보건학석사 학위논문

Thyroid hormone disruption potentials of 6:2 fluorotelomer alcohol (6:2 FTOH) in larval and adult zebrafish (*Danio rerio*)

6:2 FTOH의 치어, 성어 단계의 제브라피시 모델을 이용한 갑상선 교란 연구

2023 년 2 월

서울대학교 보건대학원

환경보건학과 환경보건학 전공

김 은 진

Thyroid hormone disruption potentials of 6:2 fluorotelomer alcohol (6:2 FTOH) in larval and adult zebrafish (*Danio rerio*)

지도교수 최 경 호

이 논문을 보건학석사 학위논문으로 제출함

2022 년 11 월

서울대학교 보건대학원

환경보건학과 환경보건학 전공

김 은 진

김은진의 석사 학위논문을 인준함

2023 년 1 월

위 원 장 조 경 덕 (인)

부 위 원 장 김 성 균 (인)

위 원 최 경 호 (인)

Abstract

Thyroid hormone disruption potentials of 6:2 fluorotelomer alcohol (6:2 FTOH) in larval and adult zebrafish (*Danio rerio*)

Eun Jin Kim
Department of Environmental Health Sciences
The Graduate School of Public Health
Seoul National University

6:2 fluorotelomer alcohol (6:2 FTOH) is used as alternatives to PFOA, which is well known for toxicity effects. While there is a growing concern about the potential adverse health concerns of alternative PFASs, little is known about the thyroid disrupting effects in aquatic organisms. This study investigated adverse effects of 6:2 FTOH in thyroid system and possible mechanisms using embryo-larval and adult male zebrafish (*Danio rerio*). Five thyroid hormones (tT3, tT4, fT3, fT4, and TSH) and related gene expression were analyzed using

enzyme-linked immunosorbent assay (ELISA) and quantitative real-time polymerase chain reaction (qRT-PCR).

6:2 FTOH significantly decreased thyroid hormone levels in zebrafish larvae. Gene expressions associated with thyroid hormone regulation, synthesis, and transport (*trh*, *trhr*, *tshβ*, *tra*, *trβ*, *tg*, and *ttr*) were decreased. Gene expressions related to thyroid hormone metabolism (*ugt1ab* and *sult1st5*) were increased in an chemical exposed group.

In adult zebrafish, 6:2 FTOH significantly increased tT3 and fT3 levels. Gene expression in adult male zebrafish liver resulted in decreased gene expression related to metabolism and transport of thyroid hormones (*sult1st5*, *dio1*, *dio2*, and *ttr*).

This study demonstrates that 6:2 FTOH have a potential for thyroid hormone disruption by interfering regulation, synthesis, transport and metabolism of thyroid hormones. Further studies are needed to investigate the additional mode of action of thyroid hormone disruption and related health effects due to 6:2 FTOH.

Keywords : Endocrine toxicity, fluorotelomer, PFAS alternatives, thyroid hormones, zebrafish
Student Number : 2021-23446

Contents

List of Tables

List of Figures

1. Introduction

Per- and Polyfluoroalkyl substances (PFASs) are collectively referred to as various types of organic compounds consisting of carbon and fluorine. PFASs has a unique characteristic of both water and oil repellent, and it is widely used in various household items (cooking utensils, water repellent clothing, and cosmetics), industrial activities (semiconductor, electronic devices, and construction), and fire-fighting foam (Glüge et al., 2020).

PFASs are difficult to decompose in the environment and have low biodegradation rate, because of strong carbon-fluorine bonds. Among PFASs, PFOS and PFOA, which had high usage rates in recent years, are restricted internationally through Stockholm Convention because of their well known toxicity. Recently, efforts to replace existing traditional PFASs have been made internationally (Coperchini et al., 2017).

Fluorotelomer is used as alternative PFASs and widely used in various household items and fire-fighting foam (Schultz et al., 2004). According to the report on the fluorotelomer market size by Global Market Insights, the market size is expected to increase by more than 12.5% by 2023 compared to 2015. The increase in market size was attributed to increased demand for fire-fighting foam, expansion of the textile industry, and increased demand for alternative PFASs

(Global Market Insights, 2016). Among fluorotelomers, 6:2 FTOH (6:2 fluorotelomer alcohol) is frequently detected in various environmental media and household products, and can be exposed to the human body through various exposure pathways (Liu et al., 2015).

PFASs are accumulative in human body (serum, kidneys, liver, and brain), and can have adverse health effects related to endocrine system and metabolism (Maras et al., 2006; Ishibashi et al., 2008). Health concerns continue to be raised for alternative PFASs, which have similar structures to PFASs. However, toxicity information on alternative PFASs is insufficient, which is also specified in the OECD report (OECD, 2022). The PFOA alternative, 6:2 FTOH, is known to cause sex hormone disruption in both *in vitro* and *in vivo* studies, but no studies have been conducted on the effects of thyroid hormone disruption (Maras et al., 2006; Ishibashi et al., 2008). There are many studies showing that alteration of thyroid function can affect sexual function. Thyroid hormone can affect sex steroid hormone axis through impact on receptors in genitals and the brain (Carosa et al., 2018). In addition, thyroid hormone play an important role in several physiological mechanisms and homeostasis in organism, and the alteration of thyroid function can lead to disruption of sex hormone regulation (Nelson et al., 2011). Therefore, further studies exploring thyroid hormone disruption effects of 6:2 FTOH are needed to reduce the knowledge gap.

The objective of this study is to investigate the thyroid disruption potential of 6:2 FTOH using a well-known animal experimental model, zebrafish (*Danio rerio*). Zebrafish has been used as experimental model that can effectively test the thyroid hormone disruption effect *in vivo*. In this study, embryo-larval and adult stage of zebrafish were used to see thyroid hormone disruption potentials. For this reason, 6:2 FTOH is exposed to embryo-larval and adult zebrafish.

