



Iopanoic acid alters thyroid hormone-related gene expression, thyroid hormone levels, swim bladder inflation, and swimming performance in Japanese medaka

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ABSTRACT

Disruption of the thyroid hormone system by synthetic chemicals is gaining attention owing to its potential negative effects on organisms. In this study, the effects of the dio-inhibitor iopanoic acid (IOP) on the levels of thyroid hormone and related gene expression, swim bladder inflation, and swimming performance were investigated in Japanese medaka. Iopanoic acid exposure suppressed thyroid-stimulating hormone β (*tsh β*), *tsh β* -like, iodotyronine deiodinase 1 (*dio1*), and *dio2* expression, and increased T4 and T3 levels. In addition, IOP exposure inhibited swim bladder inflation, reducing swimming performance. Although adverse outcome pathways of thyroid hormone disruption have been developed using zebrafish, no adverse outcome pathways have been developed using Japanese medaka. This study confirmed that IOP inhibits *dio* expression (a molecular initiating event), affects T3 and T4 levels (a key event), and reduces swim bladder inflation (a key event) and swimming performance (an adverse outcome) in Japanese medaka.

1. Introduction

In vertebrates, thyroid hormones stimulate metabolism; regulate pulse rate, body temperature, and autonomic nervous system function; and maintain energy expenditure (Mullur et al., 2014). However, in fish, they are considered to be involved in metamorphosis, the development of symmetric pelagic larvae into asymmetric benthic juveniles (Campinho et al., 2018), upwelling movement, and seawater adaptation of salmonids (Deal and Volkoff, 2020). Furthermore, thyroid hormones play a role in the development of swim bladders and eyes in fish (Stinckens et al., 2020; Gözl et al., 2022). Thus, thyroid hormones are essential for vital functions in living organisms.

Thyroid hormone levels are regulated by thyroid-stimulating hormone (TSH), which is secreted by the pituitary gland via thyroid hormone signaling through thyroid hormone receptors (Szkudlinski et al., 2002; Ortiga-Carvalho et al., 2014). Two types of thyroid hormones (T4 and T3) are secreted by the thyroid gland. T4 is mainly synthesized in the thyroid gland and converted to T3 in the liver and other organs,

where it functions as a hormone (Deal and Volkoff, 2020). The enzyme that helps this conversion is iodothyronine deiodinase, and the two enzyme types responsible are DIO1 and DIO2 (Deal and Volkoff, 2020). The associated genes are expressed during the embryonic stage in zebrafish. In addition, thyroid hormones play important roles in the development of fish from the initial stages (Parsons et al., 2020).

Research on the influence or effects of thyroid hormone-disrupting chemicals, which the European Union regulates in legislation on industrial chemicals (Registration, Evaluation, Authorization, and Restriction of Chemicals [REACH], EC, 1907/2006), plant protection products (Regulation, EC, 1107/2009), and biocide products (Regulation, 528/2012, EC, 2017a), has been increasing. Dang et al. (2021) reported that thyroid hormone-disrupting chemicals induced changes in T4 and T3 levels and thyroid hormone-related gene (including *tsh*, *dios*, and *trs*) expression, and decreased posterior swim bladder size in zebrafish.

Similar to zebrafish, the Japanese medaka is a good model fish to study the effects of chemical substances. We previously reported the

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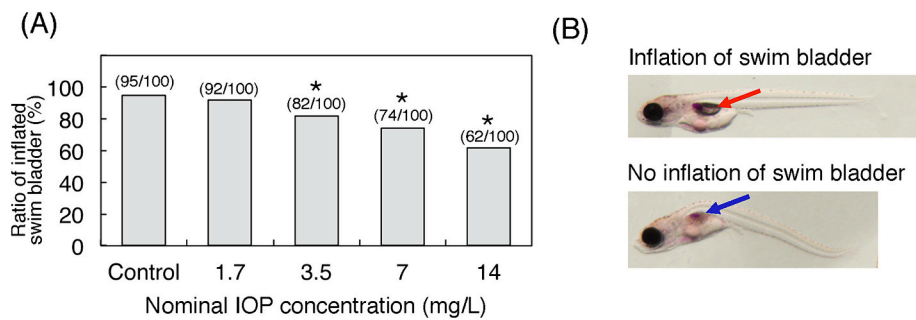


