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Horie, Yoshifumi
Yap, Chee Kong
Okamura, Hideo

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Developmental toxicity and thyroid hormone-disrupting effects of acetyl tributyl citrate in zebrafish and Japanese medaka

Yoshifumi Horie[a,*], Chee Kong Yap[b], Hideo Okamura[a]

[a] Research Center for Inland Seas (KURCIS), Kobe University, Fukaeminami-machi, Higashinada-ku, Kobe 658-0022, Japan
[b] Department of Biology, Faculty of Science, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

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ABSTRACT

Chemical pollution from plasticizers in water environments is a serious environmental problem worldwide. Phthalate plasticizers have potential endocrine-disrupting effects in vertebrates. In this study, the effects of a non-phthalate acetyl tributyl citrate (ATBC) plasticizer on endocrine hormone activity (according to thyroid-related gene expression) and the developmental toxicity of ATBC were determined in zebrafish (*Danio rerio*) and Japanese medaka (*Oryzias latipes*) embryos. ATBC exposure increased the mortality of zebrafish and Japanese medaka at concentrations of 443.05 and 1,937.41 μg/L, respectively. Body curvature, edema, and growth inhibition were also observed after ATBC exposure in both species. Importantly, both species exhibited suppressed thyroid-related gene mRNA expression after ATBC exposure. ATBC exposure significantly suppressed the expression of *thyroid-stimulating hormone beta subunit* (*tshβ*), *iodothyronine deiodinase 1* (*dio1*), *dio2*, and *thyroid hormone receptor alpha* (*trα*) in zebrafish. In Japanese medaka, ATBC exposure suppressed the mRNA expression of *dio2, trα*, and *trβ* but not *tshβ* and *dio1*. In summary, ATBC exposure inhibited thyroid-related gene expression and led to improper swim bladder inflation in the test species. This is the first report of a non-phthalate ATBC plasticizer inducing abnormal embryo development and disrupting thyroid hormone activity in fish.

1. Introduction

Plastic and microplastic pollutants and plasticizer contamination in water environments (Larsson et al., 2017; Nagorka and Koschorreck, 2020; Sun et al., 2018) have become serious environmental problems worldwide. Plasticizers are broadly classified into phthalates and non-phthalates plasticizers, and is used to make plastics flexible. Celino-Brady et al. (2021) reviewed the inhibiting effects of phthalate plasticizers on the reproduction of zebrafish (*Danio rerio*) and goldfish (*Carassius auratus*) and the growth of guppy (*Poecilia reticulata*) and flounder (*Platichthys stellatus*). Therefore, the use of phthalate plasticizers has been restricted in several countries (https://www.plasticisers.org/regulation/reach/), and efforts have been made to use safer and more environmentally friendly non-phthalate plasticizers in various industries.

Acetyl tributyl citrate (ATBC) is a non-phthalate plasticizer used as a biodegradable plasticizer in polyvinyl chloride (PVC) and cellulose derivates, such as food additives, emollients for personal care, cosmetic products, pharmaceutical coatings, lubricants, and inks (https://chemceed.com/products/acetyl-tributyl-citrate-atbc/). ATBC contamination has been detected in the following water environments: Kuwait Bay, Kuwait (0.05 μg/L) (Smith et al., 2015); Nakdong River, Korea (less than the limit of quantification to 96 ng/L) (Park and Jeon., 2021); Rur River, Germany (≤0.54 μg/L) (Schwanen and Schwarzbauer, 2022); and groundwater in England (154 μg/L) (Spurgeon et al., 2022). Although non-phthalate plasticizers are considered safe, information related to the acute toxic effects of ATBC on aquatic organisms islacking. Xu and Gye (2018) reported that the 96 h LC_{50} was 13.3 mg/L in *Xenopus laevis* embryos. Bolívar-Subirats et al. (2021) reported that the 48 h EC_{50} was 5.10 mg/L in *Daphnia magna*. In addition, Sheikh and Beg (2021) reported that ATBC was packed tightly into the human thyroxine-binding globulin ligand-binding pocket with a similar binding pattern to that of the thyroxine-binding globulin native ligand T4, suggesting that ATBC could cause thyroid dysfunction. However, it is still unclear whether ATBC is harmful to aquatic organisms in vivo.

Thyroid hormones are regulated by thyroid-stimulating hormone (TSH), which is secreted by the pituitary gland in the brain. In fish, thy-

Abbreviations: ATBC, acetyl tributyl citrate; DEHP, Bis-(2-ethylhexyl) phthalate; dio, deiodinase; dph, days post hatching; haf, hours after fertilization; OECD, organisation for Economic Cooperation and Development; RT-qPCR, Real-time quantitative polymerase chain reaction; SPE, solid-phase extraction; TDC, thyroid hormone disrupting chemicals; tr, thyroid hormone receptor; tsh, thyroid-stimulating hormone.

* Corresponding author.
E-mail address: horie@people.kobe-u.ac.jp (Y. Horie).