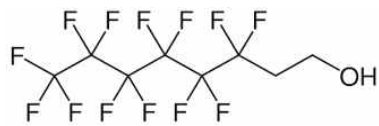
The results of this study will help identify and understand the thyroid disruption mechanisms of 6:2 FTOH in zebrafish, and estimate the potential human health effects of alternative PFASs.

2. Materials and Methods

2.1. Chemicals

6:2 fluorotelomer alcohol (6:2 FTOH, C8H5F13O, CAS No. 647-42-7, 97% purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The solvent used in this research is dimethyl sulfoxide (DMSO, CAS No. 67-68-5, 99% purity), purchased from Junsei Chemical Co. (Tokyo, Japan). All the tested chemicals were dissolved in DMSO at a concentration of 0.01% volume/volume (v/v) or weight/volume (w/v). The physicochemical properties of 6:2 FTOH is described in Table 1.

Table 1. Physicochemical properties of 6:2 FTOH

Compound	6:2 FTOH
Full name	6:2 fluorotelomer alcohol
CAS No.	647-42-7
Molecular formula	$C_8H_5F_{13}O$
Structure	
Molecular weight (g/mol)	364.10
$LogK_{ow}$	4.54

2.2. Zebrafish husbandry and experimental design

2.2.1. Zebrafish husbandry

Wild-type adult zebrafish (*Danio rerio*) pairs (> 6 months) were obtained from a commercial supplier, Greenfish (Seoul, Korea) and maintained at the Environmental Toxicology Laboratory in Seoul, Korea. All the fish were fed on brine shrimp (*Artemia salina*) and blood worms twice a day. The water temperature was maintained at 27 to 29°C. The fish were maintained under 14:10 h light:dark photo-period. Overall maintenance of fish was followed by zebrafish culture protocol (Nüsslein-Volhard et al., 2002).

2.2.2. Chemical exposure to embryo-larval stage of zebrafish

Collected fertilized eggs were exposed to 0.01% DMSO (solvent control) and concentrations of 0.3, 1, 3, 10 μM 6:2 FTOH within 3 hours post-fertilization (hpf). Zebrafish larvae were exposed until 120 hpf, following OECD test guideline 203. Through preliminary experiments, the experimental concentrations were set to a non-lethal level.

For thyroid hormone analysis, 300 embryos were exposed to 400 mL of exposure medium in 1 L beakers. For genetic analysis, 30 embryos were exposed to 50 mL of exposure medium in 50 mL beakers. All treatments had four replicates. Exposure medium was renewed daily to maintain the exposure concentration. Conductivity, pH, temperature, and dissolved oxygen of exposure medium were measured daily. During exposure, mortality of embryo and larvae, hatching rate, morphological changes of larvae were observed daily.

2.2.3. Chemical exposure to adult zebrafish

Wild-type adult male zebrafish (*Danio rerio*) (> 6 months) were exposed to 0.01% DMSO (solvent control) and concentrations of 0.1, 1, 10 μM 6:2 FTOH. Adult zebrafish were exposed for 14 days, following OECD test guideline 230. Through preliminary experiments, the experimental concentrations were set to a non-lethal level. 10 adult male zebrafish were exposed to 4 L of exposure medium in 5 L beakers. All treatments had four replicates. Exposure medium was renewed daily to maintain the exposure concentration. Conductivity, pH, temperature, and dissolved oxygen of exposure medium were measured daily. During exposure, mortality of fish were observed daily.

2.3. Thyroid hormone measurement

After 5 days of exposure to 6:2 FTOH, zebrafish larvae were sacrificed with ice. Samples were then collected (n=220) randomly per group and washed with PBS. After removing the residual moisture, the samples were weighed for weight correction of hormone levels and then stored at －80°C. For pretreatment of the samples, frozen larvae samples were thawed and homogenized with 250 μL PBS. After homogenization, samples were sonicated in 4°C water for 20 minutes. Supernatant was stored at －80°C until thyroid hormone analysis after cetrifugation at 13,000×g for 20 minutes.

In case of adult fish, adult male zebrafish were sacrificed with ice after 14 days of exposure. Blood was collected from caudal vein of the fish. After homogenization for 5,000×g for 5 minutes, plasma samples were collected from blood. Plasma samples were then diluted 6 times with PBS and stored at －80°C until thyroid hormone analysis.

Total triiodothyronine (tT3), total thyroxine (tT4), free triiodothyronine (fT3), free thyroxine (fT4), and thyroid stimulating hormone (TSH) were analyzed using commercial enzyme-linked immunosorbent assay (ELISA) kits. tT3, tT4, fT3, fT4 ELISA kits were purchased from LDN (Nordhorn, Germany) and TSH ELISA kit was purchased from Cusabio Technology LLC (Houston, TX, USA).

2.4. Gene expression analysis

The expression levels of genes involved in HPT axis (Hypothalamic-Pituitary-Thyroid axis) of zebrafish larvae were analyzed after 5 days of chemical exposure. Thirteen genes involved in the regulation, synthesis, transport, and metabolism of thyroid hormones were analyzed. The names of the target genes are as follows. Thyrotropin releasing hormone (*trh*), thyrotropin releasing hormone receptor (*trhr*), thyroid stimulating hormone subunit β (*tsh β*), thyroid stimulating hormone receptor (*tshr*), thyroid hormone receptors (*tra* and *trβ*), sodium iodide symporter (*nis*), thyrogloblin (*tg*), transthyretin (*ttr*), deiodinases (*dio1* and *dio2*), UDP glucuronosyltransferase 1 family a, b (*ugt1ab*), and sulfotransferase family 1 cytosolic sulfotransferase 5 (*sult1st5*).