Fig. 1. Effects of IOP on swim bladder inflation in Japanese medaka at 1 day post hatching (dph). (A) Numbers above each bar indicate the number of individuals with an abnormal development or inflated swim bladder among the 100 individuals examined. * $P < 0.05$ vs. control (Chi-squared test). (B) Red arrow indicates the inflation of the swim bladder. Blue arrow indicates no inflation of the swim bladder.

effects of some thyroid hormone-disrupting chemicals, such as bis(2-ethylhexyl) adipate (Horie et al., 2022a), acetyl tributyl citrate (ATBC) (Horie et al., 2022b), 6-propyl-2-thiouracil (PTU) (Horie et al., 2023a), bis(2-ethylhexyl) sebacate (Horie et al., 2023b), and diisobutyl adipate (DIBA) (Horie et al., 2023c), on swim bladder inflation, thyroid hormone-related gene (*tsh*, *dio1*, *dio2*, *tra*, and *trf*) expression, and swimming behaviors. However, the effects of thyroid hormone-disrupting chemicals on T4 and T3 levels in Japanese medaka remain unclear.

Several adverse outcome pathways (AOPs) of thyroid hormone disruption are available at AOP wiki (<https://aopwiki.org/>). Although five AOPs (155–159) of thyroid hormone disruption and swim bladder inflation have been reported, only AOPs 155 and 157, OECD AOP Series publications 22 and 24, describe the effect of inhibiting the expression of DIO types 1 and 2 on inflation of the posterior chamber in zebrafish or fathead minnows. In AOPs 155 and 157, OECD AOP Series publications 22 and 24, inhibition of *dio1* expression and that of *dio2* expression are set as molecular initiating events (MIEs) and reductions in T3 level, posterior swim bladder inflation, and swimming performance are set as key events (KEs). DIO enzymes play crucial roles in the activation and/or inactivation of thyroid hormones. Specifically, DIO isoform types 1 and 2, primarily recognized as activating enzymes, are essential for the activation of thyroid hormones by converting the prohormone T4 into its more potent form, T3. Iopanoic acid (IOP), an orally administered cholecystography agent, has been extensively studied as a DIO1 and DIO2 inhibitor (Braga and Cooper, 2001). In addition, IOP functions as a substrate for DIO1 (Renko et al., 2012). Although Stinckens et al. (2020) and Van Dingenen et al. (2023) have reported the effects of IOP on the thyroid hormone system in zebrafish, its effects on the thyroid hormone system in Japanese medaka have not been investigated. Therefore, in this study, we aimed to investigate the effects of IOP in Japanese medaka, especially on the (1) expression of thyroid hormone-related genes, (2) levels of T4 and T3, (3) expansion of the swimming bladder, and (4) swimming behavior. The toxicity of IOP in Japanese medaka as determined in this study was compared with that in zebrafish as determined in previous studies. The results of this study demonstrate that Japanese medaka and zebrafish are equally suitable for the evaluation of the effects of thyroid hormone-disrupting chemicals.

2. Material and methods

2.1. Test fish and chemicals

Japanese medaka (*Oryzias latipes*; NIES-R strain) bred at Kobe University since 2021 were used in this study. All experiments involving fish were performed in strict compliance with the existing national guidelines, including the Act on Welfare and Management of Animals of the Ministry of the Environment, Japan, and the ARRIVE guidelines 2.0. The fish were handled per the animal care guidelines established by Kobe University. This study was approved by the Institutional Animal Care

and Use Committee of the Research Center for Inland Seas of Kobe University (permission number: 2021-04). Fish before the initiation of self-feeding are not considered protected animals under the European Union directive (2010/63/EU), and Japanese medaka fries do not begin feeding until 2–3 days post hatching (dph). Therefore, 1 dph fries are not considered as protected animals.