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roid hormone is involved in the metamorphosis of flounder (Inui et al., 1995), growth of red sea bream and zebrafish (Hirata et al., 1989; Brown, 1997), skeletal formation of rainbow trout (Takagi et al., 1994), and swim bladder formation of zebrafish (Heijlen et al., 2014). The role of the thyroid axis in fish was reviewed by Deal and Volkoff (2020). Briefly, thyroid hormone is secreted via the stimulation of TSH, which is secreted from the pituitary gland, and works via the thyroid hormone receptor (tr). Thyroid hormone is secreted from the thyroid gland as the prohormone T4, which is converted into 3,5,3′-triiodothyronine (T3) which exhibits strong physiological activity in the liver and muscles. The conversion enzymes are iodothyronine deiodinases, enzymes (Dio1 and Dio2), that differ in their localization and regulation are responsible for this. Our research group found that the nonphthalate plasticizers bis-(2-ethylhexyl) adipate (DEHA) and bis-(2-ethylhexyl) sebacate (DEHS) disrupt thyroid hormone activity in medaka fish (Horie et al., 2022b; Horie et al., unpublished data); therefore, ATBC may also have thyroid hormone-disrupting effects in fish.

We assessed the adverse effects of ATBC in zebrafish and Japanese medaka (*Oryzias latipes*), which are widely used as model organisms to investigate the biological effects of chemical substances on fish as well as being designated model organisms in the OECD test methods. The endpoints in the current study were as follows: (i) mortality, (ii) embryo development, (iii) growth, and (iv) thyroid-related gene expression.

2. Experimental

2.1. Fish and chemicals

NIES-R strain zebrafish and Japanese medaka were used in this study. The NIES-R strains were supplied by the National Institute for Environmental Studies (Tsukuba, Japan) and has been breed at Kobe University since 2021. The breeding environment conditions were set at 25 ± 2 ˚C with a 16:8 h light:dark period. All experiments with fish were conducted in accordance with the relevant national guidelines (Act on Welfare and Management of Animals, Ministry of the Environment, Japan) and ARRIVE guidelines 2.0, and fish were handled in accordance with the animal care and use guidelines of Kobe University. Furthermore, the approval of the institutional animal care and use committee of the Research Center for Inland Seas, Kobe University was obtained (permission number: 2021–04). ATBC (tributyl *O*-acetyl citrate; CAS No. 77–90–7; purity: >95.0%; water solubility is 20 mg/L) purchased from Fujifilm Wako Pure Chemical Co., Ltd. (Osaka, Japan) was used in the fish exposure experiments.

2.2. Exposure tests

The nominal concentrations used in this study were as follows: 0 (control), 10, 32, 100, 320, and 1000 μg/L of ATBC for zebrafish; 0 (control), 100, 320, 1000, 3200, and 10,000 μg/L of ATBC for Japanese medaka. The highest concentration was set as the concentration at which lethal effects were observed in each species. Exposure tests were performed according to the method of Horie et al. (2022). A flowchart summarizing the experimental procedure is shown in Supplementary Figure 1. Briefly, fertilized eggs were selected after examination under a stereomicroscope, and 20 eggs were exposed to 60 mL of ATBC-spiked tap water in 100 mL glass vessels within 4 h after fertilization (haf). Four replicates were used for each concentration; hence, 80 fertilized eggs were used in total. The exposure solution was changed every 48 h. The exposure period continued until swim bladder inflation was observed, i.e., 5 days for zebrafish [from 4 haf to 2 days post hatching (dph)] and 9 or 11 days (from 4 haf to 1 dph) for Japanese medaka. Immediately after hatching, hatched larvae number were recorded and pooled with randomly, and 50 hatchlings were redistributed into 500 mL glass-beaker. Following exposure, embryo development, thyroid hormone–related gene expression, swim bladder inflation, and total body length were assessed.

Table 1
Nominal and measured concentrations of acetyl tributyl citrate (ATBC).

ATBC concentrations			
Fish species	Nominal (μg/L)	Measured (μg/L)	Standard deviation (μg/L)
Zebrafish	Control	ND	–
	10	14.0	4.9
	32	19.0	4.9
	100	71.1	8.6
	320	194.5	21.9
	1000	443.1	78.9
Japanese medaka	Control	ND	–
	100	74.7	38.1
	320	205.3	108.7
	1000	693.7	128.6
	3200	1937.4	162.2
	10,000	5408.4	412.9

2.3. ATBC chemical analysis

ATBC chemical analysis was performed after the preparation of each exposure concentration. Solid-phase extraction (SPE) was performed using a GL SPE manifold (GL Sciences, Tokyo, Japan). Samples were loaded onto silica-based nonpolar cartridges (InertSep C18 1 g; GL Sciences, Japan) that had been pre-rinsed with 10 mL each of dichloromethane, ethyl acetate, methanol, and ultrapure water. The volume of the sample injected into the column was adjusted to ensure that the amount of ATBC calculated from the prepared concentration was 500 ng. After the sample was applied, the cartridges were rinsed with 10 mL of ultrapure water and dried. The target analytes were then eluted using 10 mL of methanol. Bis-(2-ethylhexyl) phthalate (DEHP) d4 was added (20 µg/L) as the internal standard and injected into the liquid chromatography–mass spectrometry (LC–MS) system. The limits of detection and quantification were 2.9 and 7.6 µg/L, respectively. The measured concentrations of ATBC in tap water and fish exposed to this water are shown in Table 1. The LC–MS operating conditions are provided in Supplementary Table 1.

