보건학석사 학위논문

Thyroid and neurotoxicity potentials of synthetic musk compounds (MK, HHCB, AHTN) in early-life stage of zebrafish (*Danio rerio*)

생애초기발달단계의 제브라피쉬(*Danio rerio*)를 이용한 합성머스크류(MK, HHCB, AHTN)의 갑상선 및 신경독성 영향 연구

2021 년 2 월

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이 논문을 석사 학위논문으로 제출함

2020 년 11 월

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채희연의 석사 학위논문을 인준함

2021 년 1 월

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Abstract

Thyroid and neurotoxicity potentials of synthetic musk compounds (MK, HHCB, AHTN) in early-life stage of zebrafish (*Danio rerio*)

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Synthetic musk compounds (SMCs) are widely used in many applications, including consumer chemical products such as perfumes, cosmetics, soap, and fabric softener, to replace perfumes of natural origin. The potential consequences of persistence in the environment and accumulation in the biota are of concern. Their toxicities, however, are generally unknown in the aquatic environment, especially for the thyroid and neurotoxicity. In the present study, three frequently detected SMCs of musk ketone (MK), 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta[g]- benzopyran (HHCB), and 6-acetyl-1,1,2,4,4,7-hexamethyltetralin (AHTN), were chosen, and their thyroid disruption and neurotoxicity were assessed using early-life stage zebrafish (*Danio rerio*).

Zebrafish embryos were exposed to MK, HHCB, and AHTN for 5 days. At the end of the exposure, whole body larvae were collected to measure thyroid hormone, transcriptional change, and behavioral change. Daniovision® Ethovision XT was used to determine behavioral changes.

Following 120 hours of exposure to synthetic musks, significant changes in the expression of thyroid hormone and neurodevelopment related genes were observed in the zebrafish larvae. After exposure to MK, T4 was significantly decreased, and thyroid hormone-related genes such as *crhβ* were significantly up-regulated. But *ugt1ab* was significantly down-regulated, possibly explaining the observed decrease of whole-body T4 concentration. Following MK exposure, behavior changes were decreased.

After exposure to HHCB, T4 concentration was also decreased. *crhβ* gene showed an up-regulating trend, and *mbp*, *gap43,* and *syn2a* were down-regulated. HHCB exposure caused hypoactivity of the larvae was observed.

Following AHTN exposure, up-regulations of *crhβ*, *nis*, *ugt1ab*, *dio2*, and *gap43* genes were observed, while thyroid hormones were not influenced. Thyroid disruption effects of ATHN appear to be different from the other SMCs. After light stimulation, zebrafish larvae activity was decreased, and thigmotaxis had no effects.

The present observations showed that SMCs such as MK and HHCB, have the potentials to disrupt thyroid hormone or neurodevelopment in the zebrafish at the development stage. This study was conducted at concentrations several orders of magnitude higher than the environmental concentrations. Consequences of long-term exposure at the environmentally relevant concentrations in the aquatic environment warrant further investigations.

Keywords: Synthetic musk compounds, thyroid hormone, neurodevelopment, behavior, zebrafish

Student number: 2019-29555

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1. Introduction

To substitute for natural musk, a number of synthetic musks began to be used since 1890 (Gatermann et al., 2002). Depending on the structure, it can be divided into four subgroups, i.e., nitro musks, polycyclic musks, macrocyclic musks, and alicyclic musks). Among them, polycyclic musks (HHCB; 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8,-hexamethyl-cyclopenta[g]benzopyran, and AHTN; 6-Acetyl-1,1,2,4,4,7-hexamethyltetralin) are predominantly used material that accounts for 85% of the synthetic musk market, which was produced over 6000 tons in 1996, and worldwide. It is widely used in consumer chemical and hygienic products such as perfumes, cosmetics, soap, and fabric softeners (Liu et al., 2020; Wong et al., 2019). Because these musk materials are generally persistent, the possibility of bioaccumulation and ecological consequences are of concern (Gatermann et al., 2002). For example, HHCB and AHTN were measured in maternal milk at 299 and 65.1 ng/g lipids, respectively. Both musks have been detected in human milk in other countries including China (63, 16.5 ng/g lw) and Sweden (63.9, 10.4 ng/g lw) (Lee et al., 2015).

As these compounds are not completely removed from the sewage treatment plant, they have been frequently detected in the water environment (Tasselli et al., 2020). musk ketone (MK), HHCB, and AHTN were detected at up to 420, 2720, and 520 ng/L in surface water, respectively (Lee et al., 2010). In Korea, MK, HHCB, and AHTN were detected in fat tissues of women at 13, 81, and 12 ng/g lipids, respectively (Moon et al., 2012).