Iopanoic acid (CAS RN: 96–83-3, purity >98.0%) was purchased from Tokyo Chemical Industry Co., Ltd. (TCI; Tokyo, Japan).

2.2. Exposure

IOP at concentrations 0 (control), 1.7, 3.5, 7, and 14 mg/L was prepared for exposure studies. Medaka eggs were collected and screened using a stereomicroscope to select fertilized eggs. The selected fertilized eggs were used in the experiments. Twenty eggs were placed in each 100-mL glass beakers containing 80 mL IOP solution; there were 15 beakers per exposure group (with 300 eggs per exposure group). The exposure solution was changed daily. During the test period, the temperature, pH, and dissolved oxygen of the test solution in each exposure group were maintained at 25 ± 2 °C, 7.0 ± 1 , and 60% saturation, using a thermometer (CT-430WP; Custom, Tokyo, Japan), pH meter (D-55; Horiba, Kyoto, Japan), and dissolved oxygen meter (HQ30d; Hach, Loveland, CO, USA), respectively.

Nine days after fertilization (daf), the hatched fries were collected from each concentration group. Subsequently, 50 fries from each exposure group were placed in 500-mL glass beakers containing 450 mL IOP solution. For measuring swimming behavior, each hatched fry in each concentration group was placed in a well of a 24-well microplate with 1.5 mL exposure solution. The exposure period was 10 d, from post-fertilization to 1 dph (i.e., 10 daf). One day post-hatching, swim bladder inflation, swimming behavior, total body length, thyroid hormone-related gene expression, and thyroid hormone levels were assessed.

2.3. Determination of swim bladder inflation and total body length

At 1 dph, each larva was anesthetized (MS-222 at 200 mg/L). Images of larvae at all treatment concentrations were captured using a stereomicroscope (SZX 16; Olympus, Tokyo, Japan) equipped with a camera (Visualix V900FL; Visualix, Kobe, Japan). The presence of an inflated swim bladder was checked with a stereomicroscope in fifty larvae from each concentration group. The presence or absence of inflation of the swim bladder was determined as shown in Fig. 1B. The ImageJ software was used to measure total body length.

2.4. Swimming behavior

Swimming behavior/activity of Japanese medaka larvae in all treatment groups was recorded at 1 dph continuously from an aerial viewpoint using a Panasonic LUMIX GH5S camera (Panasonic, Osaka, Japan), with the images acquired at a speed of 60 FPS for 10 min with a

resolution of 1920×1080 pixels/mm. The swimming performance was assessed by measuring the total swimming distance for the final 5 min, based on the video footage and analysis using the DIPP-Motion V/2D software of DITECT (Tokyo, Japan).

2.5. Real-time quantitative polymerase chain reaction

The expression levels of six thyroid hormone-related genes (*tsh β* , *tsh β -like*, *tra*, *tr β* , *dio1*, and *dio2*) and one housekeeping gene (*ef-1 α*) were measured using a Light Cycler 96 System (Roche, Basel, Switzerland). The hatched fries at 1 dph were fixed in RNA later (Sigma-Aldrich, St. Louis, MO, USA) and stored at 4 °C. The next day, the total RNA was extracted from one whole body of the hatched fry using the RNeasy Mini Kit, comprising an on-column RNase-free DNase treatment (Qiagen, Hilden, Germany). Eight larvae were used from each treatment group, and the purity and concentration of the extracted RNA were determined using a NanoDrop One Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Next, 500 ng of RNA was reverse transcribed to cDNA using PrimeScript RT Master Mix (Perfect Real Time, Takara, Shiga, Japan). The concentration of cDNA was adjusted to 10 ng/ μ L using Easy dilution (Takara) and stored at -30 °C until quantitative polymerase chain reaction (qPCR) analysis.

