

Waterborne Exposure to Avobenzonone and Octinoxate Induces Thyroid Endocrine Disruption in Wild-Type and *Thraa*^{-/-} Zebrafish Larvae

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Research Article

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Abstract

Avobenzene and octinoxate are frequently used as organic ultraviolet filters, and these chemicals are widely detected in water. This study evaluated the potential of avobenzene and octinoxate to disrupt thyroid endocrine system in wild-type and thyroid hormone receptor alpha a knockout (*thraa*^{-/-}) zebrafish embryos/larvae. Following a 120 h exposure to various concentrations of avobenzene and octinoxate, larvae mortality and developmental toxicity in wild-type and *thraa*^{-/-} fish were assessed. Triiodothyronine (T3) and thyroxine (T4) levels as well as transcriptional levels of ten genes associated with the hypothalamus-pituitary-thyroid (HPT) axis were measured in wild-type fish. Significantly lower larvae survival rate in *thraa*^{-/-} fish exposed to $\geq 3 \mu\text{M}$ avobenzene and octinoxate suggests that the thyroid hormone receptor plays a crucial role in the toxic effects of avobenzene and octinoxate. A significant increase in the *deio2* gene level in avobenzene-exposed zebrafish supports the result of an increased ratio of T3 to T4. Significant decrease of T4 level with upregulation of *trh*, *tsh β* , and *tshr* genes indicates feedback in the hypothalamus and pituitary gland to maintain hormonal homeostasis. Our observation indicates that exposure to avobenzene and octinoxate affects the feedback mechanisms of the HPT axis.

1. Introduction

Organic and inorganic ultraviolet (UV) filters are a group of substances that either absorb or reflect UV light, preventing it from penetrating the skin (Serpone et al. 2007). The United States Food and Drug Administration has classified 22 UV filter compounds, used in sunscreen products, as Generally Recognized As Safe and Effective (GRASE) (category I), those that are not GRASE (category II), and those that do not have sufficient data to support a positive GRASE determination (category III) (US Food and Drug Administration 2019). Avobenzene (also known as butyl methoxydibenzoylmethane) and octinoxate (also known as octyl methoxycinnamate or ethylhexyl methoxycinnamate) are representative components of organic UV filters (Bratkovics et al. 2015), and are classified as category III GRASE (US Food and Drug Administration 2019). As they are often used in sunscreen products, avobenzene and octinoxate are frequently introduced into water (da Silva et al. 2022). Direct release into the marine environment may occur via recreational water activities during swimming and bathing (Labille et al. 2020). Indirect release may occur via wastewater treatment plants (WWTPs) as a result of showering and washing (Poiger et al. 2004).

Avobenzene and octinoxate are frequently detected in the aqueous environment (Ekpeghere et al. 2016; Kameda et al. 2011; Tsui et al. 2014, 2019), sediment (Sun et al. 2021), and biota (Fent et al. 2010; Peng et al. 2017). In surface waters of Hong Kong, Tokyo, New York, Los Angeles, Shantou, Chaozhou, and Bangkok, avobenzene and octinoxate have been detected at 1.37-721 ng/L and 3.84-4043 ng/L, respectively (Tsui et al. 2014, 2019). Octinoxate was detected in streams (21-260 ng/L), heavily polluted rivers (125-1040 ng/L), and sediments (2.0-101 $\mu\text{g}/\text{kg}$) (Kameda et al. 2011). In the sediments of Yalongchangpo river in China, avobenzene was detected at a concentration of 4.13-33.48 ng/g (Sun et al. 2021). In macroinvertebrate and fish samples collected from Swiss rivers, octinoxate occurred up to 337 ng/g in lipids, suggesting bioaccumulation along the food chain (Fent et al. 2010). Octinoxate was the

most abundant in three rivers, five sewage treatment plants, and four WWTPs in Korea (Ekpeghere et al. 2016).

Several studies have explored the toxic effects of avobenzene and octinoxate related to oxidative stress (Nataraj et al. 2020) and estrogenic/androgenic effects (Zhou et al. 2019). Avobenzene was confirmed to have thyroid hormone-like activity in GH3-TRE-Luc cells (Klopčič and Dolenc 2017). Production levels of triiodothyronine (T3) and thyroxine (T4), and transcription levels of genes related to type II deiodinase (*deio2*) were significantly reduced in Japanese medaka exposed to octinoxate (Lee et al. 2019). Treatment with octinoxate caused a decrease of serum thyroid hormone levels in Sprague-Dawley rats (Klammer et al. 2007; Schmutzler et al. 2004). In a recent study, significant decreases of T3 and T4 levels were reported in zebrafish larvae exposed to octinoxate for 120 h (Chu et al. 2021).

T3 and T4 are essential in promoting embryonic development and growth (Walter et al. 2019). Thyroid function depends on synthesis and transport of thyroid hormones, deiodination, iodine uptake, and ability to bind thyroid hormone receptors (Visser 2018). When the levels of T3 and T4 are insufficient, the hypothalamus and pituitary secrete thyrotropin-releasing hormone (TRH) and thyroid stimulating hormone (TSH), respectively (Song et al. 2021). If any of the hormones and enzymes located on the hypothalamus-pituitary-thyroid (HPT) axis are affected by chemical exposure, various effects can be induced at the individual level. Disruption of thyroid hormone homeostasis and developmental toxicity have been studied in fish exposed to several organic UV filters, including benzophenones (BPs; Lee et al. 2018), 4-methylbenzylidene camphor (Quintaneiro et al. 2019), and octinoxate (Chu et al. 2021; Klammer et al. 2007; Lee et al. 2019; Schmutzler et al. 2004). However, information on thyroid disruption by avobenzene is limited, and it is difficult to interpret toxic effects from a holistic point of view using *in vitro* cell experiments.