Ecotoxicity information on MK, HHCB, and AHTN is available for various species. The LC_{50} values of HHCB, and AHTN in zebrafish (*Danio rerio*) are 244-314 μg/L, and 282-452 μg/L, respectively

(Dietrich and Hitzfeld, 2004). In addition, HHCB and AHTN can cause oxidative stress and genotoxicity and may disrupt sex hormone balance such as anti-estrogenic or reduction of progesterone (Ehiguese et al., 2020; Li et al., 2013). Limited knowledge, however, is present for adverse effects of SMCs on thyroid and neurodevelopment outcomes in fish.

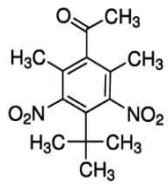
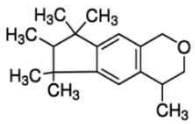
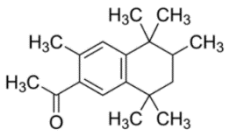
In this study, thyroid disruption and neurodevelopmental effects of major SMCs were assessed using zebrafish embryo-larvae. MK, HHCB, and AHTN were chosen as the study SMCs. The results of this study will help understand the thyroid and neurotoxicity potentials of SMCs and stimulate further investigations on their ecological effects.

2. Materials and Methods

2.1. Chemicals

Musk ketone (MK, CAS no. 81-14-1, purity: 98%), 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta-(g)-2-benzopyran (HHCB, CAS no. 1222-05-5, purity: 99%), and 7-acetyl-1,1,3,4,4,6-hexamethyltetralin (AHTN, CAS no. 1506-02-1, purity: 98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The physicochemical properties of MK, HHCB, and AHTN were described in Table 1. Dimethyl sulfoxide (DMSO, purity: 99%) was used as a solvent.

Table 1. Physicochemical properties of MK, HHCB, and AHTN

Compound	Nitro musk	Polycyclic musks	
	MK (Musk ketone)	HHCB (Galaxolide®)	AHTN (Tonalide®)
CAS No.	81-14-1	1222-05-5	1506-02-1
Structure			
Molecular formulate	$C_{14}H_{18}N_2O_5$	$C_{18}H_{26}O$	$C_{18}H_{26}O$
Molecular weight (g/mol)	294[a]	258[a]	258[a]
$LogK_{OW}$	4.30[a]	5.90[a]	5.70[a]

[a] PubChem

2.2. Zebrafish culture and exposure

For the thyroid hormone measurement and associated gene expression, zebrafish embryos were purchased from a commercial supplier (Gangnam Aquarium, Suwon, Korea). In each 500 mL beaker, 250 embryos were randomly divided with 300 mL medium. The embryos were exposed to concentrations of the test chemicals (MK 0, 0.3, 1, 3, and 8 mg/L, HHCB 0, 0.03, 0.1, 0.3, and 1 mg/L, AHTN 0, 0.01, 0.03, 0.1, and 0.3 mg/L) for 120 hours until 5 days post fertilization (5 dpf).

For the observations of behavioral change, freshly hatched larval zebrafish were used. Embryos were harvested from mating zebrafish pairs (over six months old). Adult wild-type zebrafish were used. The adult fish were obtained from a commercial supplier (Greenfish, Seoul, Korea), and in-house cultured in the Environmental Toxicology laboratory at Seoul National University (Seoul, Korea). Embryos collected within 4 hours post-fertilization (hpf) were used. The collected embryos were randomly divided into 50 mL beakers. For each treatment or control, 4 replicates with 20 embryos were used. The embryos were exposed to same concentrations of test chemicals with thyroid hormone measurment and gene expression for 120 hours. The test concentrations were determined by preliminary range-finding exposure.

Exposure media for HHCB and AHTN, DMSO (0.01% v/v) was used as a solvent because of low water solubility. For MK, no solvent was used for dilution. Exposure was conducted at room conditions of 26±1°C, under a photoperiod of 14:10 h light:dark. Temperature, conductivity, pH, and dissolved oxygen were recorded between media renewals.

2.3. Thyroid hormone measurement

At 120 hpf, zebrafish (n=200) of each replicate was weighed and whole-body samples were homogenized in 110 uL of the standard diluent included in the ELISA kit. After 10 min of sonication on the ice, the samples were centrifuged for 10 min at 5000×g, and the supernatant was collected. T4 ELISA kit (Cat no. CEA452Ge) was purchased from Cloud-Clone Corp. (Wuhan, China). The optical density of each sample was measured at 450 nm using Tecan Infinite® 200 (Tecan Group Ltd., Mändorf, Switzerland).