Real-time quantitative polymerase chain reaction was performed using FastStart SYBR Green Master Mix (Nippon Genetics Co., Ltd., Tokyo, Japan). Each reaction mixture (total 20 μ L), comprising 10 μ L of PCR Master Mix (2 \times), 0.2 μ L each of forward and reverse primers (20 μ M), 1 μ L of cDNA (10 ng), and 8.6 μ L of PCR-grade water, was used. Duplicate runs were performed to target specific genes of each sample. The LightCycler 96 SW 1.1 software (Roche) and Microsoft Excel (Microsoft, Redmond, WA, USA) were used for data analysis. Expression levels of *tsh β -like*, *tsh β* , *dio1*, *dio2*, *tra*, and *tr β* were normalized to the level of the housekeeping gene *ef1 α* , using the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen, 2001). Primer information is shown in Supplemental Table 1.

2.6. Thyroid hormone (T4 and T3) measurement

At 1 dph, 50 larvae were pooled and stored at -80 °C for measuring thyroid hormone (T4 and T3) levels from the three concentration groups: control, minimum exposure concentration (1.7 mg/L), and maximum exposure concentration (14 mg/L). For each concentration group (containing 50 larvae), three pooled samples ($n = 3$) were prepared to measure thyroid hormone levels. Fish larvae in distilled water were homogenized vigorously using ShakeMaster® NEO (Biomedical Science, Tokyo, Japan) with stainless steel beads. The homogenate samples were transferred to polypropylene (PP) tubes and spiked with isotope-labelled internal standard solutions containing $^{13}\text{C}_6$ -T4 and $^{13}\text{C}_6$ -T3. Samples were denatured with acetonitrile and equilibrated for approximately 60 min in the dark at room temperature, and then centrifuged at 3000 rpm for 3 min. The supernatants were decanted into new PP tubes and diluted with distilled water. The sample supernatants were passed through Oasis MCX cartridges (Waters, Milford, MA, USA) preconditioned with methanol, distilled water, and 1% acetic acid solution. The cartridges were washed with distilled water and then with methanol; then, the thyroid hormones were eluted with a methanol/distilled water/ammonia solution (70:30:1, v/v/v). The eluted samples were evaporated to dryness, and then reconstituted in a methanol:distilled water:pyridine solution (40:60:1, v/v/v). The dissolved samples were subjected to LC-MS/MS analysis to quantify T4 and T3. The SRM transitions were m/z 777.8/731.6 for T4, m/z 783.8/737.7 for $^{13}\text{C}_6$ -T4, m/z 651.9/605.8 for T3, and m/z 657.9/611.6 for $^{13}\text{C}_6$ -T3. The measurement ranges were 4–4000 and 0.5–500 pg/tube, and the limits of quantification were 4 and 0.5 pg/tube for T4 and T3, respectively.

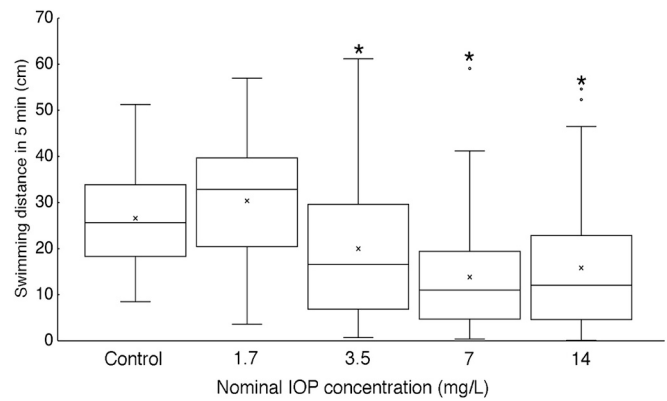


Fig. 2. Effects of IOP on the swimming performance of Japanese medaka at 1 dph. Data are presented as mean \pm SD ($n = 50$ per concentration). * $P < 0.05$ vs. control.

2.7. Data analysis

The R software was used for statistical analyses, and the data are presented as mean \pm standard deviation, with $p < 0.05$ indicating statistical significance. The chi-square test was performed to determine whether there was a significant difference in the presence or absence of swim bladder inflation by conducting a comparison with the control group. Significant differences in thyroid hormone-related gene expression, thyroid hormone levels, and swimming performance between the exposure and control groups were examined using a one-way analysis of variance with Tukey's multiple comparison test.