In the present study, the effects of avobenzene and octinoxate on zebrafish development and thyroid endocrine system were investigated at the organism-, hormonal-, and genetic-levels. By comparing the mortality rates between wild-type and thyroid hormone receptor alpha a knockout (*thraa^{-/-}*) zebrafish larvae, the contribution of both substances to the binding of thyroid hormone receptor was assessed. To fill the knowledge gap on the mechanistic basis of toxicity, the levels of two thyroid hormones and the transcription of HPT axis genes were examined in wild-type zebrafish. The results will provide integrated information elucidating the mechanism of developmental toxicity of avobenzene and octinoxate based on transcriptional and hormonal changes.

2. Materials And Methods

2.1. Chemical information

Avobenzene (CAS no. 70356-09-1) and octinoxate (CAS no. 5466-77-3) were purchased from Merck KGaA (Darmstadt, Germany). Avobenzene and octinoxate were separately dissolved in dimethyl sulfoxide at a

ratio of 0.01% to prepare both stock solutions (Junsei Chemical Co., Tokyo, Japan). The working solution was prepared by diluting the stock solution with culture medium.

2.2. Zebrafish in-house culture and chemical exposure

Wild-type (AB strain) and *thraa*^{-/-} zebrafish were maintained in a flow-through culture device (ZebTEC, Buguggiate, Italy) installed in a constant temperature room at 26 ± 1 °C under a 14 h light/10 h dark photoperiod. Adult zebrafish were fed live brine shrimp twice a day.

Embryos (wild-type and *thraa*^{-/-}) were collected from adult zebrafish pairs. Healthy embryos selected within 2 h after fertilization were randomly placed in each well of 96-well plates containing 100 μ L working solution. Ninety-six embryos were exposed to avobenzone and octinoxate (control, solvent control, 0.3, 1, 3, 10, and 30 μ M) for 120 h. The exposure concentration was selected based on the preliminary range-finding test and previous studies (Chu et al. 2021; Lee et al. 2019). At least three independent experiments were conducted to obtain individual data. Dead embryo/larvae were recorded and removed daily. Embryo coagulation, hatching, malformation, and larvae mortality were observed every 24 h. After the exposure was terminated, body length (ten larvae per replicate, n=30) and wet weight (ten larvae pooling per replicate, n=3) were measured. Ten larvae with three replicates were collected to measure changes of gene transcription and stored at -80°C until further analysis.

2.3. Measurement of thyroid hormone level

Additional experiments were performed to measure thyroid hormone levels. Three replicate groups of 250 wild-type embryos per concentration group were exposed to the test substance for 120 h, and 150 larvae per each replicate were collected after the exposure was terminated. Homogenized larvae samples were used for hormone measurement. The levels of T3 (Cat No. OKCA00348) and T4 (Cat No. OKCA00349) were analyzed using enzyme-linked immunosorbent assay kits (Aviva System Biology, San Diego, CA, USA) according to the manufacturer's recommendations. To assess the hormonal balance, the ratio of T3/T4 was calculated and normalized to the solvent control group.

2.4. Gene transcription analysis

Wild-type zebrafish larvae collected at 120 h of experiment (ten fish in triplicate per each treatment group) were used for gene transcription analysis. Ten genes associated with the HPT axis (Table S1 in supplementary data) were analyzed using quantitative real-time polymerase chain reaction (qRT-PCR). Larvae samples were homogenized and messenger RNA (mRNA) was extracted from the supernatant. Extraction of mRNA and synthesis of complementary DNA (cDNA) were conducted using the RNeasy mini kit (QIAGEN, Hilden, Germany) and iScriptTM cDNA Synthesis kit (BIORAD, Hercules, CA, USA), respectively. qRT-PCR was performed using the ABI 7500 fast real-time PCR system (Applied Biosystems, Foster City, CA, USA) after adding the reaction mixture consisting of SYBR PCR master mix (Applied Biosystems), primer, and diluted cDNA to each well of a 96-well plate. The PCR program was 50°C for 1 min, 95°C for 10 min, and 40 cycles of 95°C for 15 s and 60°C for 1 min. The procedure permitted the relative quantification of transcriptional changes (Livak and Schmittgen 2001). Threshold cycle (Ct) of each gene

was normalized based on the two reference genes (*tuba1* and *18sRNA*), and then these values from exposure group were normalized to the solvent control group.

2.5. Statistical analyses

Independent t-test compared the survival of wild-type and *thraa*^{-/-} zebrafish. For the endpoints observed at the organism, hormonal, and genetic level from wild-type zebrafish embryo/larvae and the significance of differences between solvent control and treatment groups was assessed by one-way analysis of variance using SPSS software (version 27, IBM Corp., Armonk, NY, USA). Correlation between various endpoints were assessed using Spearman correlation analysis from the SAS program (SAS Institute, Cary, NC, USA).