2.4. Embryo development, swim bladder inflation, and total body length

Embryos were observed every 24 h under a stereomicroscope (SZX 16; Olympus, Tokyo, Japan) equipped with a camera (Visualix V900FL; Visualix, Kobe, Japan), and dead embryos were removed during these observations. Swim bladder inflation and total body length measurements were performed on 50 randomly selected larvae at 2 dph for zebrafish and 1 dph for Japanese medaka. Each larva was anesthetized (MS-222 at a concentration of 200 mg/L) and was photographed under the stereomicroscope and camera described above. Total body length was calculated using ImageJ software.

2.5. Thyroid hormone-related gene expression

Real-time quantitative polymerase chain reaction (RT-qPCR) analysis was performed according to the method as described in Horie et al. (2022). At 2 dph (zebrafish) or 1 dph (Japanese medaka), larvae were placed in RNAlater (Sigma-Aldrich, St. Louis, MO, USA) and stored at 4 ˚C. The next day, total RNA was extracted from each larva using a RNeasy Mini Kit including an on-column RNase-free DNase treatment (Qiagen, Hilden, Germany) with eight larvae used for each of the five concentrations (40 larvae in total). RNA concentrations were measured using a NanoDrop One Microvolume UV–Vis Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). RNA was reverse-transcribed into cDNA using PrimeScript RT Master Mix (Perfect Real Time, Takara, Shiga, Japan), and the cDNA concentration was adjusted to 10 ng/μL. The samples were then stored at −30 ˚C. The primers used in this study for six thyroid-related genes [*thyroid-stimulating hormone*