3. Results

3.1. Influence of IOP on swim bladder inflation, swimming behavior, and total body length

In this study, IOP exposure did not affect embryonic development, hatching, or growth (Supplemental Fig. 1). Inflation of the swim bladder was observed in 95% of fries in the control group (Fig. 1A). IOP exposure reduced the number of fries with swim bladder inflation in a concentration-dependent manner (Fig. 1A & B). In the 3.5, 7, and 14 mg/L IOP groups, 82, 74, and 62 of 100 fries, respectively, and the groups showed significant differences compared to the control (Fig. 1A & B). Fig. 2 shows the results of the total swimming distance for 5 min. The swimming performance/activity of the 3.5, 7, and 14 mg/L IOP groups reduced compared with that of the control (Fig. 2).

3.2. Influence of IOP on thyroid hormone-related gene expression and thyroid hormone levels

Effects of IOP on the expression of thyroid hormone-related genes in Japanese medaka are shown in Fig. 3. The expression of *tsh β* significantly increased in the 3.5 mg/L group and significantly decreased in the 14 mg/L groups (Fig. 3) compared with that in the control. The expression of *tsh β -like* significantly decreased in all concentration groups, except the 3.5 mg/L group, compared with that in the control (Fig. 3). Additionally, the expression of *tra* and *tr β* (receptors for thyroid hormones) significantly increased in the 3.5 mg/L group compared with that in the control (Fig. 3). The expression of *dio1* significantly decreased in the 3.5 and 7 mg/L groups and that of *dio2* significantly decreased in 7 and 14 mg/L groups compared with those in the control (Fig. 3).

The T4 and T3 levels in Japanese medaka significantly increased after IOP exposure compared with those in the control group (Fig. 4).

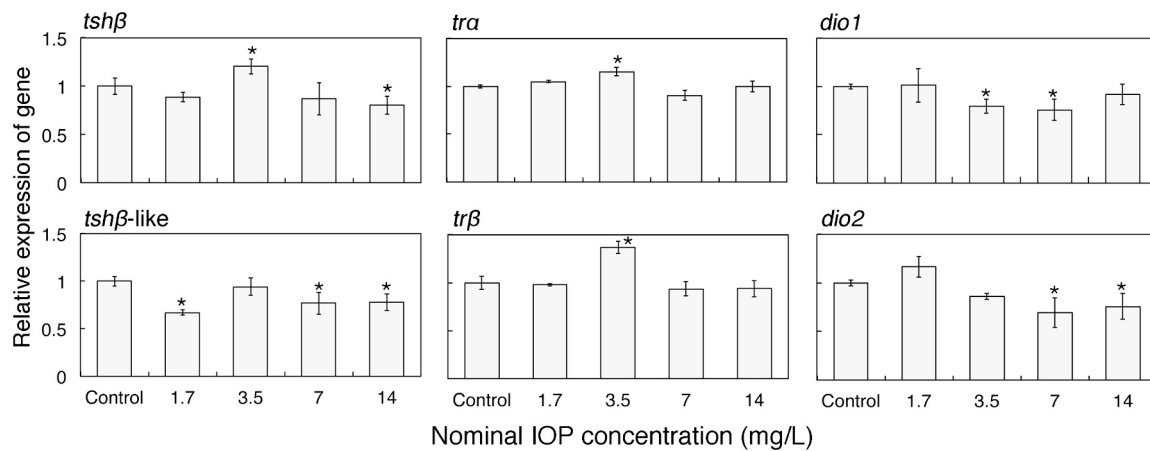


Fig. 3. Effects of IOP on the expression levels of *tshβ*, *tshβ-like*, *tra*, *trβ*, *dio1*, and *dio2* in Japanese medaka at 1 dph as analyzed using qPCR. $n = 8$; the expression levels of these genes were normalized to the level of the housekeeping gene *ef1α* and the control. * $P < 0.05$ vs. control.

