This document includes:

- Comments received on AOP 159 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 159 authors

Expert affiliation/ Country	Page #	Expert Comments	response
Germany	1	The short name should be changed to: TPO anterior swim bladder	Short names are consistent across the 5 AOPs. We prefer to keep them aligned.
	4	*This AOP is part of a larger AOP network* The numbers of the AOPs involved could already 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	an other enzyme that is important for TH synthesis **since it provides hydrogen peroxide for iodide oxidation** A similar explanatory sentence could be added on page 19 and 27 as well	This has been added where relevant.
	9	Since NIS inhibition is mentioned later on, it might be worth to include the NIS AOPs also in the graphical representation.	Since AOPs initiated by NIS inhibition have not yet been developed in fish, we prefer not to include them in the graphical representation of the fish AOP network.

Expert affiliation/ Country	Page #	Expert Comments	response
	12	1. Japanese rice fish **Medaka** (Oryzias latipes) 2. Sex differences regarding Medaka could be removed here since the AOP is not applicable to Medaka	1. This has now been changed to: "Japanese rice fish or medaka (Oryzias latipes)" 2. Anterior chamber inflation is indeed not relevant to medaka. All statements on medaka have been removed from the relevant KEs and KERs.
	13	1. important for thyroid hormone synthesis **which depends on the formation of reactive oxygen species** 2. indirect evidence from deiodinase knockdowns **(see AOPs 155-158)**	1. See previous comment on adding "**since it provides hydrogen peroxide for iodide oxidation"** 2. A reference to AOPs 155-158 has been added.
	4	"*how inhibition of TPO results in decreased synthesis of T4 in the thyroid follicles*." Also T3 is directly synthesized at respective TG-sites (e.g. https://doi.org/10.1038/s41574-019-0184-8)	T4 has been replaced by THs.
	14	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.

Expert affiliation/ Country	Page #	Expert Comments	response
	18	MMI and PTU should be introduced here. It should also be indicated that PTU also inhibits DIO1.	The abbreviations have been explained and the DIO inhibiting capacity of PTU has been added.
	19	1. MIT and DIT production – MIT and DIT are only introduced on p. 20; this should be moved here. 2. Shouldn't the guaiacol assay be briefly explained here?	1. This depends on the order in which fields are copied from the Wiki. We now explained the abbreviations in both places. 2. This is explained in the section 'how it is measured or detected'. Since this is the taxonomic domain of applicability section, we feel that the reference should be sufficient in this case.
	20	"Alterations in all of these events are not covered by some of the commonly used assays that measure "TPO inhibition" (e.g., guaiacol and AmplexUltraRed, see below)." That's absolutely right – might be specified to "Ususally just the first step of this series of events is covered by assays that measure TPO" inhibition [….]	This has been added.
	20	It would be of interest to list (some of) the reversible and irreversible inhibitors	Genistein has been added as an irreversible TPO inhibitor.
	21 and others	Thyreoperoxidase versus thyroid peroxidase While both names are used I would suggest to be consistent through this (and other) AOP documents	Due to the collaborative nature of the AOP-Wiki, standardization of terms across KEs/KERs/AOPs with different authors is not always feasible. Since these terms are synonymous and both clearly

Expert affiliation/ Country	Page #	Expert Comments	response
			represent the target, we prefer to leave this as is.
	21	Also recombinant sources of TPO are used, e.g. Schmutzler et al 2007 (PMID: 17379648), Dong et al 2020 (PMID: 31629072)	This has been added.
	27	Grave's Disease should be Graves' Disease	This typo has been corrected.
	27	and **the transport protein** pendrin	This has been added.
	28	*"The ontogeny of TPO inhibition during development by environmental chemicals is a data gap."* Some recent studies address this issue: Ramhoj et al 2022 (PMID: 34757178)	This has been rephrased: "Due to the maternal factor, the life stage specific impact of TPO inhibition after exposure to environmental chemicals is complex (Ramhoj et al., 2022)."
	36	*and a few less active iodothyronines (rT3, 3,5-T2)* Shouldn't these be introduced briefly?	The abbreviations are now introduced.

Expert affiliation/ Country	Page #	Expert Comments	response
	37	body thyroid hormone levels using LC-MS techniques (Hornung et al., 2015) **which** are increasingly used	This has been corrected.
	37	(i.e., different regulatory needs will require different levels of confidence in the AOP) It is suggested to delete this sentence; repeatability and reproducibility are required for all regulatory sectors. Moreover AOP-based testing strategies always require high confidence in the underlying mechanisms.	This phrase has been deleted.
	43	larbean metamorphoses -> larvean metamorphoses	This has been replaced by lamprey
	44	*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	This statement has been updated with the suggested information.

Expert affiliation/ Country	Page #	Expert Comments	response
		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."	
	44	*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."
	51, 83, 87, 107	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.

Expert affiliation/ Country	Page #	Expert Comments	response
	73	In Xenopus, it has been shown -> start a new paragraph and write Xenopus in bold	This has been adjusted.
	79	several studies reportedThe evidence -> reported evidence	This has been corrected.
	85	*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.