3. Results

3.1. Organism level toxicity in wild-type zebrafish

In wild-type fish, the coagulation of embryos exposed to 3 μ M or more of avobenzonone was significantly increased compared to the solvent control group (Figure 1A). Fish exposed to ≥ 3 μ M avobenzonone displayed significantly decreased hatchability, which in turn led to decreased larval survival (Figure 1A). In addition, increased malformation and decreased larvae weight were observed in wild-type fish exposed to ≥ 10 μ M avobenzonone (Figure 1A). In wild-type fish exposed to 30 μ M avobenzonone, hatching time was significantly delayed (Figure 1A). Avobenzonone did not remarkably affect larvae length compared to the solvent control group (Figure 1A).

Octinoxate induced noteworthy effects on embryo coagulation, hatchability, larvae survival, and malformation in wild-type fish exposed to ≥ 10 μ M (Figure 1B). Delayed hatching time was also observed in wild-type fish exposed to 30 μ M octinoxate (Figure 1B). Larvae length and weight were decreased in a dose-response manner, but the effects were not statistically significant (Figure 1B).

3.2. Hormone level toxicity in wild-type zebrafish

In wild-type larvae fish exposed to avobenzonone, a significant increase of T3 and significant decrease of T4 was observed at a concentration of 30 μ M and ≥ 10 μ M, respectively (Figure 2A). T3 and T4 concentrations of zebrafish larvae exposed to 30 μ M octinoxate for 120 h were significantly decreased compared to the solvent control groups (Figure 2B). The ratio of T3/T4 was significantly increased in wild-type fish exposed to 30 μ M avobenzonone (Figure 2A). However, no significant differences between groups were observed in fish exposed to octinoxate (Figure 2B).

3.3. Transcription level toxicity in wild-type zebrafish

Following the exposure to avobenzonone, significant upregulation of *trh*, *tsh β* , *tshr*, *tg*, and *deio2* genes and downregulation of *traa*, *tr β* , and *tpo* genes were observed in wild-type zebrafish larvae (Figure 3A). In relation to octinoxate, transcriptions of *trh*, *tsh β* , *tshr*, *tg*, *nis*, and *deio2* genes were significantly upregulated, while transcriptions of *traa*, *tr β* , and *tpo* genes were significantly downregulated (Figure 3B).

3.4. Comparison of survival between wild-type and *thraa*^{-/-} zebrafish

Toxicities of avobenzone and octinoxate were greater in *thraa*^{-/-} fish than in wild-type fish (Figure 1A and 1B). The extent of increase in embryo coagulation and decrease in hatchability and larval survival were greater in *thraa*^{-/-} fish than in wild-type fish at $\geq 3 \mu\text{M}$ avobenzone (Figure 1A). For octinoxate, embryo coagulation, time to hatch, hatchability, and larvae survival were significantly different between wild-type and *thraa*^{-/-} fish (Figure 1B).

3.5. Relationship between endpoints

Results of correlation analysis between organism level endpoints of survival, length, and weight; hormone level endpoints of T3, T4, and T3/T4 ratio; and gene level endpoints of ten genes related to the HPT axis are shown in Table S2 and S3 (Supplementary data). In larvae fish exposed to avobenzone, weight was positively related to survival, T4 content, and transcriptional changes of *traa*, *tr β* , *tpo*, and *nis* genes, while weight was negatively related to the T3 content, T3/T4 ratio, and transcriptional changes of *trh*, *tshr*, *tg*, and *deio2* genes. After exposure to octinoxate in zebrafish larvae, length was positively related to survival, production of T3 and T4, and transcriptional changes of *traa* and *tr β* genes, while length was negatively related to the *trh*, *tsh β* , *tshr*, *tg*, *nis*, and *deio2* genes.

4. Discussion

Avobenzone and octinoxate have received much attention due to their environmental abundance and potential toxicity. In the present study, avobenzone and octinoxate altered thyroid hormone levels and changed the transcription levels of genes associated with the HPT axis. This is the first study using *thraa*^{-/-} zebrafish to elucidate that these two UV filters interfere with the binding of thyroid hormone receptors, resulting in thyroid endocrine disruption. More importantly, thyroid endocrine disruption induced by avobenzone and octinoxate ultimately delayed hatching and decreased larval weight.

The ratio of the T3 and T4 thyroid hormones is important to maintain homeostasis (Eales 2019). In the present study, avobenzone exposure ultimately increased the T3/T4 ratio. This was attributed to a decrease in T4 and an increase in T3 levels. Avobenzone belongs to the BP group along with deoxybenzone, sulisobenzene (also known as BP-4), and oxybenzone (also known as BP-3) (Kullavanijaya and Lim 2005). The changes in the levels of thyroid hormones, such as the decrease in T4 level, are consistent with the effects of other BPs, including BP-1, BP-3, and BP-8 (Lee et al. 2018). Upregulation of the *deio2* gene in larvae exposed to avobenzone may also contribute to the increase in T3/T4 ratio. T4 can be converted to T3 through outer ring deiodination of deiodinase type I (*deio1*) and II (*deio2*) (Walpita et al. 2009) or the glucuronidation enzymes *ugt1ab* (Parsons et al. 2020). Upregulated *deio2* transcription due to avobenzone exposure may enhance secretion of T3 and consequently increase the T3/T4 ratio. Although no significant difference in the T3/T4 ratio was observed in larvae exposed to octinoxate, the decrease in T4 level could be supported in part by a significant upregulation of the *deio2*

gene. Previous studies also reported that octinoxate upregulates transcription of the *deio2* gene and decreases T4 level, which supports our findings (Chu et al. 2021; Lee et al. 2019).

