This document includes:

- Comments received on AOP 155 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 155 authors

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Germany	4	Shouldn't the similarity to AOP 156 (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	*Therefore the current AOP is may be of higher biological relevance compared to AOP 155.* Should be AOP 157?	Indeed, this has been changed now.
	9	Only iopanoic acid is listed as inhibitor, which is, however, not specific for DIO2. Are there other stressor that could be mentioned? It should be mentioned already here that iopanoic acid is an inhibitor of all three DIO isoforms	The following clarification has now been added to the abstract, since it is not possible to add such general considerations to the stressor section: DIO2 inhibitors are often also inhibitors of DIO1 (Olker et al., 2019; Stinckens et al. 2018). 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.

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			The uncertainties related to DIO1 versus DIO2 inhibition have also been the subject of discussion during the review led by NC3Rs. It was decided to keep DIO1 and DIO2 as separate MIEs initiating distinct AOPs while mentioning the uncertainties related to the relative importance of both isoforms. 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

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			description of the evidence. We added a phrase explaining that IOP inhibits all three DIO isoforms.
	10	Inhibition of deiodinase (DIO) therefore impacts swim bladder inflation Deiodinase 1 and 2?	Since this is mentioned on the AOP page of AOP 155, we now changed this to DIO2.
	13	MIE and TH level are also relevant for other organisms than fish which could be stated here	We added the following clarification to the section 'Considerations for Potential Applications of the AOP': While the AOP is only applicable to fish, some of the upstream KEs are relevant across vertebrates. The taxonomic domain of applicability call of the KEs can be found on the respective pages.
	16	*Key Event Component* "Object" should be type II	Indeed, this has now been changed.
	17	1.It would be of interest here to list more of the DIO2-specific inhibitors. 2. PFOA is a strong inhibitor of both DIO1 and DIO2 which should be indicated here	1. 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: "DIO2 inhibitors are often also inhibitors of DIO1 (Olker et al., 2019; Stinckens et al. 2018). In the ToxCast DIO2 inhibition single concentration assay, 304 out of 1820 chemicals were positive and 177 of these

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			were also positive for DIO1 inhibition (viewed on 5/7/2022)." See comment related to page 9 for response about mentioning more stressors. 2. We added a clarification on the DIO1 and 2 inhibitory capacity of PFOA
	18, 54, 76	*Based on these results, DIO2 seemed to be more important than DIO1* Is this really relevant? Six of seven strong DIO1 inhibitors impaired posterior chamber inflation	We added the following clarification: "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." There is currently not enough evidence for a solid conclusion. This has been clearly indicated in several sections in the AOP.

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	18	*Houbrechts et al. (2016) did however confirm decreased DIO2 activity in a DIO1-DIO2 knockdown zebrafish* It is unclear what is meant here. Houbrechts et al. describe the effects of permanent DIO2 deficiency indicating that early development is perturbed, e.g. defects in swim bladder inflation were observed which should be mentioned here. Check also a similar statement on page 19	We understand that this was confusing and we now added some clarification to the section taxonomic domain of applicability of MIE 1002: "Evidence for fish (e.g., zebrafish and fathead minnow) is mostly indirect since DIO enzyme activity is usually not measured in chemical exposure experiments. Houbrechts et al. (2016) showed decreased DIO2 activity in a DIO1-DIO2 knockdown zebrafish at the ages of 3 and 7 days post fertilization together with impaired swim bladder inflation, showing that the enzyme is present, the activity is measurable and impairing its activity has negative effects. Noyes confirmed decreased outer ring deiodination activity in fathead minnows exposed to BDE-209. Walpita et al. (2007) showed decreased DIO2 activity in the liver of Nile tilapia injected with dexamethasone." The statement on page 19 is part of the section 'How it is measured or detected'. Houbrechts et al. is included here as an example of measurements in fish.
	18	*In mammals, DIO2 controls the intracellular concentration of T3.* Is there evidence, that in teleost this is not true? In case DIO2 controls the intracellular levels in fish, the KE 1003 may not fully reflect the sequence of events. DIO2 inhibition may directly affect local TH levels independent of changes in serum levels (which would also differentiate to e.g. TPO-related effects)	There is uncertainty related to the role of DIO2 in regulating local versus systemic thyroid hormone levels and potential cross-species differences. Because of this uncertainty and because this issue has also been raised during the review of a related AOP (AOP 363), we decided to remove 'serum' from the KE 1003 title and change it to