2.4. RNA isolation and RT-PCR

After 120 h of exposure, each group of 15 larvae was pooled and total RNA was extracted. The pooled zebrafish larval samples were stored at -80°C until further analysis. Four independent tests with different batches of fish were conducted and the results of each test were averaged and used for the presentation in this study.

Whole-body samples of zebrafish larvae were homogenized with lysis buffer using a tissue grinder. After grinding, samples were centrifuged for 3 min at 16000×g. The supernatant was collected in an e-tube, and the mRNA was immediately extracted using an RNeasy mini kit (Qiagen). The 260/280 ratio and mRNA concentration were verified with Gen5 2.05 (BioTek, Winooski, USA). After quantification, mRNA samples were diluted to 10 or 20 ng/μL. Then, complementary DNAs were synthesized by using a cDNA synthesis kit (BioRad, Hercules, CA, USA). To analyze the data using quantitative real-time PCR (qRT-PCR), a total of 20 μL of reaction mix was used. The reaction mix was composed of 0.1 μL of each PCR primer, 7.8 μL of nano pure water, 10 μL of Light Cycler® 480 SYBR Green I master mix (Roche Diagnostics Ltd., Lewes, UK), and 2 μL cDNA. Primer sequences of target genes are shown in Table 2. *rpl8* was used as the house-keeping gene. For quantitative analysis, a $2^{-\Delta\Delta}$Ct method was used, and Ct values for the target gene were normalized with the housekeeping gene (Livak and Schmittigen, 2001).

Table 2. Primer sequences of zebrafish used in the present study

Gene		Primer Sequence (5'-3')	
		Forward	Reverse
Housekeeping gene			
	rpl8	ttgttggtgttgttgctggt	ggatgctcaacagggttcat
Thyroid hormone related			
Central regulation	*crh*	ttcgggaagtaaccacaagc	ctgcactctattcgccttcc
	tshβ	ccagaccctccagacagaca	agaagcccatgcagatggtg
Synthesis	*nis*	ggtggcatgaaggctgtaat	gatacgggatccattgttgg
	tg	ctctatcctttcggctggtatg	gaaggagagcggagactaaat
Metabolism	*ugt1ab*	gccagctttgatgaacttgcc	aactcctccagttccttggtt
	sult1st5	cccatccaacttttgcctcg	ggatccccatcacaattgtcct
	dio1	aacttggaggagaggcttgct	agcgcatggagggtcttctt
	dio2	cgcgaaatgggcttgct	ccaggcaaaatctgcaaagtta
Transportation	*ttr*	cgggtggagtttgacacttt	gctcagaaggagagccagtg
	klf9	gggtgactacgatgacggac	ctcctcgccggttagtttgt
Neurotoxicity related			
Neurodevelopment	*mbp*	cagcaggttcttcggaggag	acgaggagaggacacaaagc
	gap43	aaatagacaaaccagacgctgc	cgaacataaagcaggctgtcg
	gfap	ggatgcagccaatcgtaat	ttccaggtcacaggtcag
	c-fos	tgcagcacggcttcaccgag	cgggcatgaagagatcgccgt
	syn2a	gttctgatccggcaacatgc	cagacatgcaaatgcccagg

2.5. Behavioral effects

For the behavioral change, approximately 120 hpf (hours post fertilization) exposed larvae were used. A 96-well plate was used with each well filled with 300 μL medium. In each concentration, a total of 16 larvae per treatment (or control) were randomly selected and transferred to a 96-well plate (1 larva/well) using a pasteur pipet. Before the behavior test, the larvae were acclimated to the experimental condition for >1 hour.

The light and dark transition of each larva was recorded for 70 min with a 10 min acclimation to a dark state, followed by 20 min of 'light-1', 20 min of 'dark-1', and 20 min of 'light-2'. The behavioral parameters, such as total distance moved (mm) and mean velocity (mm/s) were measured. Thigmotaxis was assessed for 60 min. The behavioral parameters such as total distance moved (mm), mean velocity (mm/s), and zone cumulative duration (s), were analyzed.

Behaviors were recorded and analyzed using a Daniovision video tracking system (Noldus, Netherlands), and were simultaneously measured during the testing periods using the Ethovision XT 8.5 software (Noldus).

2.6. Statistical analysis

One-way analysis of variance (ANOVA) with Dunnett's test was conducted to compare the differences between the control and the exposure. Spearman's rank correlation was conducted to understand the direction of the trend. In all statistical results, $p < 0.05$ was considered significant. For the data analysis, SPSS statistical software 25 (IBM Corporation, New York, USA) was used.