TRH secreted from the hypothalamus stimulate the secretion of TSH from the pituitary, which subsequently stimulates the synthesis and secretion of thyroid hormones from the thyroid gland (Zhang et al. 2018). In the present study, a significant increase in the transcription levels of *trh*, *tsh β* , and *tshr* genes was observed in fish exposed to avobenzene and octinoxate. These data suggest that avobenzene and octinoxate may affect TRH and TSH directly or indirectly by negative feedback responses to produce more T4. The results of correlation analysis also support the essential roles of the transcription of the *trh*, *tsh β* , and *tshr* genes in thyroid hormone regulation. The upregulation of *tsh β* and *tshr* genes by exposure to avobenzene and octinoxate observed in this study is similar to that of previous studies that reported elevation of TSH-related genes by BPs and octinoxate (Chu et al. 2021; Lee et al. 2018).

Thyroid hormones function by binding to their corresponding receptors (*traa* or *tr β*) (Deal and Volkoff 2020). Especially, the highly bioactive T3 hormone binds to the corresponding receptor, moves to the target tissue, and induces the intended effects. Therefore, downregulation of the *traa* and *tr β* genes in fish larvae exposed to avobenzene and octinoxate may also suggest possible activities to inhibit thyroid hormone receptor binding. The observation of higher mortality in *thraa*^{-/-} than in wild-type fish is interesting, given that BPs and octinoxate have been reported to inhibit receptor binding capacity in previous studies (Chu et al. 2021; Lee et al. 2018). Our observation also supports the central role of the thyroid hormone receptor in the toxicity of avobenzene and octinoxate.

Thyroid peroxidase (*tpo*) is an enzyme that attaches iodine to thyroglobulin (*tg*), an important protein in the production of thyroid hormones (Nishihara et al. 2017). Decreased transcription of the *tpo* gene after avobenzene and octinoxate exposure indicates that these substances inhibit *tpo* activity in a manner similar to BP-2, thereby reducing thyroid hormone production (Lee et al. 2018). The sodium iodide symporter (*nis*) transports iodide (essential for thyroid hormone production) across the thyroid epithelium (Holloway et al. 2021). The results of upregulation of the *nis* gene in zebrafish larvae exposed to octinoxate are consistent with the results of other studies (Chu et al. 2021).

Overall, decrease in T4 contents induced by avobenzene and octinoxate exposure is potentially associated with genes along with the HPT axis, which eventually affects development. In addition, these two UV filters could affect survival or development of zebrafish larvae by interfering with the binding to *traa*, which provides clues to the contribution of both substances to the binding of thyroid hormone receptors. Our observation of possible thyroid hormone perturbation in larvae shows that some UV filters may have detrimental consequences for aquatic organisms. Although developmental delay and thyroid endocrine disturbance were observed in fish exposed to avobenzene and octinoxate, levels of detection in the environment were relatively lower than those associated with effects measured in this study. Given the importance of thyroid hormone homeostasis in early development, and the results based on a short-term exposure of 120 h, the effects of long-term exposure on thyroid function should be further investigated.

Declarations

Acknowledgement

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Data availability statements

All data generated or analyzed during this study are included in this published article and its supplementary data files.

Animal research (ethics)

This study was approved by the Institutional Animal Care and Use Committee of Yongin University, Korea (YUIACUC-2021-7).

Consent to participate (ethics)

Not applicable.

Consent to publish (ethics)

Not applicable.

Plant reproducibility

Not applicable.

Clinical trials registration

Not applicable.

Author contributions statement

All authors contributed to the study conception and design. Material preparation, data collection and analysis, and writing the first draft of the manuscript were performed by Yujin Ka. Editing on previous version of the manuscript was conducted by Kyunghee Ji. All authors read and approved the final manuscript.

Declaration of interest

There is no conflict of interest associated with this work.