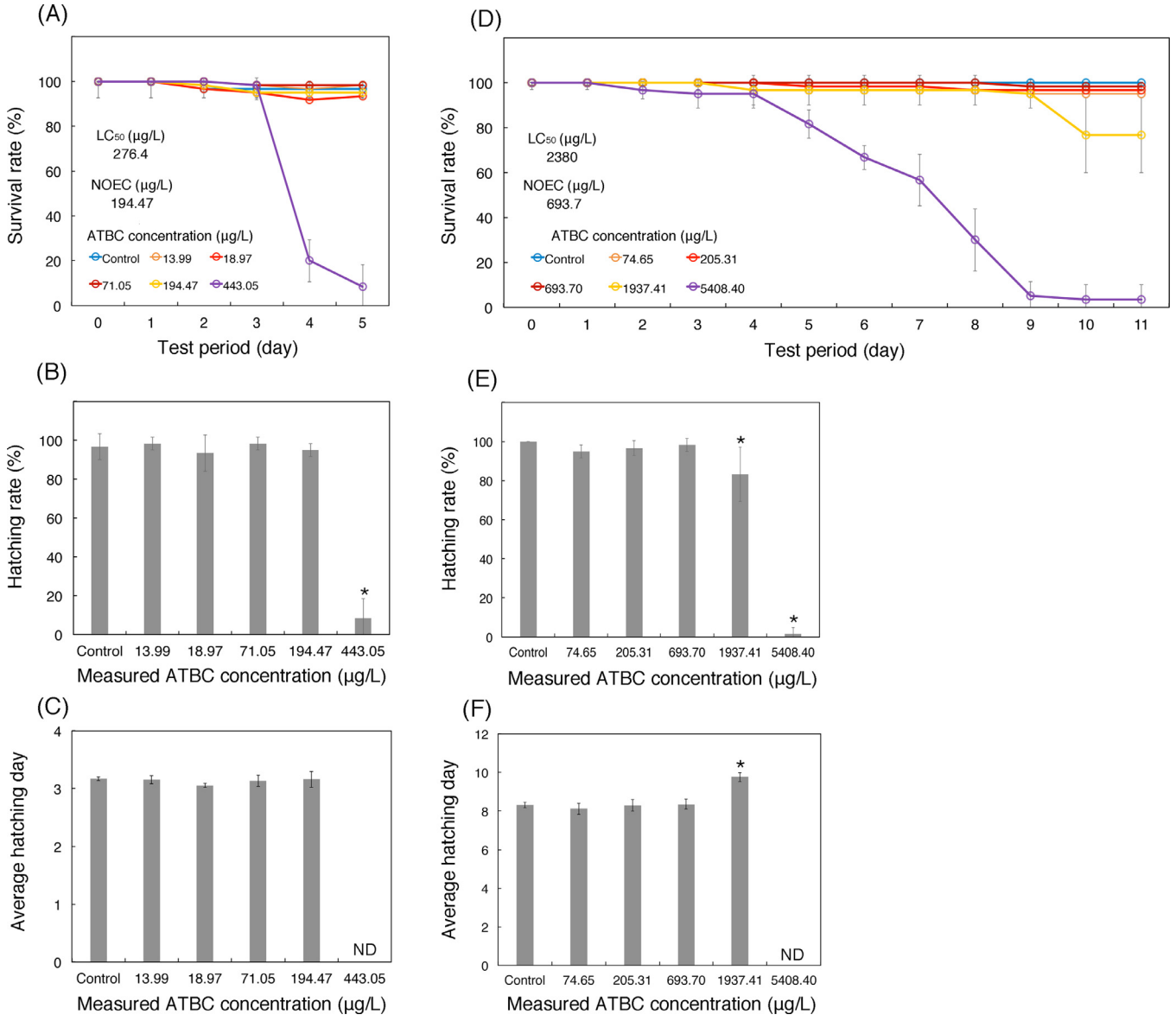

Fig. 1. Effects of acetyl tributyl citrate (ATBC) on the survival rate (A, D) and hatching (B, C, E, F) of zebrafish (A–C) and Japanese medaka (D–F). Error bars show ± 1 SD ($n = 4$). $^{*}P < 0.05$ vs. control (Dunnett's or Steel's test).

beta subunit (*tshβ*), *iodothyronine deiodinase 1* (*dio1*), *dio2, thyroid hormone receptor alpha* (*trα*), and *trβ*] are listed in Supplementary Table 2. RT-qPCR was performed via a LightCycler 96 System (Roche, Basel, Switzerland) using FastStart SYBR Green Master (Nippon Genetics Co., Ltd, Tokyo, Japan). Each reaction mixture (20 μL) contained 10 μL of PCR Master Mix (2×), 0.2 μL of each 20 μM primer, 1 μL of 10 ng/μL cDNA, and 8.6 μL of PCR-grade water. Each sample for each target gene was run in duplicate. The resultant data were analyzed using LightCycler 96 SW 1.1 software (Roche) and exported to Microsoft Excel (Microsoft, Redmond, WA, USA). Transcript levels were normalized to that of the housekeeping gene *beta-actin* (*β-actin*) or *elongation factor 1 alpha* (*ef1α*) using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.6. Statistical analysis

Statistical analyses were performed as reported previously (Horie et al., 2017a). The homogeneity of variance of the data was tested using Bartlett's test (significance level: 5%) via R (http://www.R-project.org/) and the R package Rcmdr (Fox and Bouchet-Valat, 2018). If the homogeneity of variance was not rejected, differences in the test results among treatments were tested using Dunnett's test or Steel's test (Dunnett., 1964; Steel, 1959).

3. Results and discussion

3.1. Effects of ATBC on embryo and larval development

Fig. 1 shows the effects of ATBC on mortality, hatching rate, and hatching day in both zebrafish and Japanese medaka. The hatching rate of zebrafish in the 443.1 μg/L concentration group was significantly lower than that in the control group (Fig. 1B), and individuals started to die from day 4 without hatching (Fig. 1A). All concentration groups except the 443.1 μg/L group hatched on day 3, which was similar to the control group hatching day (Fig. 1C); hatching in the 443.1 μg/L group could not be calculated owing to the small number of hatched individuals (Fig. 1C). Body curvature and edema were observed in the 443.1 μg/L concentration group both at the embryo (Fig. 2A and B) and larval stages (Fig. 2C and E).

