This document includes:

- Comments received on AOP 158 following a request for endorsement by written procedure sent by the OECD Secretariat to the WNT and WPHA with the deadline of 10 June 2022,

- Responses from AOP 158 authors

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Germany	4	Shouldn't the similarity to AOP 157 (only difference posterior vs. anterior swim bladder inflation) be discussed here as well? All AOPs involved in the network so far, could be mentioned here.	We prefer not to clutter the abstract too much with references to other AOPs. This will not be sustainable with future additions of AOPs to the network. The AOP network is described in the section 'Background' just below.
	4	*and converting rT3 to the inactive thyroid hormone 3,3' T2* Isn't this statement misleading since rT3 is an inactive metabolite as well? rT3 should be introduced as reverse T3	We agree that this was confusing, this has now been changed to: "type I deiodinase is capable of both converting T4 into T3 and converting rT3 to 3,3' T2" An explanation of the abbreviation has been added for rT3 in the section 'KE description', p17.
	10	*"based on available evidence DIO2 seems to be more important than DIO1 in providing sufficient"* Statement is repeated several times, but "more" is quite relative – there is no evidence, that Dio1 inhibition alone leads to disturbed swim bladder inflation, see on page 11 *"There is no specific evidence for the essentiality of DIO1 inhibition independent of DIO2 inhibition and"*	We agree that this is not exactly defined, but it accurately reflects the current state of the art. It was agreed during the revision process to clearly mention that DIO2 seems to be more important, to include an explanation in the uncertainties/inconsistencies section, and to indicate a low level of evidence for the upstream linkages in the DIO1 AOPs.

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	10	"Inhibition of deiodinase (DIO) therefore" May need a more precise description regarding isoforms	Since this is AOP 158, this has been changed to DIO1.
	10	Japanese rice fish **Medaka** (Oryzias latipes)	This has now been changed to: "Japanese rice fish or medaka (Oryzias latipes)"
	14	The wording for DIO1 in AOP 189 is different from AOP 157 and 158, maybe this could be harmonised AOP 189: Type I iodothyronine deiodinase (DIO1) inhibition AOP 157/148: Deiodinase 1 inhibition.	We are not the authors of AOP 189. Standardization of terms across AOPs with different authors is not always feasible. Since these terms are synonymous and both clearly represent the target, we prefer to leave this as is.
	14	Is there any prototypic DIO1 inhibitor in fish? If not that could be stated here.	The text in the section 'Evidence for Perturbation by Stressor' is already providing more explanation based on Olker et al. (2019). The following clarification has now been added: "DIO1 inhibitors are often also inhibitors of DIO2 (Olker et al., 2019; Stinckens et al. 2018). In the ToxCast DIO1 inhibition single concentration assay, 219 out of 1820 chemicals were positive and 177 of these were also positive for DIO2 inhibition (viewed on 5/7/2022)." The following clarification has also been added to the abstract, since it is not possible to add such general considerations to the stressor section: "DIO1 inhibitors are often also inhibitors of DIO2 (Olker et al., 2019; Stinckens et al. 2018). In the

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			ToxCast DIO1 inhibition single concentration assay, 219 out of 1820 chemicals were positive and 177 of these were also positive for DIO2 inhibition (viewed on 5/7/2022). This complicates the distinction between the relative contribution of DIO1 and DIO2 inhibition to reduced swim bladder inflation." The question to add more stressors was also raised during the review process and it was decided against. When a stressor is known to target the MIE of an AOP, this does not necessarily mean that there is evidence for the perturbation of every KE along the AOP. Adding stressors was not our focus during AOP development. We tend to add stressors only when we have specific and extensive experience with the chemical. Revisions to the Users Handbook to better define the role of stressors in AOP descriptions are under development. The term stressor as applied to an AOP is to be replaced with "prototypical stressor" – defined as: A stressor that is known to trigger the molecular initiating event (MIE) (or the earliest key event in the pathway) and for which there is an extensive database with respect to its impacts on the downstream key events (KEs) such that experimental evidence related to that stressor's effects provided considerable support for key event relationships (KERs) along the pathway and the AOP as a whole. Other stressors that may provide empirical evidence for a given KER, etc. should simply be noted in the description of the evidence.