3. Results

3.1. Thyroid hormone changes

In the whole body zebrafish larvae, the T4 level measured tended to decrease following a 5 day-exposure to SMCs (MK, HHCB, and AHTN) (Fig. 1). Statistically significant decreases of T4 concentration were observed for MK and HHCB exposure.

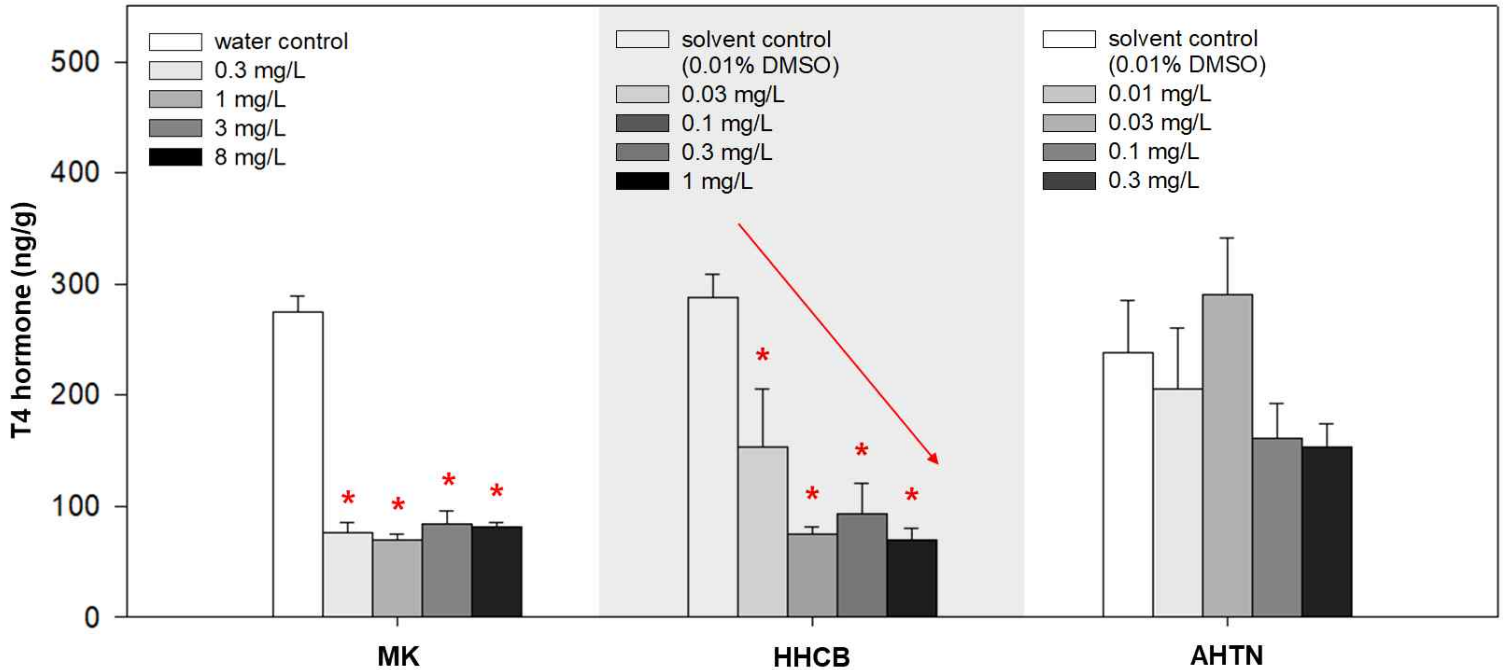


Figure 1. T4 hormone level in the whole body of zebrafish larvae at 120 hpf after exposure to MK, HHCB, or AHTN. All data are shown as the mean ± SEM. Asterisks (*) indicate significant differences ($p<0.05$) from the response of the control.

3.2. Transcriptional changes of key genes

3.2.1. Thyroid hormone related genes

The transcription changes of thyroid hormone related genes in zebrafish larvae samples, following the exposure to each chemical, are shown in Fig. 2.

After exposure to MK, *crhβ* was significantly up-regulated, and *ugt1ab* was significantly down-regulated. Thyroid hormone synthesis related genes such as *nis* and *tg* were down-regulated (Fig. 2A). Following HHCB exposure, *crhβ* and *klf9* genes were up-regulated (Fig. 2B). Other genes such as *tshβ*, *ugt1ab*, *dio1*, and *ttr* were down-regulated. Following exposure to AHTN, most of the thyroid regulating genes including *crhβ*, *nis*, *ugt1ab*, *sult1st5*, and *dio2* were up-regulated (Fig. 2C).