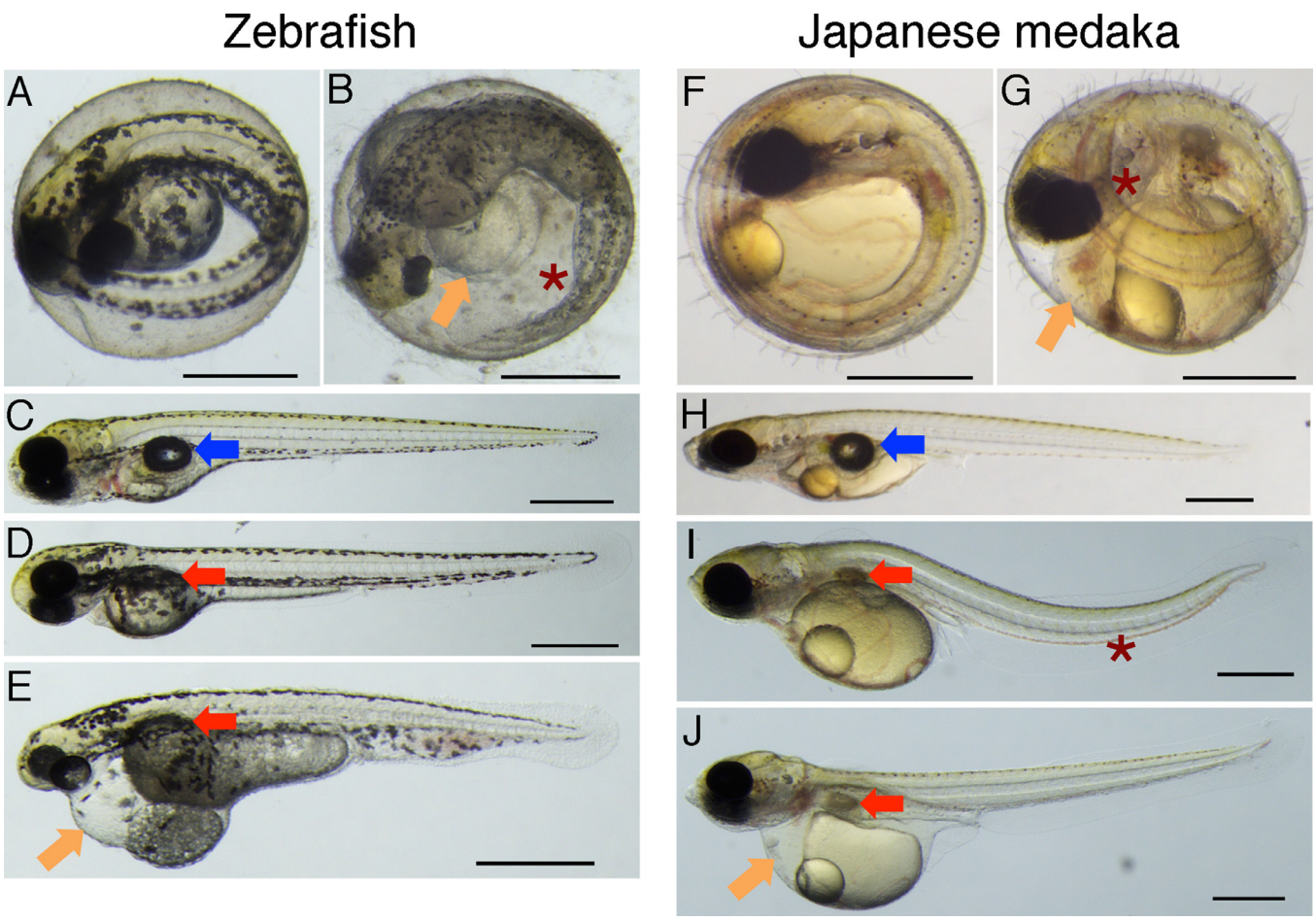

Fig. 2. Photographs showing zebrafish (A–E) and Japanese medaka (F–J) embryos (A, B, F, G) and larvae (C–E, H–J) from the control group (A, C, F, H) and ATBC exposure groups (443.1 μg/L exposure group; B, D, E), (1937.4 μg/L exposure group; G, I, J). Blue and red arrows indicate inflated swim bladders and swim bladders lacking inflation, respectively. Orange arrows indicate edema. Asterisks indicate body curvature. Scale bar=0.5 mm.

The hatching rate of Japanese medaka in the 1937.4 and 5408.4 μg/L concentration groups was significantly lower than that in the control group (Fig. 1E), and individuals started to die from day 5 without hatching in the 5408.4 μg/L group (Fig. 1D). Hatching was delayed in the 1937.4 μg/L concentration group (Fig. 1F), and the hatching day in the 5408.4 μg/L concentration group could not be calculated owing to the small number of hatched individuals (Fig. 1F). Body curvature and edema were observed in the 1937.4 μg/L concentration group at the embryo (Fig. 2F and G) and larval stage (Fig. 2H–J).

Little is known about the acute toxic effects of ATBC on aquatic organisms. Xu and Gye (2018) reported that the 96 h LC_{50} was 13.3 mg/L in *Xenopus laevis* embryos. Bolívar-Subirats et al. (2021) reported that the 48 h EC_{50} was 5.10 mg/L in *Daphnia magna*. The LC_{50} values at the embryo and early larval stage in zebrafish and Japanese medakas were 276.4 μg/L (95% confidence interval: 152.0–502.4 μg/L) and 2380 μg/L (95% confidence interval: 1483.3–3820 μg/L) (Fig. 1A and D). These values were lower than those of *X. laevis* or *D. magna*, indicating that ATBC sensitivity is higher in fish than in amphibians or crustaceans. The reason for increased sensitivity remains unclear through the outcomes of the present study; however, one possible reason is that the exposure period is longer in fish than in amphibians or crustaceans. Thus, zebrafish and Japanese medaka may be appropriate model organisms for the assessment of plasticizer toxicity.

Regarding ecological risk assessment of chemicals, elucidating the no observed effect concentration (NOEC) and the lowest observed effect concentrations (LOEC) of chemicals on aquatic organisms and actual concentrations of chemicals in the aquatic environment is of paramount importance. High level of ATBC contamination was reported from groundwater in England (154 μg/L) (Spurgeon et al., 2022). The LOECs of ATBC in zebrafish obtained in the present study (mortality being 443.1 μg/L) was approximately 2–3 times greater than the detected ATBC levels in the groundwater in England. These results indicated that ATBC may not be affecting fish populations in the aquatic environment. However, in the actual aquatic ecosystem, fish populations currently exist under more severe conditions than under laboratory conditions in terms of sunshine hours, pH, dissolved oxygen, and feed. Therefore, ecological impact concentration of ATBC in the nature environment is possibly lower than the value of LOEC obtained. We need to continue to survey ATBC levels in the aquatic environment.

3.2. *Effects of ATBC on growth and swim bladder inflation*

Fig. 3 shows the effects of ATBC on growth and swim bladder inflation in both zebrafish and Japanese medaka. The swim bladder inflation of zebrafish decreased in a concentration-dependent manner and was significantly lower than the control in the 19.0, 71.1, and 194.5 μg/L concentration groups (Fig. 3A). The swim bladder inflation of Japanese medaka was significantly lower than the control in the 693.7 and 1937.4 μg/L concentration groups (Fig. 3C). ATBC-induced growth inhibition was observed in zebrafish in the 194.5 μg/L concentration group (Fig. 3B). ATBC exposure induced growth inhibition in all concentration groups in Japanese medaka (Fig. 3D).