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References

1. Bratkovics S, Wirth E, Sapozhnikova Y, Pennington P, Sanger D (2015) Baseline monitoring of organic sunscreen compounds along South Carolina's coastal marine environment. *Mar Pollut Bull* 101(1):370–377. <https://doi.org/10.1016/j.marpolbul.2015.10.015>
2. Chu S, Kwon BR, Lee YM, Zho KD, Choi K (2021) Effects of 2-ethylhexyl-4-methoxycinnamate (EHMC) on thyroid hormones and genes associated with thyroid, neurotoxic, and nephrotoxic responses in adult and larval zebrafish (*Danio rerio*). *Chemosphere* 263:128176. <https://doi.org/10.1016/j.chemosphere.2020.128176>
3. da Silva ACP, Santos BAMC, Castro HC, Rodrigues CR (2022) Ethylhexyl methoxycinnamate and butyl methoxydibenzoylmethane: toxicological effects on marine biota and human concerns. *J Appl Toxicol* 42(1):73–86. <https://doi.org/10.1002/jat.4210>
4. Eales JG (2019) The relationship between ingested thyroid hormones, thyroid homeostasis and iodine metabolism in humans and teleost fish. *Gen Comp Endocrinol* 280:62–72. <https://doi.org/10.1016/j.ygcen.2019.04.012>
5. Ekpeghere KI, Kim UJ, O SH, Kim HY, Oh JE (2016) Distribution and seasonal occurrence of UV filters in rivers and wastewater treatment plants in Korea. *Sci Total Environ* 542:121–128. <https://doi.org/10.1016/j.scitotenv.2015.10.033>
6. Holloway N, Riley B, MacKenzie DS (2021) Expression of the sodium iodide symporter (NIS) in reproductive and neural tissues of teleost fish. *Gen Comp Endocrinol* 300:113632. <https://doi.org/10.1016/j.ygcen.2020.113632>
7. Fent K, Zenker A, Rapp M (2010) Widespread occurrence of estrogenic UV-filters in aquatic ecosystems in Switzerland. *Environ Pollut* 158:1817–1824. <https://doi.org/10.1016/j.envpol.2009.11.005>
8. Kameda Y, Kimura K, Miyazaki M (2011) Occurrence and profiles of organic sun-blocking agents in surface waters and sediments in Japanese rivers and lakes. *Environ Pollut* 159:1570–1576. <https://doi.org/10.1016/j.envpol.2011.02.055>
9. Klammer H, Schlecht C, Wuttke W, Schmutzler C, Gotthardt I, Köhrle J, Jarry H (2007) Effects of a 5-day treatment with the UV-filter octyl-methoxycinnamate (OMC) on the function of the hypothalamo-pituitary-thyroid function in rats. *Toxicology* 238:192–199. <https://doi.org/10.1016/j.tox.2007.06.088>
10. Klopčič I, Dolenc MS (2017) Endocrine activity of AVB, 2MR, BHA, and their mixtures. *Toxicol Sci* 156(1):240–251. <https://doi.org/10.1093/toxsci/kfw253>
11. Kullavanijaya P, Lim HW (2005) Photoprotection. *J Am Acad Dermatol* 52:937–958. <https://doi.org/10.1016/j.jaad.2004.07.063>

12. Labille J, Slomberg D, Catalano R, Robert S, Apers-Tremelo ML, Boudenne JL, Manasfi T, Radakovitch O (2020) Assessing UV filter inputs into beach waters during recreational activity: a field study of three French Mediterranean beaches from consumer survey to water analysis. *Sci Total Environ* 706:136010. <https://doi.org/10.1016/j.scitotenv.2019.136010>
13. Lee I, Lee J, Jung D, Kim S, Choi K (2019) Two-generation exposure to 2-ethylhexyl 4-methoxycinnamate (EHMC) in Japanese medaka (*Oryzias latipes*) and its reproduction and endocrine related effects. *Chemosphere* 228:478–484. <https://doi.org/10.1016/j.chemosphere.2019.04.123>
14. Lee J, Kim S, Park YJ, Moon HB, Choi K (2018) Thyroid hormone-disrupting potentials of major benzophenones in two cell lines (GH3 and FRTL-5) and embryo-larval zebrafish. *Environ Sci Technol* 52(15):8858–8865. <https://doi.org/10.1021/acs.est.8b01796>
15. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. *Methods* 25(4):402–408. <https://doi.org/10.1006/meth.2001.1262>
16. Nataraj B, Maharajan K, Hemalatha D, Rangasamy B, Arul N, Ramesh M (2020) Comparative toxicity of UV-filter octyl methoxycinnamate and its photoproducts on zebrafish development. *Sci Total Environ* 718:134546. <https://doi.org/10.1016/j.scitotenv.2019.134546>
17. Nishihara E, Amino N, Kudo T, Ito M, Fukata S, Nishikawa M, Nakamura H, Miyauchi A (2017) Comparison of thyroglobulin and thyroid peroxidase antibodies measured by five different kits in autoimmune thyroid diseases. *Endocr J* 64(10):955–961
18. Parsons AE, Lange A, Hutchinson TH, Miyagawa S, Iguchi T, Kudoh T, Tyler CR (2020) Expression dynamics of genes in the hypothalamic-pituitary-thyroid (HPT) cascade and their responses to 3,3',5-triiodo-1-thyronine (T3) highlights potential vulnerability to thyroid-disrupting chemicals in zebrafish (*Danio rerio*) embryo-larvae. *Aquat Toxicol* 225:105547. <https://doi.org/10.1016/j.aquatox.2020.105547>
19. Peng X, Fan Y, Jin J, Xiong S, Liu J, Tang C (2017) Bioaccumulation and biomagnification of ultraviolet absorbents in marine wildlife of the Pearl River Estuarine, South China Sea. *Environ Pollut* 225:55–65. <https://doi.org/10.1016/j.envpol.2017.03.035>
20. Poiger T, Buser HR, Balmer ME, Bergqvist PA, Müller MD (2004) Occurrence of UV filter compounds from sunscreens in surface waters: regional mass balance in two Swiss lakes. *Chemosphere* 55(7):951–963. <https://doi.org/10.1016/j.chemosphere.2004.01.012>
21. Quintaneiro C, Teixeira B, Benedé JL, Chisvert A, Soares AMVM, Monteiro MS (2019) Toxicity effects of the organic UV-filter 4-methylbenzylidene camphor in zebrafish embryos. *Chemosphere* 218:273–281. <https://doi.org/10.1016/j.chemosphere.2018.11.096>
22. Schmutzler C, Hamann I, Hofmann PJ, Kovacs G, Stemmler L, Mentrup B, Schomburg L, Ambrügger P, Grüters A, Seidlova-Wuttke D, Jarry H, Wuttke W, Köhrle J (2004) Endocrine active compounds affect thyrotropin and thyroid hormone levels in serum as well as endpoints of thyroid hormone action in liver, heart and kidney. *Toxicology* 205:95–102. <https://doi.org/10.1016/j.tox.2004.06.041>