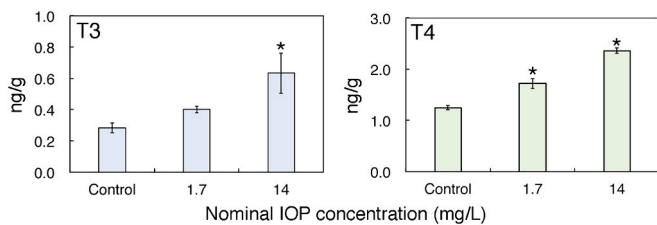


Fig. 4. Effects of IOP on the whole-body T3 and T4 levels in Japanese medaka at 1 dph. Data are presented as mean \pm SD ($n = 3$ per concentration). * $P < 0.05$ vs. control.

4. Discussion

In this study, Japanese medaka fries were exposed to IOP, an inhibitor of DIO expression. A reduction in swim bladder inflation was observed in fries exposed to >3.5 mg/L IOP. In addition, fries exposed to >3.5 mg/L IOP showed reduced swimming behavior and alterations in thyroid hormone-related gene expression and thyroid hormone levels.

Van Dingenen et al. (2023) reported that DIO inhibition interferes with the formation of the posterior swim bladder tissue layers during early embryonic development in zebrafish and that the knockdown of *dio* expression in zebrafish induced abnormal swim bladder development (Heijlen et al., 2014). These findings indicate that DIO plays an important role in thyroid hormone-mediated swim bladder inflation and development. Studies have reported the relationship between thyroid hormone-disrupting chemicals and DIO expression in zebrafish and Japanese medaka. For example, Baumann et al. (2016) reported that PTU exposure upregulated *dio2* expression but did not change *dio1* expression in zebrafish. In a study by Horie et al. (2023a), neither *dio1* nor *dio2* was expressed in Japanese medaka exposed to PTU. In previous studies, TBBPA exposure did not change the expression of *dio1* or *dio2* in zebrafish (Baumann et al., 2016) or Japanese medaka (Horie et al., 2023a). Bis(2-ethylhexyl) phthalate (DEHP) exposure reportedly upregulates *dio2* expression, but does not alter *dio1* expression in zebrafish (Jia et al., 2016); increased expression of *dio1* and *dio2* has been observed in Japanese medaka exposed to DEHP (Horie et al., 2022a). Farías-Serratos et al. (2021) and Cavallin et al. (2017) have reported that IOP upregulates *dio2* expression in zebrafish larvae and fathead minnows larvae, respectively; however, in this study, the expression of *dio1* and *dio2* was decreased by IOP treatment in Japanese medaka. Thus, determining whether chemicals have potential thyroid hormone-disrupting activity based solely on *dio* expression is difficult because *dio* expression differs depending on the thyroid hormone-disrupting

chemicals. Furthermore, the changes in *dio* expression may vary among fish species, even when exposed to the same thyroid hormone-disrupting chemicals.

The TSH-specific β subunit forms one of the two subunits, α (common with alpha subunits) and β , which comprise thyroid hormones. Two paralogs of medaka Tsh β (*tshβ*, XM_011477157; *tshβ-like*, XM_004068796) have been listed in the NCBI database (<https://www.ncbi.nlm.nih.gov/>); however, the role of these two paralogs remains unknown (Maugars et al., 2016; Fleming et al., 2019). Thyroid hormones function through thyroid hormone receptors (*tra* and *trβ*). Campbell and Langlois (2018) reported a decrease in *trβ* expression without a change in *tra* expression in *Silurana tropicalis* after exposure to IOP. To the best of our knowledge, this is the first report of IOP-induced downregulation of *tshβ-like* and *tshβ* expression, but no change in *tra* and *trβ* expression, in a dose-dependent manner in Japanese medaka. Our research group has also reported the effects of several thyroid hormone-disrupting chemicals on *tshβ*, *tshβ-like*, *tra*, and *trβ* expression in Japanese medaka. For example, upregulation of *tshβ-like* expression but no changes in *tshβ*, *tra*, and *trβ* expression after exposure to PTU (Horie et al., 2023a); upregulation of *tshβ-like* and *tshβ* expression and downregulation of *tra* and *trβ* expression with TBBPA (Horie et al., 2023a) and DIBA exposure (Horie et al., 2023c); and downregulation of *tra* and *trβ* expression but no change in *tshβ* expression after exposure to ATBC (Horie et al., 2022b). Thus, determining whether chemicals have potential for thyroid hormone-disruption based on thyroid hormone-related gene expression alone is difficult because thyroid hormone-related gene expression differs depending on the thyroid hormone-disrupting chemicals.