The effects of thyroid hormone disrupting chemicals (TDCs) have been studied intensively in recent years, motivated partly by the

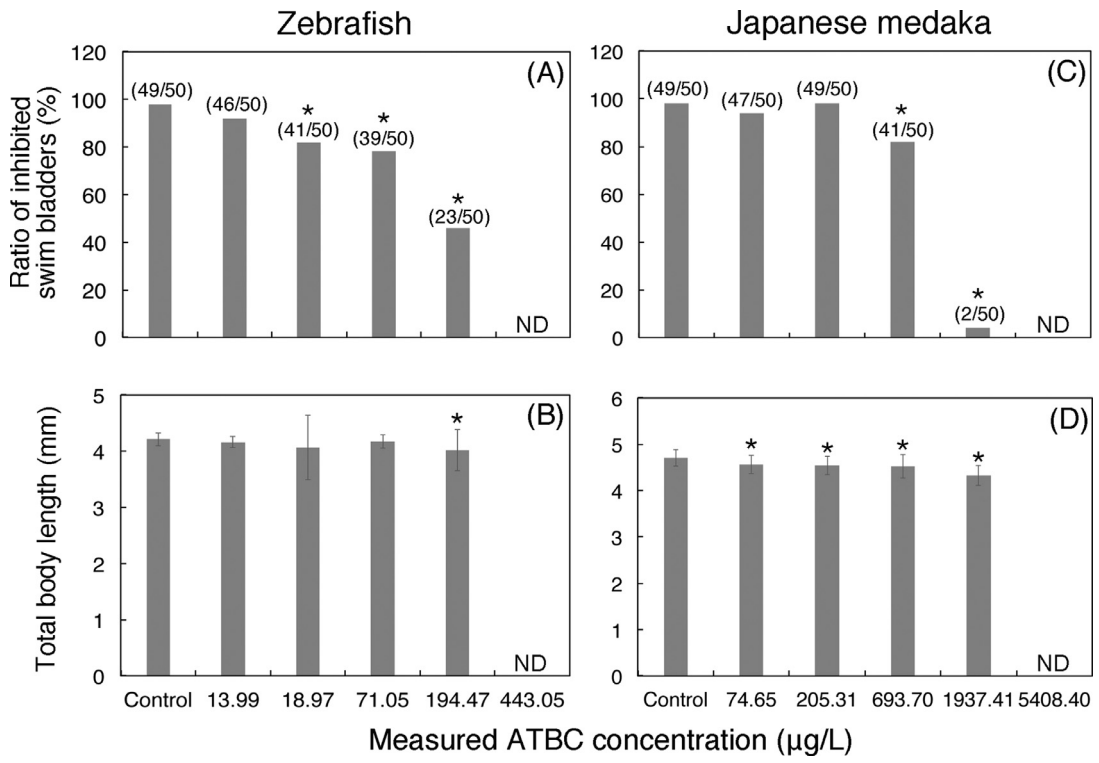

Fig. 3. Effects of ATBC on the swim bladder inflation (A and C) and total body length (B and D) of zebrafish (A and B) and Japanese medaka (C and D). Numbers above each bar indicate the number of individuals with an inflated swim bladder among the 50 individuals examined. In A and B, $^{*}P < 0.05$ vs. control (Chi-squared test). In all other panels, data are means ± SDs ($n = 50$); ND, No data; * $P < 0.05$ vs. control (Dunnett's or Steel's test).

European Union legislation regulating industrial chemicals (Registration, Evaluation, Authorization and Restriction of Chemicals, EC, 1907/2006), plant protection products (Regulation, EC, 1107/2009), and biocide products (Regulation, 528/2012, EC, 2017a). The adverse outcome pathways of TDCs have been reported in zebrafish (Knapen et al., 2020) in the following order: Dio1/Dio2 expression inhibited → T3 levels in serum decreased → swim bladder inflation reduced → swimming performance reduced → population size decreased. In both zebrafish and Japanese medaka, swim bladder inflation and population size were reduced, indicating that ATBC potentially induces thyroid hormone activity. Indeed, Zughaibi et al. (2022) revealed that ATBC showed commonality with the TR*α* native ligand T3 in terms of the interacting amino acid residues in the TR*α* ligand-binding pocket; thus, ligand-complex formation was successful and stable, indicating the potential of thyroid hormone activity. Sheikh and Beg (2021) reported that ATBC bound stably in the TBG substrate–binding pocket and formed a series of important interactions with the amino acids lining the binding pocket, with the majority of the interacting residues showing commonality with those of the native ligand T4, which also indicates the potential of thyroid hormone activity. Thus, this is the first study to show that ATBC disrupts the thyroid hormone system in an in vivo assay with fish.