After 5 days of exposure to 6:2 FTOH, zebrafish larvae were sacrificed with ice. Samples were then collected (n=30) randomly per group and washed with PBS. After removing the residual moisture, the samples stored at －80°C. For RNA extraction, frozen larvae samples were thawed and homogenized with lysis buffer. RNeasy mini kit (Qiagen) was used for RNA extraction.

The expression levels of genes involved in HPT axis of adult male zebrafish organ (brain, thyroid, and liver) were analyzed after 14 days of chemical exposure. Thirteen genes involved in the regulation, synthesis, transport, and metabolism of thyroid hormones were analyzed. The names of the target genes are as follows. Thyrotropin releasing hormone (*trh*), thyrotropin releasing hormone receptor (*trhr*), thyroid stimulating hormone subunit β (*tshβ*), corticotropin releasing hormone b (*crhb*), sodium iodide symporter (*nis*), thyroid peroxidase (*tpo*), thyrogloblin (*tg*), transthyretin (*ttr*), monocarboxylate transporter 8 (*mct8*), deiodinases (*dio1* and *dio2*), UDP glucuronosyltransferase 1 family a, b (*ugt1ab*), and sulfotransferase family 1 cytosolic sulfotransferase 5 (*sult1st5*).

After 14 days of exposure to 6:2 FTOH, adult zebrafish were sacrificed with ice. Collected organ samples (n=6) were stored in two separate tubes (n=3 per tube) at - 80°C. For RNA extraction, frozen organ samples in each tube (n=3) were thawed and homogenized with lysis buffer, and each tube was separately performed. RNeasy Plus mini kit (Qiagen) was used for RNA extraction.

The ratio of absorbance at 260 nm and 280 nm for purity assessment and concentration of RNA samples were measured using Nanodrop ND-1000 spectrometer (Nanodrop Technologies, Wilmington, DE, USA). cDNAs were synthesized from RNA templates using iScript^{TM} cDNA synthesis kit (BioRad, Hercules, CA, USA). For

target gene expression analysis, quantitative real-time PCR (qRT-PCR) assay was used. qRT-PCR was performed using Power SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA). The function and primer sequences of housekeeping gene and target genes used for larval and adult zebrafish organ analysis were listed in Table 2 and Table 3.

The qRT-PCR thermal cycling was comprised of pre-incubation at 95°C for 10 min, 40 cycles of amplification at 95°C for 10 s, 60°C for 20 s, and 72°C for 20s. Ribosomal protein L8 (*rpl8*) was chosen as larval zebrafish sample housekeeping gene, and ribosomal protein L13 (*rpl13*) was chosen as adult zebrafish organ sample housekeeping gene. The relative expression levels of target genes were normalized using the $2^{-\Delta\Delta Ct}$ method (Pfaffl M. W. 2001).

Table 2. Genes used in larval zebrafish analysis

Function	Abbreviation	Full name	Primer Sequence (5' to 3')
Housekeeping	*rpl8*	Ribosomal Protein L8	F: ttgttggtgttgttgctggt R: ggatgctcaacagggttcat
Thyroid hormone regulation	*trh*	Thyrotropin Releasing Hormone	F: gctctctccgtcggtctgtt R: gcgagatccgtgctgatga
	trhr	Thyrotropin Releasing Hormone Receptor	F: cagtgccatcaaccctctga R: ggcagcgcggaacttct
	tshβ	Thyroid Stimulating Hormone Subunit β	F: gcagatcctcacttcacctacc R: gcacaggtttggagcatctca
	tshr	Thyroid Stimulating Hormone Receptor	F: gcgagaagggagaggaggtt R: tcctcgcaagggttgaactc
	tra	Thyroid Hormone Receptor α	F: gccgcttcctgcacatg R: agcggcgggaacagttc
	trβ	Thyroid Hormone Receptor β	F: tggcatggctacagacttggt R: tcagcttccgcttggctaa

Table 2. (Continued)

Function	Abbreviation	Full name	Primer Sequence (5' to 3')
Thyroid hormone synthesis	*nis*	Sodium Iodide Symporter	F: ggtggcatgaaggctgtaat R: gatacgggatccattgttgg
	tg	Thyroglobulin	F: gtctcttgagtgttcgaatgacaag R: aaaggcgggccattaagg
Thyroid hormone transport	*ttr*	Transthyretin	F: cgggtggagtttgacacttt R: gctcagaaggagagccagtg
Thyroid hormone metabolism	*dio1*	Deiodinase 1	F: aacttggaggagaggcttgct R: agggcatggagggtcttctt
	dio2	Deiodinase 2	F: cgcgaaatgggcttgct R: ccaggcaaaatctgcaaagtta
	ugt1ab	UDP Glucuronosyltransferase 1 Family a, b	F: gccagctttgatgaacttgcc R: aactcctccagttccttggtt
	sult1st5	Sulfotransferase Family 1 Cytosolic Sulfotransferase 5	F: cccatccaacttttgcctcg R: ggatccccataccaattgtcct