The effects of thyroid hormone-disrupting chemicals on the levels of T4 and T3 in zebrafish have been summarized by Dang et al. (2021), although these thyroid hormone-disrupting chemicals alter the levels of T4 and T3 through various molecular mechanisms—not only through DIO inhibition but also direct or indirect mechanisms. For example, DEHP (Jia et al., 2016) and decabromodiphenyl ethane (Wang et al., 2019) increase both T4 and T3 levels. Bisphenol AF (Tang et al., 2015), bisphenol S (Zhang et al., 2017), methimazole (MMI) (Stinckens et al., 2020), and PTU (Stinckens et al., 2020) decrease both T4 and T3 levels. Tris(1,3-dichloro-2-propyl)phosphate (TDCPP) (Wang et al., 2013) and monoethylhexyl phthalate (Zhai et al., 2014) reportedly increase T3 levels and decrease T4 levels. TBBPA (Zhu et al., 2018) and triadimefon (Liu et al., 2011) decrease T3 levels and increase T4 levels. Thus, thyroid hormone-disrupting chemicals induce changes in T4 and T3 levels; however, the trend varies depending on the chemicals used. Cavallin et al. (2017) reported that pooled whole-body T4 and T3 levels significantly increased after IOP exposure in fathead minnow embryos at 4 dpf, although there was no significant difference in the T4 levels at 6 dpf.

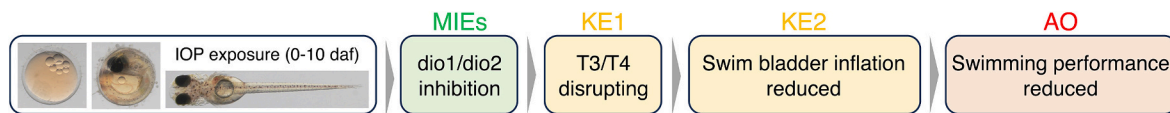


Fig. 5. AOP pathway of thyroid hormone disruption in Japanese medaka after IOP exposure. KE1, T3 and T4 levels increase; KE2, Swim bladder inflation reduction; AO, Swimming performance reduction.

These results are consistent with those of this study on Japanese medaka. In contrast, Van Dingenen et al. (2023) reported that IOP exposure upregulated and downregulated T3 and T4 levels in zebrafish, respectively. Thus, the trend of the change patterns in T4 and T3 levels after IOP exposure differs among studies; however, the reason for this difference remains unclear. Thus, further studies should investigate how thyroid hormone-disrupting chemicals affect T4 and T3 levels depending on chemical concentrations, exposure periods, or exposure timing in the developmental stages (embryo, larval, or juvenile).