Phthalate acid esters have been shown to induce abnormal development and skeletal morphogenesis in zebrafish (Pu et al., 2020). In addition, DEHP exposure was shown to induce growth inhibition in Japanese medaka (Yang et al., 2018) and guppy (Zanotelli et al., 2010). The present study is the first to show that ATBC exposure induces abnormal embryo development, skeletal morphogenesis, and growth inhibition in zebrafish (194.5 *μ*g/L) and Japanese medaka (1937.4 *μ*g/L) (Fig. 2). Our research group revealed previously that such sublethal effects (abnormal embryo development, skeletal morphogenesis, and the lack of swim bladder inflation) ultimately lead to death (Horie et al., 2017a, b); therefore, it is expected that ATBC is a lethal hazard risk even at concentrations of 194.5 and 1937.4 *μ*g/L in zebrafish and Japanese medaka, respectively.

3.3. Effects of ATBC on thyroid-related gene expression

Fig. 4 shows the effects of ATBC on the expression levels of *tshβ, dio1, dio2, trα*, and *trβ* in both zebrafish and Japanese medaka. The expression of *tshβ* in zebrafish was significantly suppressed in the 19.0 and 194.5 *μ*g/L concentration group, *trα* expression levels was significantly suppressed in the 194.5 *μ*g/L group, and *dio1* and *dio2* expression levels were significantly suppressed in the 194.5 *μ*g/L group and in the 71.1 and 194.5 *μ*g/L groups, respectively. The expression of *trβ* was not altered by ATBC exposure.

In Japanese medaka, the expression of *tshβ* and *dio1* was not changed by ATBC exposure. In contrast, the expression of *dio2* was suppressed significantly in the 74.7, 205.3, and 693.7 *μ*g/L concentration groups. The expression of *trα* was significantly suppressed at all ATBC concentration levels. The expression of *trβ* was significantly suppressed in the 74.7, 693.7, and 1937.4 *μ*g/L concentration groups.

ATBC exposure suppressed the thyroid-related gene expression in both zebrafish and Japanese medaka. Jia et al. (2016) reported an increase in the expression of *tshβ* and *dio2* but not *dio1, trα*, and *trβ* in zebrafish exposed to the phthalate plasticizer DEHP. Mono-(2-ethylhexyl) phthalate is also known to increase *tshβ, dio1*, and *dio2* expression levels in zebrafish (Zhai et al., 2014). However, our research group has found that exposure to the non-phthalate plasticizer bis-(2-ethylhexyl) adipate suppresses the expression of *dio2* in Japanese medaka (Horie et al., 2022b) and exposure to bis-(2-ethylhexyl) sebacate suppresses the expression of *tshβ, dio2,* and *trβ* in the same species (Horie et al., 2022, unpublished data). ATBC exposure suppressed the expression of *tshβ, dio1, dio2, trα*, and *trβ* in zebrafish and/or Japanese medaka. Phthalate and non-phthalate plasticizers tend to increase and decrease the expression levels of thyroid-related genes, respectively, and their chemical structures are different. In future studies, other phthalate and non-phthalate plasticizers should be tested to clarify the relationship between chemical structures and thyroid-related gene expression.