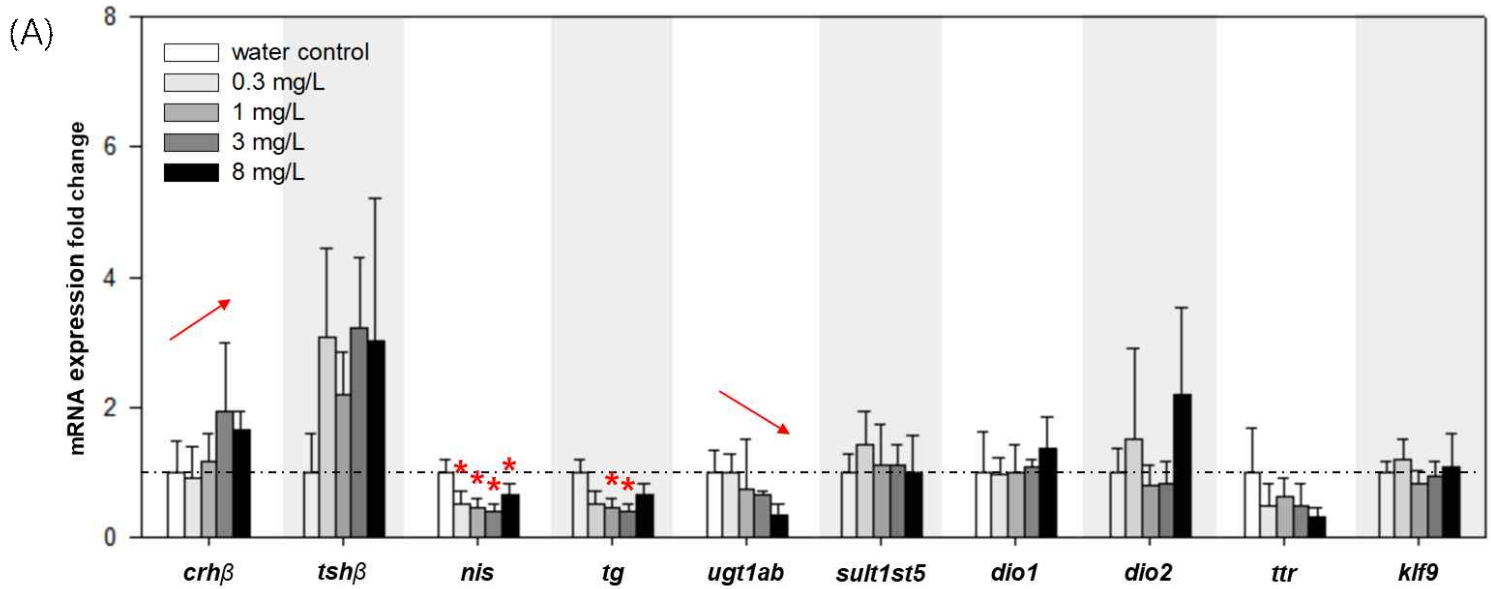


Figure 2. Changes in gene expression related to thyroid system in zebrafish larvae following exposure to MK (A), HHCB (B), or AHTN (C). All data are shown as the mean ± STDEV. Asterisks (*) indicate significant differences ($p<0.05$) from the responses of the water control or solvent control (SC, 0.01% DMSO v/v). Arrow indicates significant trends ($p<0.05$) of each group, according to Spearman's rank correlation analysis.