23. Serpone N, Dondi D, Albini A (2007) Inorganic and organic UV filters: their role and efficacy in sunscreens and skincare products. *Inorg Chim Acta* 360:794–802. <https://doi.org/10.1016/j.ica.2005.12.057>
24. Song J, Lu Y, Cheng X, Shi C, Lou Q, Jin X, He J, Zhai G, Yin Z (2021) Functions of the thyroid-stimulating hormone on key developmental features revealed in a series of zebrafish dysmorphogenesis models. *Cells* 10: 1984. <https://doi.org/10.3390/cells10081984>
25. Sun Y, Lu G, Li J, Dang T, Xue C, Liu J, Yan Z (2021) Multimedia distribution and trophic transfer of PPCPs in the middle and lower reaches of the Yarlung Zhanbo River. *Environ Pollut* 271:116408. <https://doi.org/10.1016/j.envpol.2020.116408>
26. Tsui MMP, Chen L, He T, Wang Q, Hu C, Lam JCW, Lam PKS (2019) Organic ultraviolet (UV) filters in the South China sea coastal region: environmental occurrence, toxicological effects and risk assessment. *Ecotoxicol Environ Saf* 181:26–33. <https://doi.org/10.1016/j.ecoenv.2019.05.075>
27. Tsui MMP, Leung HW, Wai TC, Yamashita N, Taniyasu S, Liu W, Lam PKS, Murphy MB (2014) Occurrence, distribution and ecological risk assessment of multiple classes of UV filters in surface waters from different countries. *Water Res* 67:55–65. <https://doi.org/10.1016/j.watres.2014.09.013>
28. US Food and Drug Administration (2019) Sunscreen Drug Products for Over-the-Counter Human Use. Federal Register. <https://www.federalregister.gov/documents/2019/02/26/2019-03019/sunscreen-drug-products-for-over-the-counter-human-use>. Accessed 16 December 2021
29. Visser TJ (2018) Regulation of Thyroid Function, Synthesis, and Function of Thyroid Hormones.. In: In: Vitti P, Hegedüs L (eds) *Thyroid Diseases*. Endocrinology. Springer, Cham. https://doi.org/10.1007/978-3-319-45013-1_1
30. Walpita CN, Crawford AD, Janssens EDR, Van der Geyten S, Darras VM (2009) Type 2 iodothyronine deiodinase is essential for thyroid hormone-dependent embryonic development and pigmentation in zebrafish. *Endocrinol* 150(1):530–539. <https://doi.org/10.1210/en.2008-0457>
31. Walter KM, Miller GW, Chen X, Yaghoobi B, Puschner B, Lein PJ (2019) Effects of thyroid hormone disruption on the ontogenetic expression of thyroid hormone signaling genes in developing zebrafish (*Danio rerio*). *Gen Comp Endocrinol* 272:20–32. <https://doi.org/10.1016/j.yggen.2018.11.007>
32. Zhang S, Guo X, Lu S, Sang N, Li G, Xie P, Liu C, Zhang L, Xing Y (2018) Exposure to PFDoA causes disruption of the hypothalamus-pituitary-thyroid axis in zebrafish larvae. *Environ Pollut* 235:974–982. <https://doi.org/10.1016/j.envpol.2018.01.015>
33. Zhou R, Lu G, Yan Z, Bao X, Zhang P, Jian R (2019) Bioaccumulation and biochemical effects of ethylhexyl methoxycinnamate and its main transformation products in zebrafish. *Aquat Toxicol* 214:105241. <https://doi.org/10.1016/j.aquatox.2019.105241>