Table 3. Genes used in adult zebrafish organ analysis

Function	Abbreviation	Full name	Primer Sequence (5' to 3')
Housekeeping	*rpl13*	Ribosomal Protein L13	F: gctgaaggaataccgcacca R: tccagtaagctgtgttgccat
Thyroid hormone regulation	*trh*	Thyrotropin Releasing Hormone	F: catgctagaggaccccactg R: gagcagcatcaggtagcgtt
	trhr	Thyrotropin Releasing Hormone Receptor	F: cagtgccatcaaccctctga R: ggcagcgcggaacttct
	tshβ	Thyroid Stimulating Hormone Subunit β	F: gcagatcctcacttcacctacc R: gcacaggtttggagcatctca
	crhb	Corticotropin Releasing Hormone b	F: caattacgcacagattctcctcg R: gaagtactcctcccccaagc
Thyroid hormone synthesis	*nis*	Sodium Iodide Symporter	F: ccactgaagatcggcagaat R: cagccaagcccatagaaca
	tpo	Thyroid Peroxidase	F: tgatatctccttcacgccgc R: tctgtatggggaagcaggga
	tg	Thyroglobulin	F: acaatccactgggtgtgtgtt R: gagagcaaaagacctgccct

Table 3. (Continued)

Function	Abbreviation	Full name	Primer Sequence (5' to 3')
Thyroid hormone transport	*ttr*	Transthyretin	F: cgggtggagtttgacacttt R: gctcagaaggagagccagtg
	mct8	Monocarboxylate Transporter 8	F: cttcggatgtcggaaaacgg R: cccagagtcgtggcgaag
Thyroid hormone metabolism	*dio1*	Deiodinase 1	F: gttcaaacagcttgtcaaggact R: agcaagcctctcctccaagtt
	dio2	Deiodinase 2	F: tggatgcctacaaacaggtga R: gtcttaccgctgatgctcca
	ugt1ab	UDP Glucuronosyltransferase 1 Family a, b	F: gccagctttgatgaacttgcc R: aactcctccagttccttggtt
	sult1st5	Sulfotransferase Family 1 Cytosolic Sulfotransferase 5	F: gaaagaggaccctgctcgtg R: tttgccatggggttttctcg

2.5. Statistical analysis

All of the data are presented as the mean±standard error of the mean (SEM). For comparison among the solvent control and experimental groups, one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test was used. Spearman's rank correlation test was used to see linear trends of each concentration effects. A p-value <0.05 was considered statistically significant. The statistical analyses were performed using IBM SPSS Statistics 26 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Thyroid hormone disruption in larval zebrafish

3.1.1. Zebrafish larvae thyroid hormone levels

Exposure to 6:2 FTOH for 5 days showed an overall decline in thyroid hormone levels in zebrafish larvae (Figure 1). Hormone levels of tT4, fT3, fT4, and TSH decreased statistically significantly with concentration dependent. In particular, in the case of tT4 and fT3, a statistically significant decrease was observed in every chemical exposed group when compared to the solvent control group.

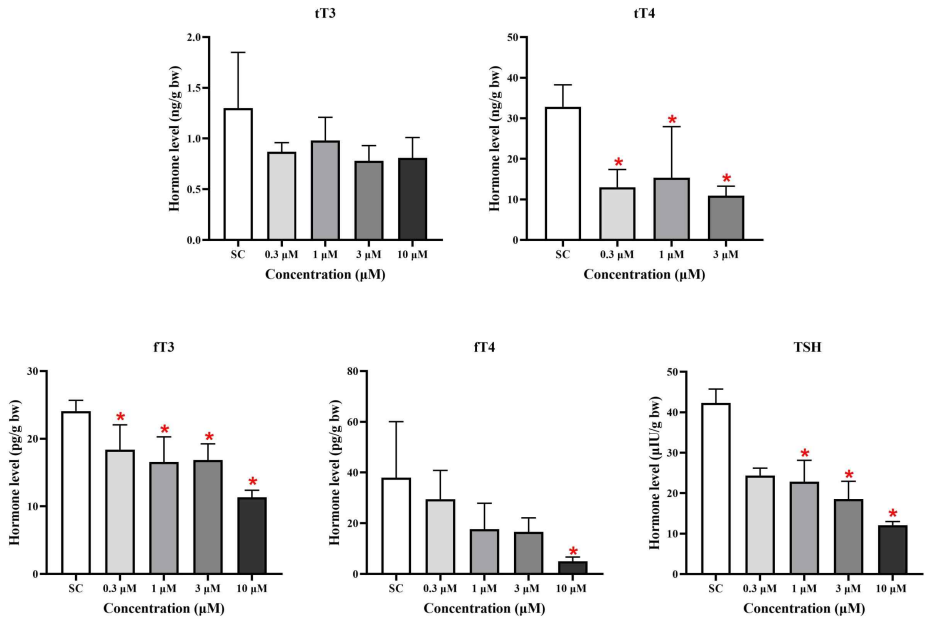


Figure 1. Levels of tT3, tT4, fT3, fT4, and TSH in the whole body zebrafish larvae following 120 h exposure to 6:2 FTOH. The results were shown as mean±SEM with four replicates, n=220 per group. Asteriks (*) indicate statistically significant differences ($p<0.05$) from the response of the solvent control determined by ANOVA analysis.

3.1.2. Zebrafish larvae gene expression levels

The expression of genes involved in the HPT axis of zebrafish larvae after exposure to 6:2 FTOH for 5 days were analyzed (Figure 2). Exposure to 6:2 FTOH resulted in concentration dependent decreasing trend of genes related to thyroid hormone regulation, synthesis, and transport (*trh*, *trhr*, *tshβ*, *tra*, *trβ*, *tg*, and *ttr*) after statistical analysis. In the case of *tra* gene, a statistically significant decrease was observed in every chemical exposed group compared to the solvent control group. The expression of genes related to thyroid hormone metabolism (*ugt1ab* and *sult1st5*) was increased statistically significantly when exposed to 0.3 μM of 6:2 FTOH compared to the solvent control group.