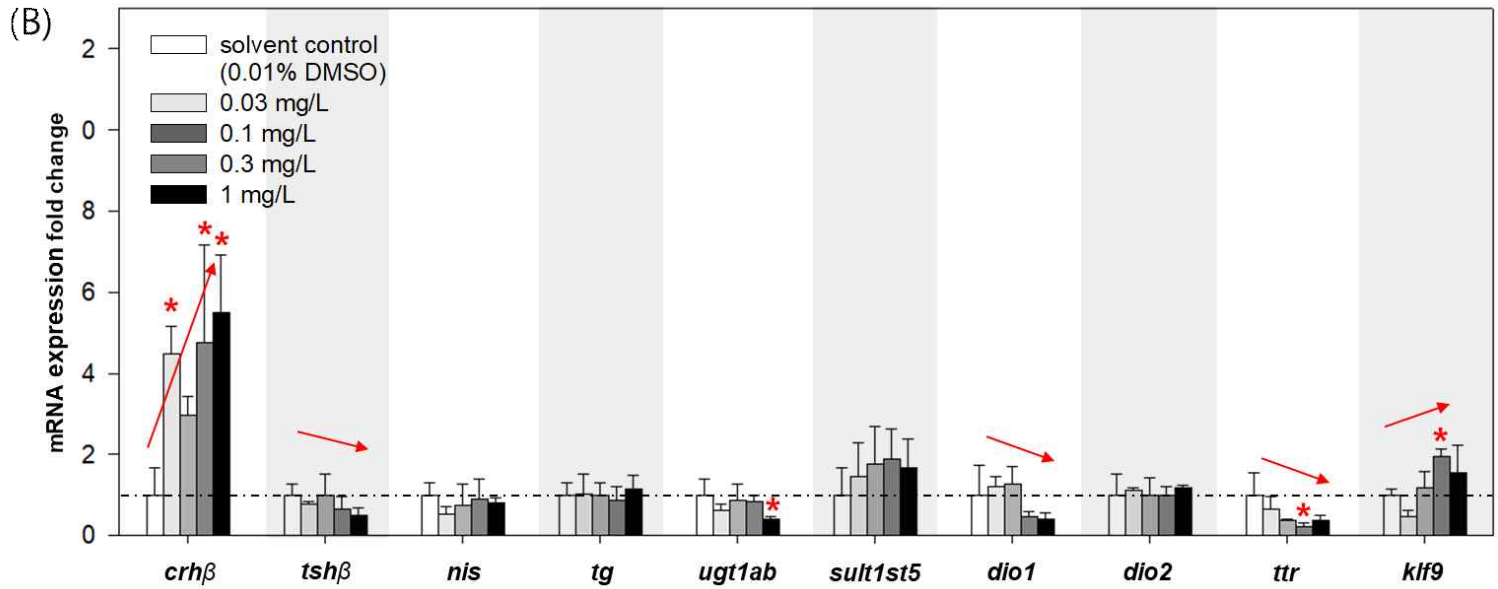


Figure 2. continued.

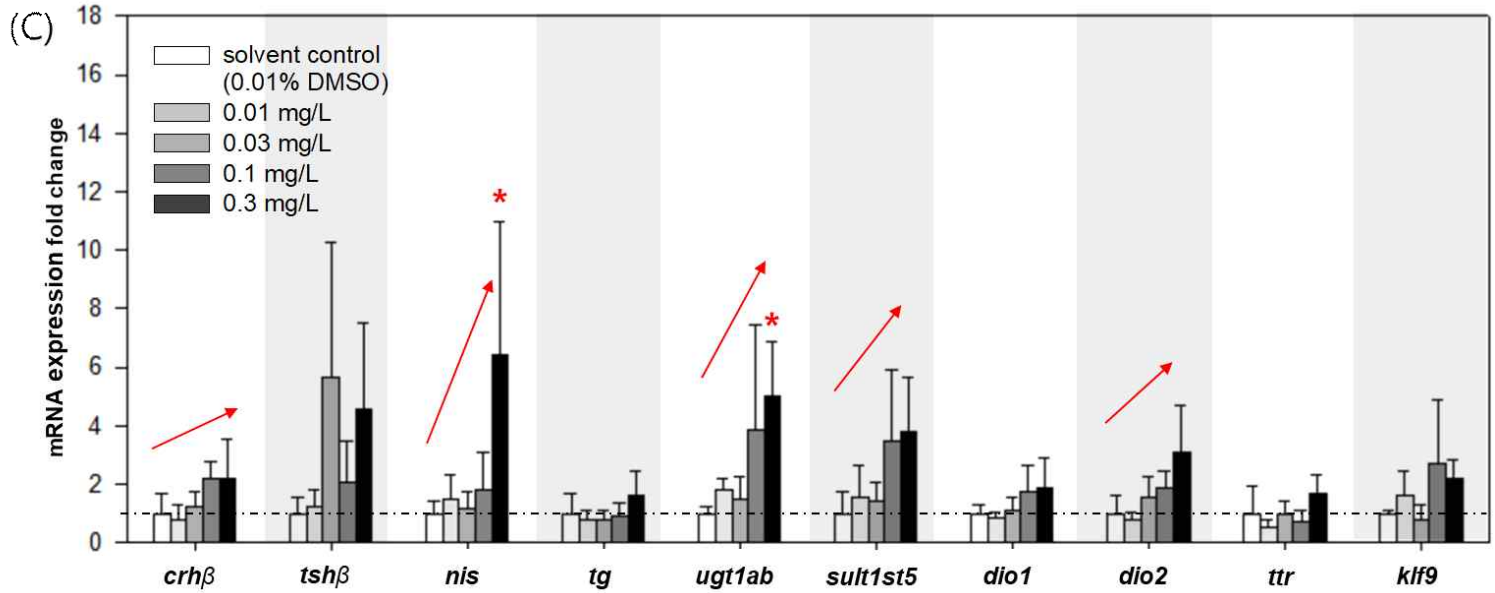


Figure 2. continued.

