	-.*	0.35, 1 mg/L ^c	-	0.35, 1, 2 mg/L	0.35, 1, 2 mg/L	-	0.35, 1, 2 mg/L	0.35, 1, 2 mg/L	-		
Stinckens et al. (2020)	zebrafish	ipanoic acid	DIO1 and 2 inhibition	0-32 dpf	32 dpf	0.35, 1, 2 mg/L	n/a	n/a	n/a	0.35, 1, 2 mg/L ^c	-.*	0.35, 1, 2 mg/L ^c	-	0.35, 1, 2 mg/L	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	450, 500 mg/L	-.*	-	125, 250, 500 mg/L ^c	-	250, 500 mg/L ^c	250, 500 mg/L ^c	200, 250, 300, 350, 400, 450 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	1,000, 2,000, 4,000, 8,000, 16,000, 32,000, 64,000, 128,000, 256,000 mg/L	1200, 1400, 1600, 1800, 2000, 2200, 2400 mg/L ^c	-.*	600, 1200, 2200 mg/L ^c	1200, 2200 mg/L ^c	800, 1000, 1200, 1400, 1600 n/a	n/a	n/a	-	-	-	-.*	-.*	-.*		
Wang et al. (2020)	zebrafish	PFO4DA	unknown	0-5 dpf	5 dpf	150, 180, 210, 240 mg/L	-.*	240 mg/L ^c	-.*	60, 120, 240 mg/L ^c	(lower α 60, 90, 120, 150, 180, 21 n/a	n/a	n/a	-	-	-.*	-.*	-.*			
Wang et al. (2020)	zebrafish	PFO5DoDA	unknown	0-5 dpf	5 dpf	30, 35, 40 mg/L	-.*	10, 20, 40 mg/L ^c	-	10, 20, 40 mg/L ^c	10, 20, 40 mg/L ^c	20, 25, 30, 35, 40 mg/L ^c	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	n/a	-	-		

Legend

n/a: not measured

* based on increased mRNA levels of the target as indirect measurement of MIE

\$ based on thyroid histopathology

E based on whole body measurement

§ based on visual evaluation of graphs because no statistics have been reported

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