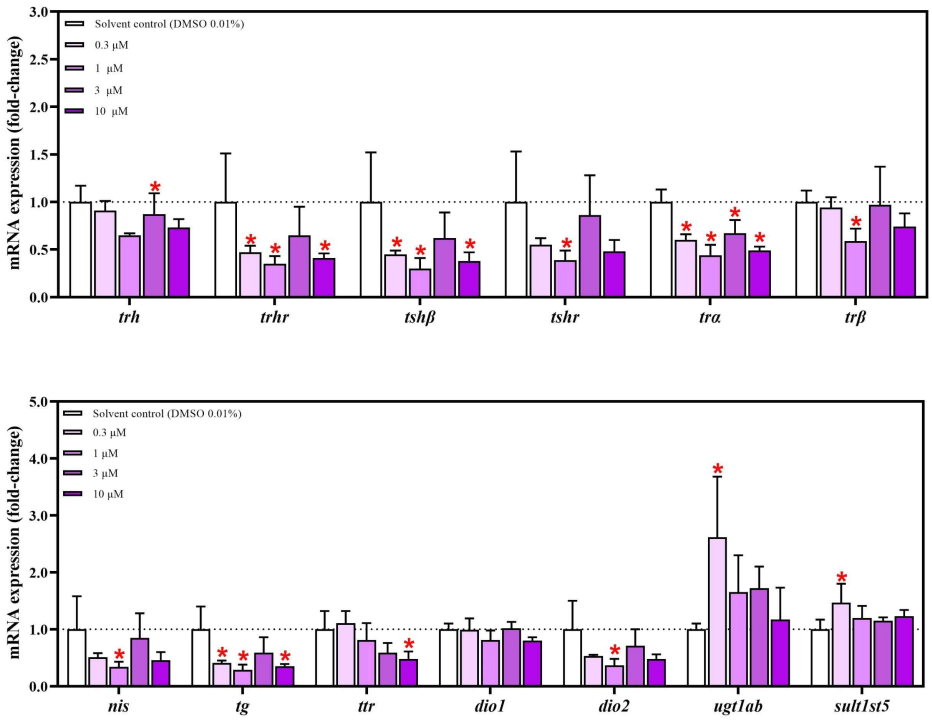


Figure 2. Gene expression levels of in the whole body zebrafish larvae following 120 h exposure to 6:2 FTOH. The results were shown as mean±SEM with four replicates, n=30 per group. Asteriks (*) indicate statistically significant differences ($p<0.05$) from the response of the solvent control determined by ANOVA analysis.

3.2. Thyroid hormone disruption in adult zebrafish

3.2.1. Adult zebrafish thyroid hormone levels

Exposure to 6:2 FTOH for 14 days altered thyroid hormone levels in adult male zebrafish. tT3 and fT3 levels showed a concentration dependent increasing trend after statistical analysis, yet the values were not noticeable changes (Figure 3). tT3 and fT3 levels in fish exposed to 1 μM of 6:2 FTOH were statistically significantly increased compared to the solvent control group. TSH levels did not change significantly after statistical analysis.

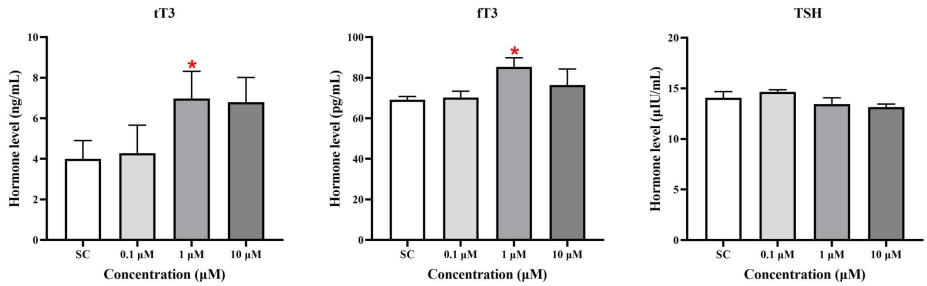


Figure 3. Levels of tT3, fT3, and TSH in the adult male zebrafish plasma following 14 d exposure to 6:2 FTOH. The results were shown as mean±SEM with four replicates, n=10 per group. Asteriks (*) indicate statistically significant differences ($p<0.05$) from the response of the solvent control determined by ANOVA analysis.

3.2.2. Adult zebrafish organ gene expression levels

Gene expression levels in adult zebrafish organ (brain, thyroid, and liver) related to HPT axis were altered after exposure to 6:2 FTOH for 14 days (Figure 4-6). In brain samples, statistically significant changes were observed in the *crhb* and *mct8* genes in the 1 μM and 0.1 μM exposed group, respectively (Figure 4). However, the fold change was 1.62 and 0.72, respectively, which was not very noticeable change. Changes in gene expression in thyroid samples were not statistically significant (Figure 5). There was a statistically significant change in gene expression in the 10 μM exposed group in the liver samples (Figure 6). In liver samples, exposure to 6:2 FTOH resulted in concentration dependent decreasing trend of thyroid hormone metabolism gene (*sult1st5*), deiodinase genes (*dio1* and *dio2*) and transthyretin gene (*ttr*) after statistical analysis.

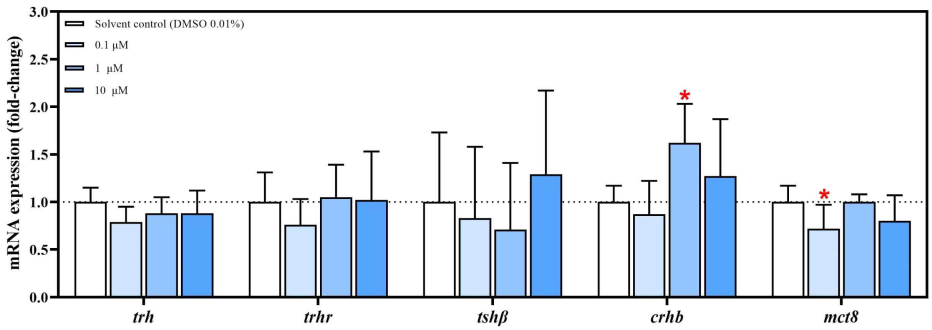


Figure 4. Gene expression changes in adult zebrafish brain following 14 d exposure to 6:2 FTOH. The results were shown as mean±SEM with four replicates, n=6 per group. Asteriks (*) indicate statistically significant differences ($p<0.05$) from the response of the solvent control determined by ANOVA analysis.