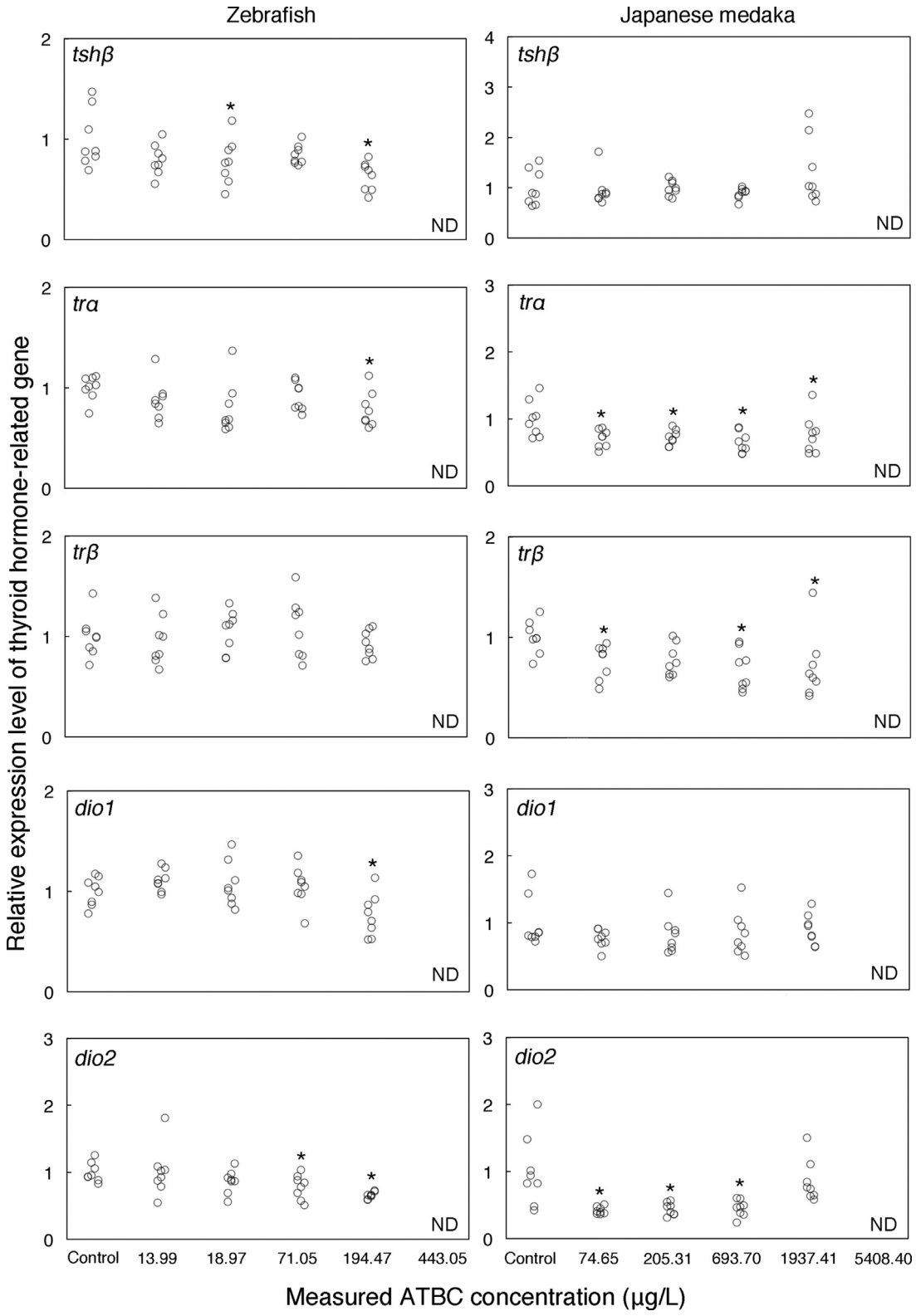

Fig. 4. Effects of ATBC on the expression levels of *tshβ, dio1, dio2, trα*, and *trβ* in zebrafish (left panel) and Japanese medaka (right panel) according to real-time quantitative PCR analysis. The expression levels of *tshβ*-like, *tshβ, dio1, dio2, trα*, and *trβ* were normalized to that of *β-actin* (for zebrafish) or *ef1α* (for Japanese medaka) as housekeeping genes and to the control. ND, No data; $^{*}P < 0.05$ vs. control (Dunnett's or Steel's test).

The effects of ATBC exposure on *tshβ, dio1*, and *trβ* expression differed between zebrafish and Japanese medaka. Some of the effects of TDCs on thyroid-related gene expression have been reported previously. For example, heptafluorobutanoic acid exposure induced the upregulation of *trβ* expression in zebrafish but not in Japanese medaka (Horie et al., 2022a). In addition, tris(1,3-dichloro-2-propyl) phosphate exposure induced the downregulation of *trα* expression and the upregulation of *tshβ* and *trβ* expression in zebrafish (Liu et al., 2019) but not in Japanese medaka (Horie et al., 2022a). Even when the chemical, model organism (zebrafish), and exposure period are the same, TDCs can have different effects on thyroid-related gene expression. For instance, tetrabromobisphenol A exposure induced the upregulation and downregulation of *tshβ* and *trβ* expression, respectively, in one study (Zhu et al., 2018), whereas the expression levels of *tshβ* and *trβ* did not differ in another study (Baumann et al., 2016). In addition, propylthiouracil exposure induced the upregulation of *dio2* expression in one study (Baumann et al., 2016), but no difference in *dio2* expression was observed in another study (Liu et al., 2013). It is not clear from the present results why these responses differ, but the same phenomenon occurs in other cases. Investigating whether the same phenomenon occurs at the blood thyroid hormone levels is necessary.

The adverse outcome pathway framework, consisting of a molecular initiating event, key event, and adverse outcome, is well suited to the development of tiered testing approaches that seek to provide evidence for the association between perturbations of a toxicological pathway and downstream responses. Noyes et al. (2019) and Knapen et al. (2020) summarized the adverse outcome pathway framework for TDCs in fishes, showing that the inhibition of thyroid-related genes (molecular initiating event), leads to the improper inflation of the swim bladder (key event), which in turn leads to reduced swimming performance and/or increased mortality (adverse outcome) after TDC exposure. This framework is largely consistent with the present results on zebrafish and Japanese medaka exposed to ATBC, i.e., *tshβ, dio1, dio2, trα*, and *trβ* expression was inhibited in zebrafish and/or Japanese medaka, the swim bladders of these species were improperly inflated, and mortality levels increased in both tested species.

4. Conclusions

Here, we confirmed that ATBC has endocrine-disrupting activity and lethal toxicity in zebrafish and Japanese medaka. First, ATBC exposure induced lethal effects in both fish species, this was despite the fact that although the value of LOEC for mortality is higher than the actual concentrations of ATBC in the aquatic environment. ATBC exposure suppressed thyroid-related gene expression and led to a lack of swim bladder inflation, indicating that ATBC has the potential for thyroid hormone-disrupting activity in fish. This is possibly the first report of a nonphthalate ATBC plasticizer inducing abnormal embryo development and disrupting thyroid hormone activity in fish.

Funding information

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Declaration of Competing Interest

The authors have no conflicts of interest related to this research.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hazadv.2022.100199.

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