AOP 159: Thyroperoxidase inhibition leading to increased mortality via 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: 309 Thyroperoxidase, Inhibition (KE 279) leads to TH synthesis, Decreased (KE 277)	**High** 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** 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** 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) in serum (KE 1003)	**Moderate** 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 serum 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 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 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 1039:	**Moderate**			

T4 in serum, Decreased (KE 281), leads to Reduced, Anterior swim bladder inflation (KE 1007)	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. Together with empirical evidence, it is plausible to assume that anterior inflation is under thyroid hormone regulation but scientific understanding is incomplete. Since most of the more biologically active T3 originates from the conversion of T4, decreased circulatory T4 levels are plausibly linked to reduced anterior chamber inflation.

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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 rats (Cooper et al., 1982; 1983; AOP 42), but not in fish.			
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). Chopra et al. (2019) showed that knockdown of dual oxidase, important for thyroid hormone synthesis, reduced anterior swim bladder inflation.			
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; Sawin et al., 1998; Axelstad et al., 2008; Shibutani et al., 2009; Lasley and Gilbert, 2011; AOP 42), but not in fish.			
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, confirming a direct link between T3 levels and the swim bladder system in general. (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. Less information is available about the essentiality of reduced T3 levels for reduced anterior chamber inflation. • 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. It should be noted that dual oxidase also plays a role in oxidative stress. 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.			
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	**Moderate** Overall, the confidence in the supporting data for essentiality of KEs within the AOP is moderate. There is indirect evidence that reduced thyroid hormone synthesis causes reduced anterior swim bladder inflation from a study where a similar MIE was targeted: Chopra et al. (2019) showed that knockdown of dual oxidase, important for thyroid hormone synthesis, reduced anterior swim bladder inflation. Additionally, there is indirect evidence from deiodinase knockdowns supporting the downstream part of the AOP linking decreased T3 levels to reduced swim bladder inflation (targeted at posterior chamber inflation, not specifically at anterior chamber inflation). There is also evidence that alleviation of the effect on anterior chamber inflation reduces the effect on swimming performance.			

	It should be noted that dual oxidase is not only involved in thyroid hormone synthesis, but also in the production of reactive oxygen species (ROS) (Flores et al. 2010; Niethammer et al. 2009). In zebrafish, ROS can also be induced e.g. by copper (Zhou et al. 2016), which has also been shown to impair swim bladder development (Xu et al. 2017). Impaired production of ROS after dual oxidase knockdown may contribute to an impairment of swim bladder development.

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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: 309 Thyroperoxidase, Inhibition (KE 279) leads to TH synthesis, Decreased (KE 277)	**Low** 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** 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** 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) in serum (KE 1003)	**Moderate** 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.			
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 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 1039: T4 in serum, Decreased (KE 281), leads to Reduced, Anterior swim bladder inflation (KE 1007)	**Moderate** Several studies show both T4 decrease and reduced anterior chamber inflation including significant linear relationships between T4 levels and anterior chamber surface and some support for dose concordance.

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
							dose and temporal concordance										uncertainties, inconsistencies			
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, 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	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, 1600	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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