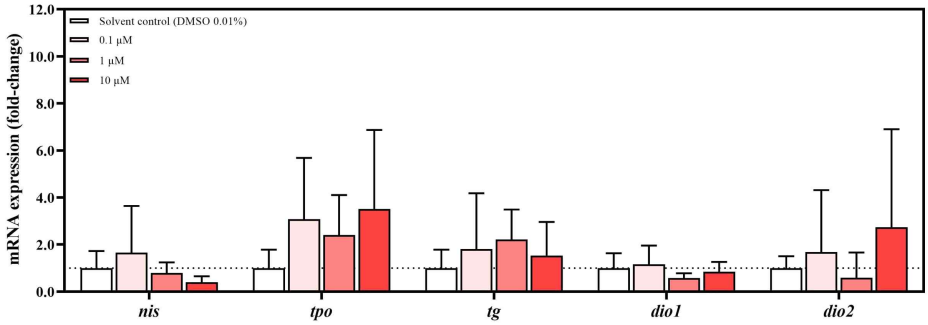


Figure 5. Gene expression changes in adult zebrafish thyroid following 14 d exposure to 6:2 FTOH. The results were shown as mean±SEM with four replicates, n=6 per group.

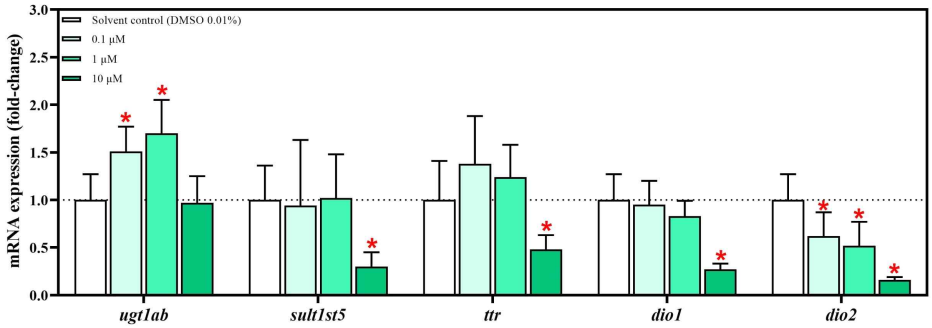


Figure 6. Gene expression changes in adult zebrafish liver following 14 d exposure to 6:2 FTOH. The results were shown as mean±SEM with four replicates, n=6 per group. Asteriks (*) indicate statistically significant differences ($p<0.05$) from the response of the solvent control determined by ANOVA analysis.

4. Discussion

In this study, thyroid hormone levels were decreased in zebrafish larvae after 5 days of exposure to 6:2 FTOH (Figure 1). Following genetic analysis shows decreasing trend of *trh*, *trhr*, *tshβ*, *tra*, *trβ*, *tg,* and *ttr* (Figure 2). Decreased level of thyroid hormones in zebrafish larvae can be explained by decreased gene expression related to regulation, synthesis, and transport of thyroid hormone (Zhang et al., 2018; Yu et al., 2010). The *trβ* gene is involved in thyroid hormone regulation by acting as a receptor using thyroid hormone as a ligand. The decreased expression of this gene can lead to decreased amount of thyroid hormone (Liu and Chan, 2002). The *ttr* gene encodes transthyretin, the major transport protein of thyroid hormones in zebrafish (Yu et al., 2010). When transthyretin decreases, thyroid hormone can be easily metabolized and removed from the body, which reduces the amount of thyroid hormone (Fernie et al., 2005). Decreased level of thyroid hormones in zebrafish larvae can also be explained by increased gene expression related to thyroid hormone metabolism (*ugt1ab* and *sult1st5*). Studies show over expression of gene related to metabolism can lead to decreased level of tT4 levels in zebrafish larvae (Zhang et al., 2017; Huang et al., 2016). In this study, we can see up-regulation of *ugt1ab* and *sult1st5* after exposure to 0.3 μM of 6:2 FTOH. Despite the low concentration, up-regulation of these genes can be proposed for the decreased tT4 levels.

After exposure to 6:2 FTOH for 14 days in adult zebrafish, plasma tT3 and fT3 levels were increased with concentration dependent manner (Figure 3). This can be explained by decreased transcription level of *sult1st5* and *ttr* in liver samples (Figure 6). *Sult1st5* is a gene related to sulfation and plays a role to promote the elimination of thyroid hormones in adult zebrafish (Liang et al., 2021). Decreased level of this gene can lead to increased level of tT3 and fT3. Decreased gene expression of *ttr* would have caused decreased amount of transthyretin binding to free thyroid hormone (Zhai et al., 2014). This consequently could have led to increased level of fT3. Significant down regulation of deiodinase genes, *dio1* and *dio2* were also observed in liver samples, and this can be explained by decreased level of *sult1st5* (Figure 6). Sulfation promotes the deiodination of tT3 by the type 1 deiodinase in liver, which leads to thyroid hormone inactivation (Visser et al., 1994). Considering this, decreased sulfation can be suggested to a decrease in deiodinase gene expression in liver.