Figures

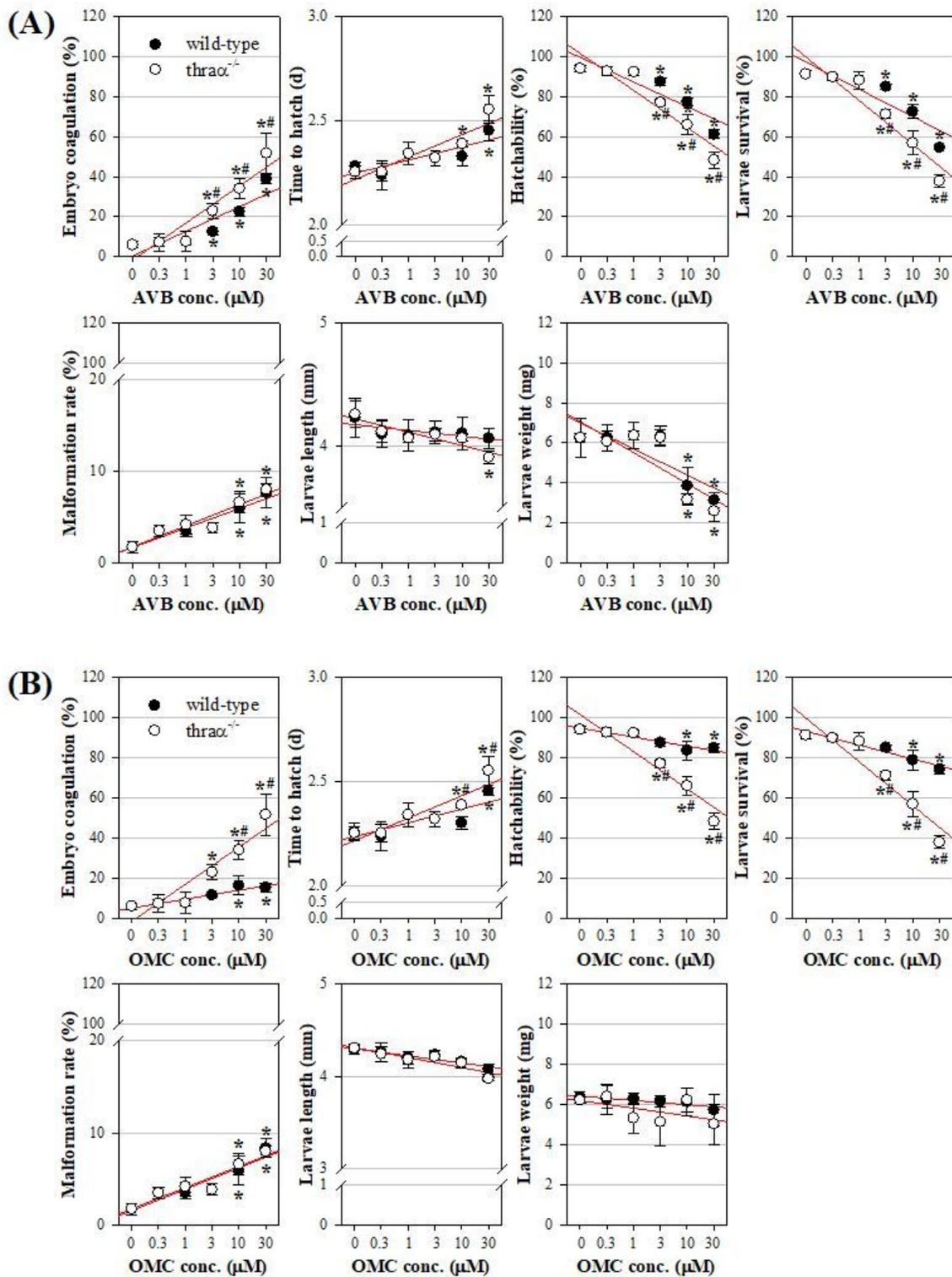


Figure 1

Effects of (A) avobenzone and (B) octinoxate on embryo coagulation, time to hatch, hatchability, larvae survival, malformation rate, larvae length, and larvae weight. The results are shown as mean \pm standard deviation of three replicates. Asterisk (*) indicates significant difference between solvent control and treatment groups, and # indicates significant difference between wild-type and *thraa*^{-/-} groups.

