AOP 158: Deiodinase 1 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 1037: Inhibition, Deiodinase 1 (KE 1009) leads to Decreased, Triiodothyronine (T3) in serum (KE 1003) | **Low**<br>DIO1 is capable of converting T4 to the more biologically active T3, but its role in this process in a physiological situation is likely limited. The importance of DIO1 inhibition in altering serum T3 levels further depends on the relative role of different deiodinases in regulating serum versus tissue T3 levels and in negative feedback within the HPT axis. Finally, 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. In summary, there is a plausible link but the physiological relevance remains uncertain. | | | |
| Relationship 1035: Decreased, Triiodothyronine (T3) in serum (KE 1003) leads to Reduced, Anterior swim bladder inflation (KE 1007) | **Moderate**<br>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**<br>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**<br>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**<br>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 1009 (MIE): Inhibition, deiodinase 1 | 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. 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 suggests that DIO1 is less important than DIO2 in 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 perhaps also Dio1 (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, confirming a direct link between T3 levels and the swim bladder system in general.<br>(1) from zebrafish knockdown/knockout studies:<br>• 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.<br>• 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).<br>(2) from chemical exposures:<br>• 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.<br>• 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.<br>Less information is available about the essentiality of reduced T3 levels for reduced anterior chamber inflation.<br>• 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.<br>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 | **Low**<br>Overall, the support for essentiality of the KEs is low since there is limited direct evidence from specifically designed experimental studies illustrating essentiality. This includes evidence from combined DIO1 and DIO2 knockdown studies in zebrafish showing the link with reduced posterior chamber inflation, 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 no specific evidence for the essentiality of DIO1 inhibition independent of DIO2 inhibition and DIO2 seems more important than DIO1 in providing sufficient T3 for proper swim bladder inflation. Therefore the overall evidence for essentiality is considered low. | | | |

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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 1037: Inhibition, Deiodinase 1 (KE 1009) leads to Decreased, Triiodothyronine (T3) in serum (KE 1003) | **Low**<br>Although direct measurements of both KEs in the same organisms are not available in fish, several studies have shown that chemicals able to inhibit DIO1 in vitro, reduce T3 levels. The relative importance of DIO1 versus DIO2 is uncertain, and 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**<br>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**<br>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**<br>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**<br>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. | | | |

| | | | | | | | 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£ | -£ | 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 co | 60, 120, 240 mg/L£ (lower co | 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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