Previous studies reported the thyroid disruption effects of various PFASs, but there were differences between experimental animals and life stages. After exposure of PFOS in zebrafish larvae for 15 days, T3 levels were significantly increased and the related gene expression was altered (Shi et al., 2009). This was different from the influence of mammals that had significantly decreased thyroid hormone levels due to exposure of PFOS (Lau et al., 2003; Thibodeaux et al., 2003).

After exposure to PFHxA in zebrafish embryos until 96 h after fertilization, T4 and T3 levels was increased, which was the opposite of the results from the 28-day oral toxicity study in rats (NTP, 2019; Zhang et al., 2022). In this study, the thyroid hormone levels after exposure to 6:2 FTOH in larvae and adult zebrafish were differed (Figure 1, 3). Previous study showed the changes in thyroid hormone expression by the developmental stage of the zebrafish (Chang et al., 2012). In detail, whole body T4 and T3 levels remained stable until 3 dpf (days post-fertilization), and at 10 dpf, T3 level reached the highest, while T4 level peaked at 21 dpf. Thyroid hormones were declined during later development. These alterations of thyroid hormones in zebrafish life cycle can help to understand the difference of thyroid disruption effects in the larval and adult zebrafish after exposure to 6:2 FTOH. Further studies are needed to fully understand the difference of thyroid disrupting effects due to 6:2 FTOH among life stages.

5. Conclusion

Following exposure to 6:2 fluorotelomer alcohol (6:2 FTOH), larval and adult zebrafish HPT axis regulation was altered. The results of this study show potential thyroid hormone disrupting effects of 6:2 FTOH. After exposure of 6:2 FTOH, tT4, fT3, fT4, and TSH levels were significantly decreased in larval zebrafish. There was an increasing trend of tT3 and fT3 levels after exposure to 6:2 FTOH in adult zebrafish. It is interesting that there were differences in the tendency of thyroid hormones due to 6:2 FTOH depending on the life stages of zebrafish.

This study demonstrates that 6:2 FTOH have a potential thyroid disruption effects in embryo-larval and adult stage of zebrafish. Further studies are needed to investigate the additional mode of action of thyroid hormone disruption and related health effects due to 6:2 FTOH.

References

Carosa, E., Lenzi, A., & Jannini, E. A. (2018). Thyroid hormone receptors and ligands, tissue distribution and sexual behavior. *Molecular and cellular endocrinology*, *467*, 49-59.

Chang, J., Wang, M., Gui, W., Zhao, Y., Yu, L., & Zhu, G. (2012). Changes in thyroid hormone levels during zebrafish development. *Zoological science*, *29*(3), 181-184.

Coperchini, F., Awwad, O., Rotondi, M., Santini, F. E. R. R. U. C. C. I. O., Imbriani, M., & Chiovato, L. (2017). Thyroid disruption by perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA). *Journal of endocrinological investigation*, *40*(2), 105-121.

Fernie, K. J., Shutt, J. L., Mayne, G., Hoffman, D., Letcher, R. J., Drouillard, K. G., & Ritchie, I. J. (2005). Exposure to polybrominated diphenyl ethers (PBDEs): changes in thyroid, vitamin A, glutathione homeostasis, and oxidative stress in American kestrels (Falco sparverius). *Toxicological Sciences*, *88*(2), 375-383.

Global Market Insights (2016). Fluorotelomers Market Size By Product (Fluorotelomer Iodide, Fluorotelomer Acrylate, Fluorotelomer Alcohols), By Application (Textiles, Stain resistant, Food packaging, Fire fighting foams), Industry Analysis Report, Regional Outlook, Application Potential, Price Trends, Competitive Market Share & Forecast, 2016 – 2023. Report ID: GMI407.

Glüge, J., Scheringer, M., Cousins, I. T., DeWitt, J. C., Goldenman, G., Herzke, D., ... & Wang, Z. (2020). An overview of the uses of per-and polyfluoroalkyl substances (PFAS). *Environmental Science: Processes & Impacts*, *22*(12), 2345-2373.

Huang, G. M., Tian, X. F., Fang, X. D., & Ji, F. J. (2016). Waterborne exposure to bisphenol F causes thyroid endocrine disruption in zebrafish larvae. *Chemosphere*, *147*, 188-194.

Ishibashi, H., Yamauchi, R., Matsuoka, M., Kim, J. W., Hirano, M., Yamaguchi, A., ... & Arizono, K. (2008). Fluorotelomer alcohols induce hepatic vitellogenin through activation of the estrogen receptor in male medaka (Oryzias latipes). *Chemosphere*, *71*(10), 1853-1859.

Lau, C., Thibodeaux, J. R., Hanson, R. G., Rogers, J. M., Grey, B. E., Stanton, M. E., ... & Stevenson, L. A. (2003). Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: postnatal evaluation. *Toxicological Sciences*, *74*(2), 382-392.

Liang, Y. Q., Situ, Y., Xie, L., Huo, J., Dong, Z., Li, C., & Lin, Z. (2021). Exposure of adult zebrafish to androstenedione alters thyroid hormone levels and the transcriptional expression of genes related to the hypothalamus-pituitary-thyroid axis. *Aquaculture Reports*, *21*, 100966.

Liu, X., Guo, Z., Folk IV, E. E., & Roache, N. F. (2015). Determination of fluorotelomer alcohols in selected consumer products and preliminary investigation of their fate in the indoor environment. *Chemosphere*, *129*, 81-86.

Liu, Y. W., & Chan, W. K. (2002). Thyroid hormones are important for embryonic to larval transitory phase in zebrafish. *Differentiation*, *70*(1), 36-45.