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	16	There is evidence for sex- and age-differences of Dio1 in mice, e.g PMID: 17937617	This information has been added to sex and life stage applicability.
	16	rT3 should be introduced here.	An explanation of the abbreviation has been added for rT3 in the section 'KE description', p17.
	16	Use of recombinant human DIO enzyme (all Isotypes) in 96Well format using the SK-reaction was first demonstrated by Renko et al 2015.	This has been adapted to: "Renko et al. (2015), Hornung et al. (2018) and Olker et al. (2019) on the other hand used an adenovirus expression system to produce the DIO1 enzyme and developed an assay for nonradioactive measurement of iodide released using the Sandell-Kolthoff method in a 96 well plate format."
	17	*Measurements of in vivo deiodinase activity in tissues collected from animal experiments are scarce* Renko (2022) (doi: 10.3389/ftox.2022.822993) could be referenced here as well.	The following information has been added: "Renko et al. (2022) showed tissue-specific changes in DIO1 activity in hyper- and hypothyroid mice."
	21	larbean metamorphoses -> larvean metamorphoses	This has been replaced by lamprey.

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	22	*Until recently, it was believed that all of the effects of TH were mediated by the binding of T3 to the thyroid nuclear receptors (TRa and TRb), a notion which is now questionable due to the increasing evidence that support the non-genomic action of TH.* This statement might not be up to date, different mechanisms are discussed in DOI:https://doi.org/10.1530/JOE-17-0708: "four types of thyroid hormone signaling are defined: type 1 is the canonical pathway in which liganded TR binds directly to DNA; type 2 describes liganded TR tethered to chromatin-associated proteins, but not bound to DNA directly; type 3 suggests that liganded TR can exert its function without recruitment to chromatin in either the nucleus or cytoplasm; and type 4 proposes that thyroid hormone acts at the plasma membrane or in the cytoplasm without binding TR, a mechanism of action that is emerging as a key component of thyroid hormone signaling."	This statement has been updated with the suggested information.
	23	*Many transporter proteins have been identified up to date but the monocarboxylate transporters (Mct8, Mct10) and the anion-transporting polypeptide (OATP1c1) show the highest degree of affinity towards TH (Jansen et al., 2005)* There is more recent literature available, e.g. a review from 2015 (https://doi.org/10.1038/nrendo.2015.66)	Additional information has been added to the KE description of KE 1003: "Many transporter proteins have been identified to date. The monocarboxylate transporters (Mct8, Mct10) and the anion-transporting polypeptide (OATP1c1) show the highest degree of affinity towards TH (Jansen et al., 2005) and mutations in these genes have pathophysiological effects in humans (Bernal et al., 2015). Unlike humans with an MCT8 deficiency, MCT8 knockout mice do not have neurological impairment. One explanation for this discrepancy could be differences in expression of the T4 transporter OATP1C1 in the blood–brain barrier. This shows that cross-species

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			differences in the importance of specific transporters may occur."
	29, 46, 50	In Medaka, sex can be morphologically distinguished as soon as 10 days post fertilization Since the KE is only applicable to physostomous fish, statements to Medaka could be removed here.	Anterior chamber inflation is indeed not relevant to medaka. All statements on medaka have been removed from the relevant KEs and KERs.
	43	Any clear data on Dio1 knockdown and T4/T3 levels? Walpita et al report no effect on swim bladder inflation by Dio1 knockdown alone.	Walpita is already mentioned in uncertainties/inconsistencies: It has been shown that a morpholino knockdown targeting DIO1 mRNA alone did not affect embryonic development in zebrafish, while knockdown of DIO2 delayed progression of otic vesicle length, head-trunk angle and pigmentation index (Houbrechts et al., 2016; Walpita et al., 2010, 2009). DIO1 inhibition may only become essential in hypothyroidal circumstances, for example when DIO2 is inhibited or in case of iodine deficiency, in zebrafish (Walpita et al., 2010) and mice (Galton et al., 2009; Schneider et al., 2006). We have set the evidence level to 'low' for KER 1037, because the relative importance of DIO1 and DIO2 is unclear and it seems that DIO2 is more important. This is indicated in several places throughout the AOP. We further clarified the relevant statement in the uncertainties:

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			"Six out of seven DIO1 inhibitors impaired posterior chamber inflation, but almost all of these compounds also inhibit DIO2. TCBPA, the only compound that inhibits DIO1 and not DIO2, had no effect on the posterior swim bladder. Exposure to strong DIO2 inhibitors on the other hand affected posterior chamber inflation and/or surface area in all cases. In the ToxCast DIO2 inhibition single concentration assay, 304 out of 1820 chemicals were positive and 177 of these were also positive for DIO1 inhibition (viewed on 5/7/2022). This complicates the distinction between the relative contribution of DIO1 and DIO2 inhibition to reduced swim bladder inflation."
	49	*The authors suggested impaired muscle function as an additional key event between decreased T3 levels and reduced swim bladder inflation* Why hasn't this been included?	This suggestion was based solely on gene expression analysis. There is currently insufficient evidence for impaired muscle function to be added as a key event. Therefore, this information is included in KER 1027 linking decreased T3 to reduced posterior swim bladder inflation.