3.2.2. Neurodevelopment related genes

The transcriptional changes of Neurodevelopment related genes in zebrafish larvae, following the exposure to each chemical, are shown in Fig. 3.

After exposure to MK, *mbp*, and *c-fos* showed a trend of a down-regulation (Fig. 3A). Following to HHCB exposure, *mbp*, *gap43* and *syn2a* exhibited down-regulation trend (Fig. 3B). Following exposure to AHTN, however, *gap43* showed significant up-regulating change (Fig. 3C).

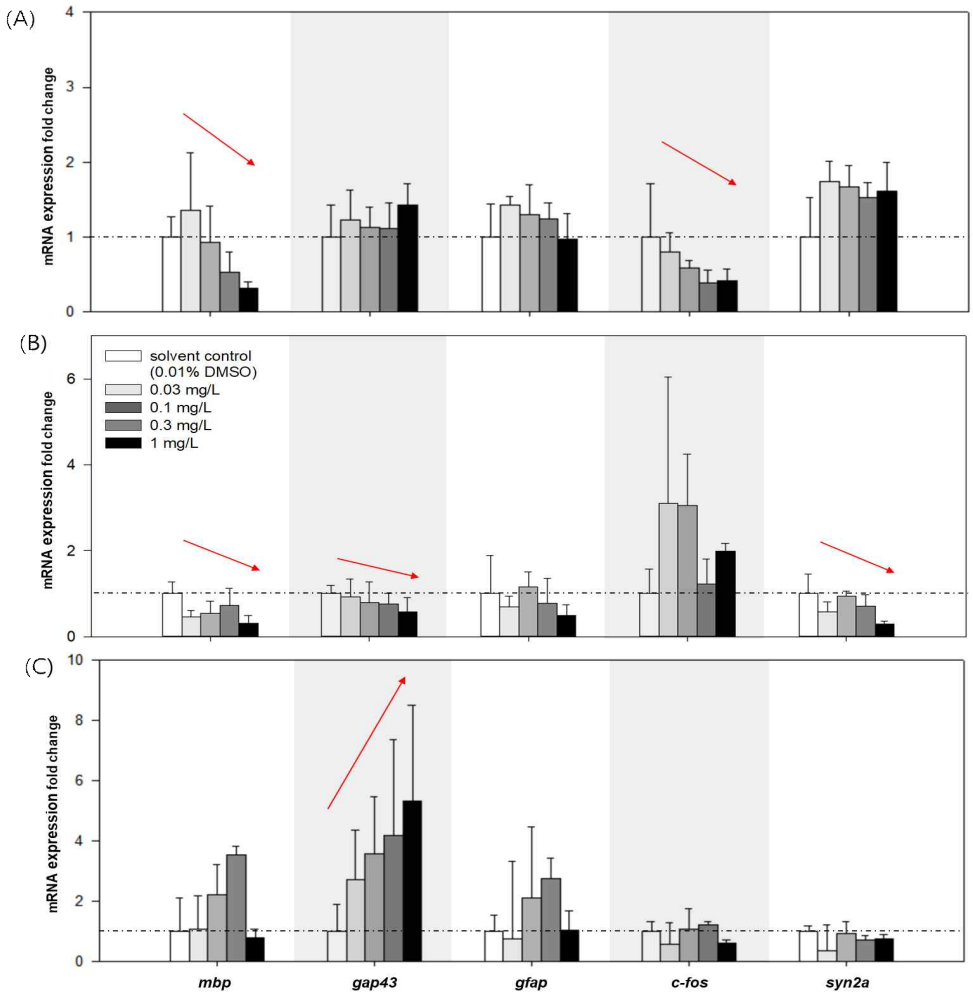


Figure 3. Effects on gene expression related to neurodevelopment in zebrafish larvae exposure to MK (A), HHCB (B), or AHTN (C). All data are shown as the mean ± STDEV of triplicates. Asterisks (*) indicate significant differences ($p<0.05$) from the responses of the water control or solvent control (SC, 0.01% DMSO v/v). Arrows indicate significant trends ($p<0.05$) of each group, according to Spearman's rank correlation analysis. Arrow indicates significant trends ($p<0.05$) of each group, according to Spearman's rank correlation analysis.