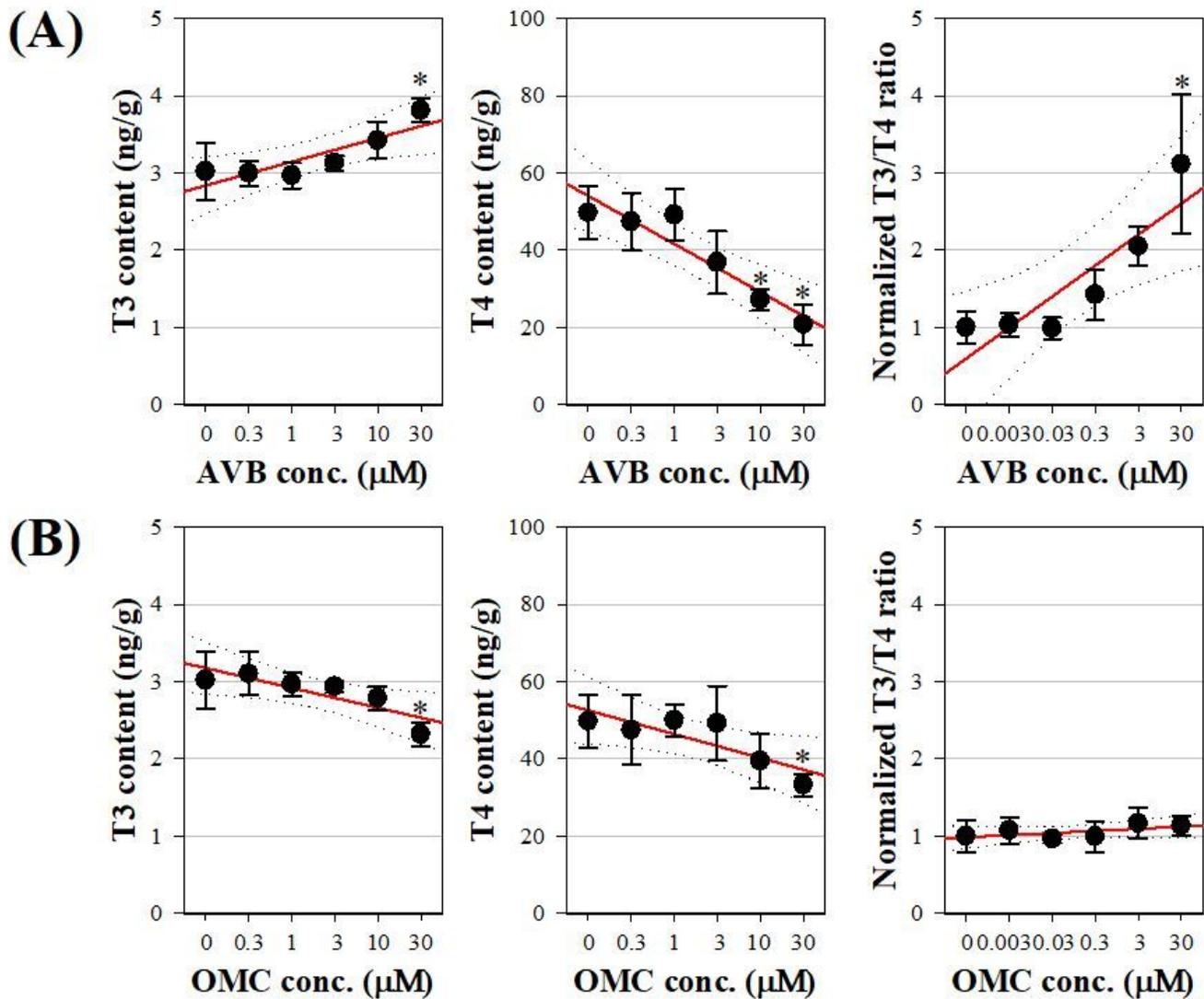


Figure 2

Effects of (A) avobenzone and (B) octinoxate on triiodothyronine (T3), thyroxine (T4), and normalized T3/T4 ratio in zebrafish larvae. The results are shown as mean \pm standard deviation of three replicates. Asterisk indicates significant difference from solvent control.

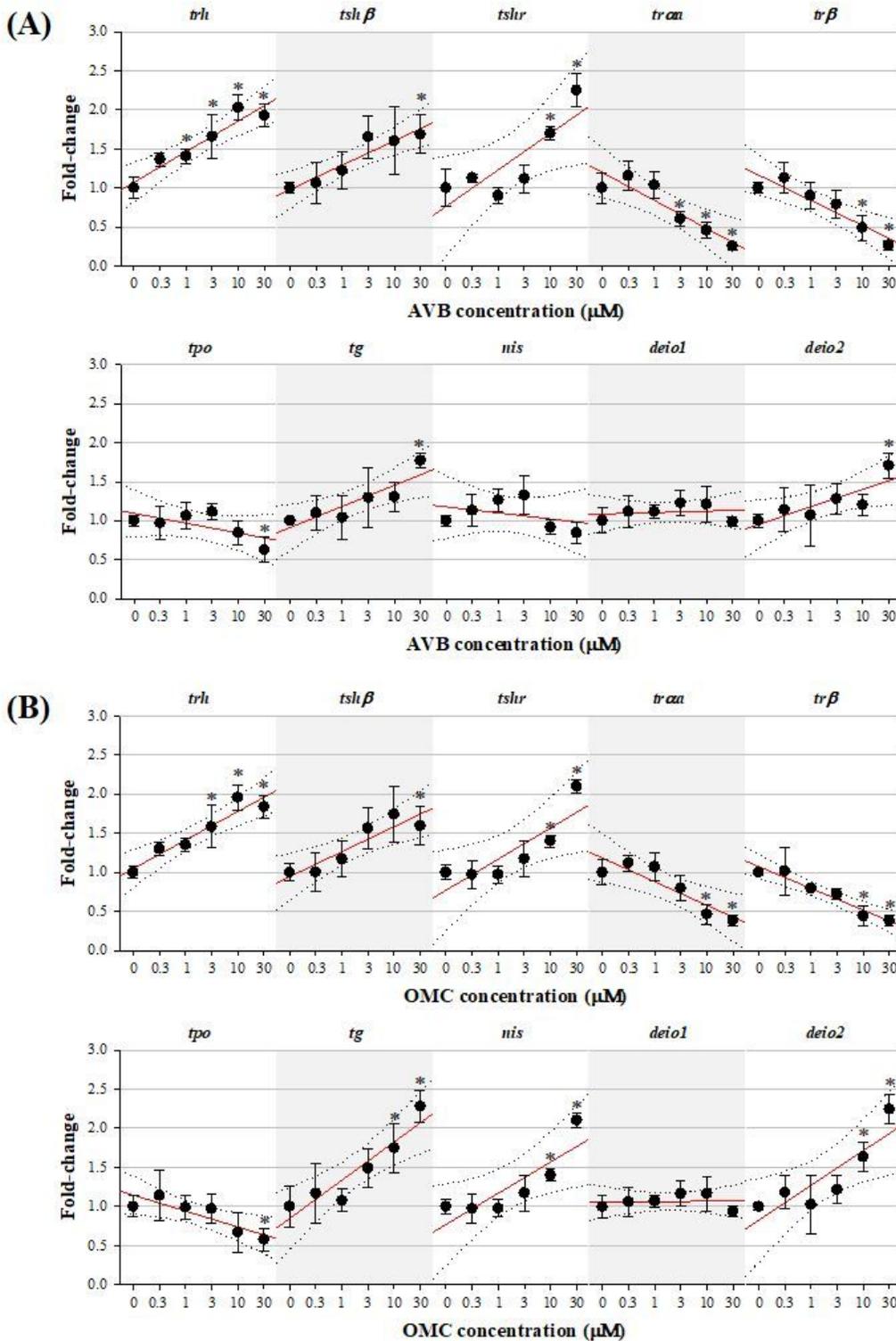


Figure 3

Transcriptional response of genes related to the hypothalamus-pituitary-thyroid (HPT) axis after exposure to avobenzone and octinoxate. The results are shown as mean \pm standard deviation of three replicates. Asterisk indicates significant difference from solvent control.

Supplementary Files

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- [Supplementarydata.docx](#)