The swim bladder is a buoyancy regulator that allows fish to remain in place with minimal effort, neither floating nor sinking; therefore, they do not spend energy in the water. In zebrafish, a physostomous fish, the swim bladder is composed of two chambers. The posterior chamber inflates 1–2 days after hatching (i.e., 4–5 days after fertilization). On the contrary, the anterior chamber inflates around 2–3 weeks after hatching. In case of fathead minnow, also a physostomous fish, the swim bladder is composed of two chambers; the posterior chamber inflates 1–2 days after hatching (i.e., 5–6 days after fertilization) and the anterior chamber inflates around 14 days after hatching (i.e., 9 days after fertilization). Contrarily, Japanese medaka, which is a physoclistous fish, has only one chamber and conceptually corresponds to the posterior chamber of zebrafish and fathead minnow. In zebrafish and fathead minnows, thyroid hormones are involved in swim bladder development, and the reduction in swim bladder inflation is a KE of IOP for thyroid hormone-disrupting chemicals (OECD, 2022a, 2022b, 2022c, 2022d). Although thyroid hormone-disrupting chemicals inhibit/decrease swim bladder inflation, the molecular mechanisms vary. For example, PTU (Stinckens et al., 2020), IOP (Stinckens et al., 2020), MMI (Jomaa et al., 2014), TDCPP (Godfrey et al., 2017), and perfluorobutanoic acid (PFBA) (Hagenaars et al., 2011) reduce swim bladder inflation in zebrafish. Similarly, in Japanese medaka, several thyroid hormone-disrupting chemicals, such as PTU (Horie et al., 2023a), IOP (this study), MMI (Godfrey et al., 2019), TDCPP (Horie et al., 2022c), and PFBA (Horie et al., 2022c), reduce swim bladder inflation. Cavallin et al. (2017) reported that the IOP-induced inflation of the posterior swim bladders was significantly low in fathead minnows. These findings reveal that the presence or absence of swim bladder inflation in zebrafish and Japanese medaka is a useful endpoint for determining the thyroid hormone-disrupting activity of chemicals.

In this study, IOP affected swimming performance in the exposure group in which the inflation of the swim bladder was inhibited. Van Dingenen et al. (2023) also reported decreased swimming performance of zebrafish after IOP exposure. Studies have demonstrated that exposure to thyroid hormone-disrupting chemicals reduced swimming performance in both zebrafish and Japanese medaka. For example, PTU (zebrafish- Stinckens et al., 2020; Japanese medaka- Horie et al., 2023a, 2023b, 2023c), MMI (zebrafish- Stinckens et al., 2020), and TBBPA (zebrafish- Zhu et al., 2018) reduce swimming performance. These studies indicate that swimming performance is also a useful endpoint for determining the thyroid hormone-disrupting activity of chemicals in Japanese medaka.

The AOP is composed of MIEs, KEs, and adverse outcomes, and describes and structures the causal sequence of events at different levels of biological organization, leading to ecotoxicological effects. An AOP is a central element of a toxicological knowledge framework built to support the risk assessment of chemicals based on mechanistic reasoning. Recently, the OECD established an AOP for thyroid hormone-disrupting chemicals in zebrafish and fathead minnows (OECD, 2022a, 2022b,

2022c, 2022d). Based on the results of this study, an AOP for the thyroid hormone-disrupting chemical IOP was developed using Japanese medaka (Fig. 5). These results are consistent with the AOP of zebrafish and fathead minnows. This is the first study on AOP in Japanese medaka, indicating medaka is a suitable model organism for evaluating the thyroid hormone-disrupting activity of chemicals.

5. Conclusion

In this study, the effects of IOP, a thyroid hormone-disrupting chemical, were evaluated based on thyroid hormone-related gene expression, thyroid hormone (T4 and T3) levels, swim bladder inflation, and swimming performance of Japanese medaka. *Tsh β* , *tsh β -like*, *dio1*, and *dio2* expressions was suppressed by IOP treatment, and pooled whole-body T4 and T3 levels increased following exposure. Exposure to IOP also disrupted swim bladder inflation, ultimately affecting swimming performance. Furthermore, for IOP exposure, the MIE (*dio1* and *dio2* expression decrease), KEs (increase in T3 and T4 levels and reduction in swim bladder inflation), and adverse outcomes (reduction in swimming behavior) were successfully identified in Japanese medaka.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cbpc.2024.109930>.

CRedit authorship contribution statement

Yoshifumi Horie: Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Ayaka Sawada:** Formal analysis, Data curation. **Uaciquete Dorcas:** Writing – original draft, Methodology. **Babu Rajendran Ramaswamy:** Writing – review & editing. **Taisen Iguchi:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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