Maras, M., Vanparys, C., Muylle, F., Robbens, J., Berger, U., Barber, J. L., ... & De Coen, W. (2006). Estrogen-like properties of fluorotelomer alcohols as revealed by MCF-7 breast cancer cell proliferation. *Environmental health perspectives*, *114*(1), 100-105.

National Toxicology Program. 2011. Testing Status of Agents at

NTP.

Nelson, E. R., Allan, E. R., Pang, F. Y., & Habibi, H. R. (2011). Auto-regulation of thyroid hormone receptors in the goldfish ovary and testis. *General and comparative endocrinology*, *172*(1), 50-55.

Nüsslein-Volhard, C. and Dahm, R. (2002) Zebrafish: a practical approach. New York: Oxford University Press. :303p.

OECD (2022). PFASs and Alternatives in Food Packaging (Paper and Paperboard) Report on the Commercial Availability and Current Uses, OECD Series on Risk Management, No. 58, Environment, Health and Safety, Environment Directorate, OECD.

Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT–PCR. *Nucleic acids research*, *29*(9), e45-e45.

Schultz, M. M., Barofsky, D. F., & Field, J. A. (2004). Quantitative determination of fluorotelomer sulfonates in groundwater by LC MS/MS. *Environmental science & technology*, *38*(6), 1828-1835.

Shi, X., Liu, C., Wu, G., & Zhou, B. (2009). Waterborne exposure to PFOS causes disruption of the hypothalamus–pituitary–thyroid axis in zebrafish larvae. *Chemosphere*, *77*(7), 1010-1018.

Thibodeaux, J. R., Hanson, R. G., Rogers, J. M., Grey, B. E., Barbee, B. D., Richards, J. H., ... & Lau, C. (2003). Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. I: maternal and prenatal evaluations. *Toxicological Sciences*, *74*(2), 369-381.

Visser, T. J. (1994). Role of sulfation in thyroid hormone metabolism. *Chemico-biological interactions*, *92*(1-3), 293-303.

Yu, L., Deng, J., Shi, X., Liu, C., Yu, K., & Zhou, B. (2010). Exposure to DE-71 alters thyroid hormone levels and gene

transcription in the hypothalamic - pituitary - thyroid axis of zebrafish larvae. *Aquatic toxicology*, *97*(3), 226-233.

Zhai, W., Huang, Z., Chen, L., Feng, C., Li, B., & Li, T. (2014). Thyroid endocrine disruption in zebrafish larvae after exposure to mono-(2-ethylhexyl) phthalate (MEHP). *PLoS One*, *9*(3), e92465.

Zhang, D. H., Zhou, E. X., & Yang, Z. L. (2017). Waterborne exposure to BPS causes thyroid endocrine disruption in zebrafish larvae. *PLoS One*, *12*(5), e0176927.

Zhang, S., Guo, X., Lu, S., He, J., Wu, Q., Liu, X., ... & Xie, P. (2022). Perfluorohexanoic acid caused disruption of the hypothalamus-pituitary-thyroid axis in zebrafish larvae. *Ecotoxicology and Environmental Safety*, *232*, 113283.

Zhang, Y. F., Ren, X. M., Li, Y. Y., Yao, X. F., Li, C. H., Qin, Z. F., & Guo, L. H. (2018). Bisphenol A alternatives bisphenol S and bisphenol F interfere with thyroid hormone signaling pathway in vitro and in vivo. *Environmental pollution*, *237*, 1072-1079.

국문초록

6:2 FTOH의 치어, 성어 단계의 제브라피시 모델을 이용한 갑상선 교란 연구

김 은 진
환경보건학과 환경보건전공
서울대학교 보건대학원

6:2 fluorotelomer alcohol (6:2 FTOH)은 유해성이 잘 알려진 PFOA를 대체하여 사용되는 신종 과불화화합물이다. 대체 과불화화합물의 잠재적 건강 악영향에 대한 우려가 커지고 있지만, 갑상선 교란 영향에 대한 연구가 매우 부족하다. 따라서, 본 연구에서는 제브라피시 (*Danio rerio*) 배아-치어와 수컷 성어를 이용하여 6:2 FTOH의 갑상선 교란 영향을 조사하였다. 본 연구에서는 enzyme-linked immunosorbent assay (ELISA)와 quantitative real-time polymerase chain reaction (qRT-PCR) 기법을 이용하여 다섯 종류의 갑상선 호르몬 (tT3, tT4, fT3, fT4 및 TSH)과 관련 유전자 발현을 분석하였다.

6:2 FTOH는 제브라피시 치어에서 통계적으로 유의한 수준으로 갑상선 호르몬 수치를 감소시켰다. 또한, 갑상선 호르몬 조절, 합성, 수송 관련 유전자 (*trh*, *trhr*, *tshβ*, *tra*, *trβ*, *tg* 및 *ttr*) 발현을 감소시켰다. 반면,

일부 농도 군에서 갑상선 호르몬 대사 관련 유전자 (*ugt1ab* 및 *sult1st5*) 발현을 증가시켰다.

6:2 FTOH는 제브라피시 성어에서 통계적으로 유의한 수준으로 tT3와 fT3 수치를 증가시켰고, 간에서 갑상선 호르몬 대사와 수송에 관련된 유전자 (*sult1st5*, *dio1*, *dio2* 및 *ttr*) 발현을 감소시켰다.

본 연구 결과는 6:2 FTOH가 갑상선 호르몬 조절, 합성, 수송 및 대사 기전을 방해하여 갑상선 교란 가능성이 있음을 시사한다. 6:2 FTOH로 인한 갑상선 교란 영향과 관련 건강 영향을 확인하기 위한 추가 연구가 필요할 것이다.

주요어 : 내분비계 독성, 플루오르텔로머, 과불화화합물 대체물질, 갑상선 호르몬, 제브라피시
학번 : 2021-23446