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			'Decreased, triiodothyronine (T3)'. This does not change the AOP, but enables a broader interpretation to include local T3 changes. This way, in accordance with the OECD Users' handbook, we are describing the KE as generally as possible and we are adding the tissue specificity in the downstream linkages. Cfr. Developer tip from the OECD Users' handbook: The biological context of the KE (e.g., the tissue type/taxa/life stage/sex etc.) should only be restricted (e.g., "enzyme activity in liver, decreased" or "hormone concentration in females, increased") to the extent that function changes with context. If the function is equivalent in both sexes, do not restrict the context by sex. If the function is equivalent in all cell types, do not restrict to a specific cell type. We made appropriate changes to the relevant KE and KER descriptions, evidence sections and uncertainties to accommodate this change. We have also added more information to the taxonomic domain of applicability section of KE 1002 (p 18) to explain the uncertainty related to the role of DIO2 in regulating local versus systemic thyroid hormone levels and potential cross-species differences.

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	18	1. **decabromodiphenyl ether (**BDE-209**)** 2. *as well as to convert rT3* Shouldn't rT3 be introduced here? It could be stated here that deiodination is not only a mechanism for activation T4 by conversion to T3, but it is also an important mechanism for degrading both T4 and T3 to inactive compounds. It could be stated here as well that the relative contribution of the DIOs varies amongst species, developmental stages and tissues.	1. An explanation of the abbreviation has been added for BDE. 2. An explanation of the abbreviation has been added for rT3 in the section 'KE description', p19. Activation versus inactivation in general is explained in the paragraph above. Further details on the exact conversions by the different DIO isoforms are also given. A sentence on the relative contribution of the DIOs has been added.
	19	The Sandell-Kolthoff method could be explained, e.g. a photometric method based on Ce^{4+} reduction. Renko et al. 2012 (https://doi.org/10.1210/en.2011-1863) could be cited here.	More detail on the Sandell-Kolthoff method has been added.
	23	larbean metamorphoses -> larvean metamorphoses	Larbean has been replaced by lamprey.
	24	*sequential outer or inner ring monodeiodination of T4 by the deiodinating enzymes* should be "T4 and T3", as both are de novo products of the thyroid	This has been adjusted in the KE description of KE 1003.

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	24	*that the changes measured in the TH concentration reflect mainly the changes* *should be "in the **free** TH concentrations"?*	This has been changed to free TH concentrations.
	24	*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.
	25	*"regulating the production of pro-hormone T4 and in a lesser extent of T3, which is the biologically active TH."* A little bit misleading –T4/T3-production of the thyroid is regulated to the	This has now been clarified in the KE description of KE 1003 (p 26): "The TRH and the TSH regulate the production of thyroid hormones. Less T3 (the biologically more active TH) than T4 is produced by the thyroid gland. The rest of the required amount of

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		same extend (imho), but under normal conditions less T3 than T4 is produced by the thyroid.	T3 is produced by outer ring deiodination of T4 ..”
	25	*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 differences in the importance of specific transporters may occur.”
	32	Medala (Oryzias latipes) -> Medaka (Oryzias latipes) To which group does Medaka belong (physostomous and physoclistous)? Life stage: Could Medaka be added here as well?	This typo has been corrected. Medaka is now listed as physoclistous species in the first paragraph of the taxonomic domain of applicability section (p 33) We also added a recent study: “Horie et al. (2022) eludicated the

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			timing of swim bladder inflation in medaka and compared effects on the swim bladder after exposure of zebrafish and medaka to PFBA and TDCPP." We added the following information to life stage applicability: "In medaka, the swim bladder inflates around 2 hours post hatch (hatching occurs around 8 dpf) (Horie et al., 2022)."
	47	Regarding Taxonomy: It could be mentioned, that D2 and D3 expression customize the timing and intensity of TH signalling in an organ/tissue-specific way (ref. e.g. Russo et al 2021)	We added the suggested information to the KER description of KER 1026.
	49	*Known Feedforward/Feedback loops influencing this KER* It might be worth to include, that expression of all three DIO are regulated by TH and that iodine supply, as a consequence, also affects their regulation (for mDio2 e.g. Wagner MS 2007)	Regulation of DIO by THs was added.
	54	*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.