3.3. Behavioral changes

3.3.1. Behavioral changes by light stimulation

Following exposure to MK, HHCB, or AHTN, the total distance moved (mm) showed concentration-dependent decreases regardless of light condition. The larval fish moved more in the dark condition. The trend of mean velocity (mm/s) showed a similar pattern of changes to the total distance moved (Fig. 4).

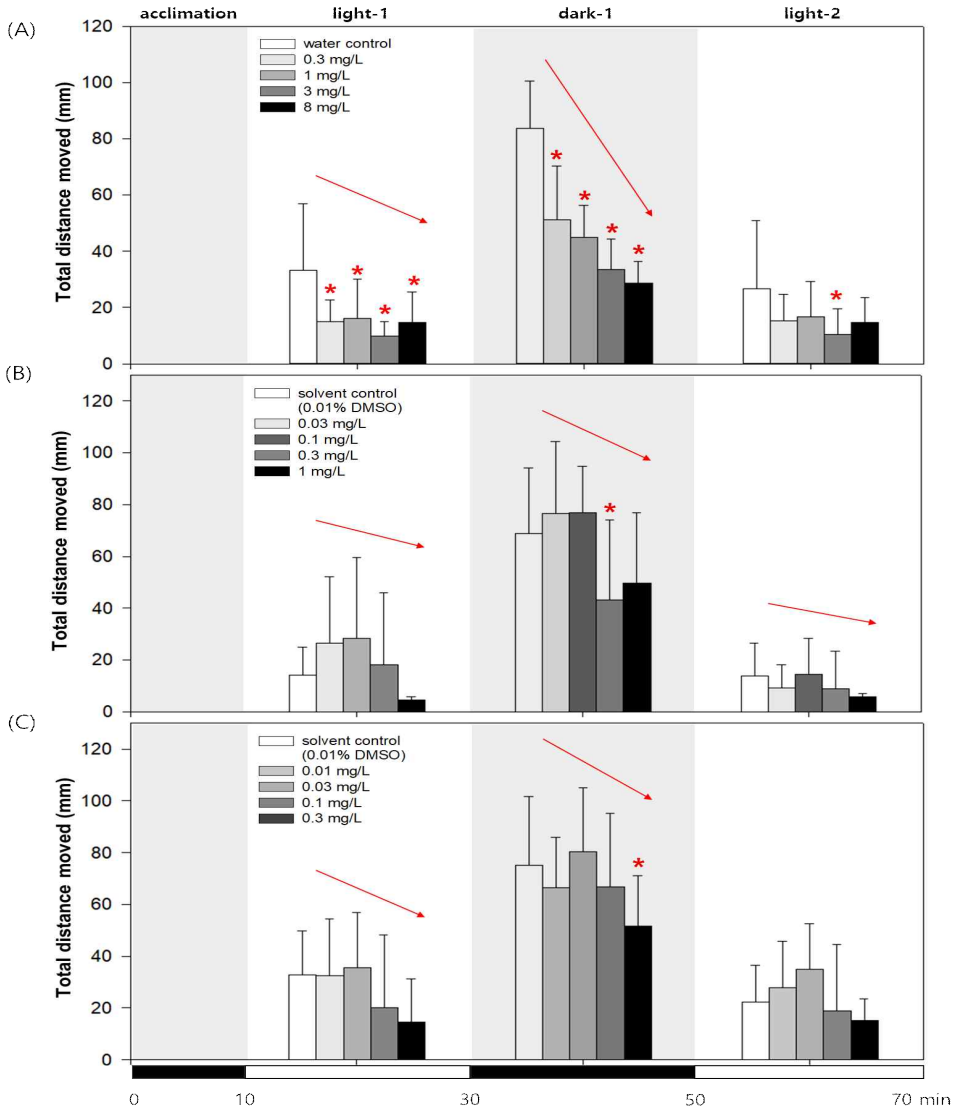


Figure 4. Effects on total distance moved (mm) by light stimulation in zebrafish larvae following exposure to MK (A), HHCB (B), or AHTN (C). Fish were situated under light (light-1), dark (dark-1), and light (light-2) for Aall data are shown as the mean ± STDEV. Asterisks (*) indicate significant differences ($p<0.05$) from the responses of the control. Arrow indicates significant trends ($p<0.05$) of each group, according to Spearman's rank correlation analysis.

3.3.2. Thigmotaxis

With increasing concentrations of MK, HHCB, or AHTN, the larval fish tended to move more in the center compared to the peripheral area of the well. The ratio of total distance moved between peripheral and center is decreased by increasing concentrations of the SMC (Fig. 5). Statistical significance was not observed.

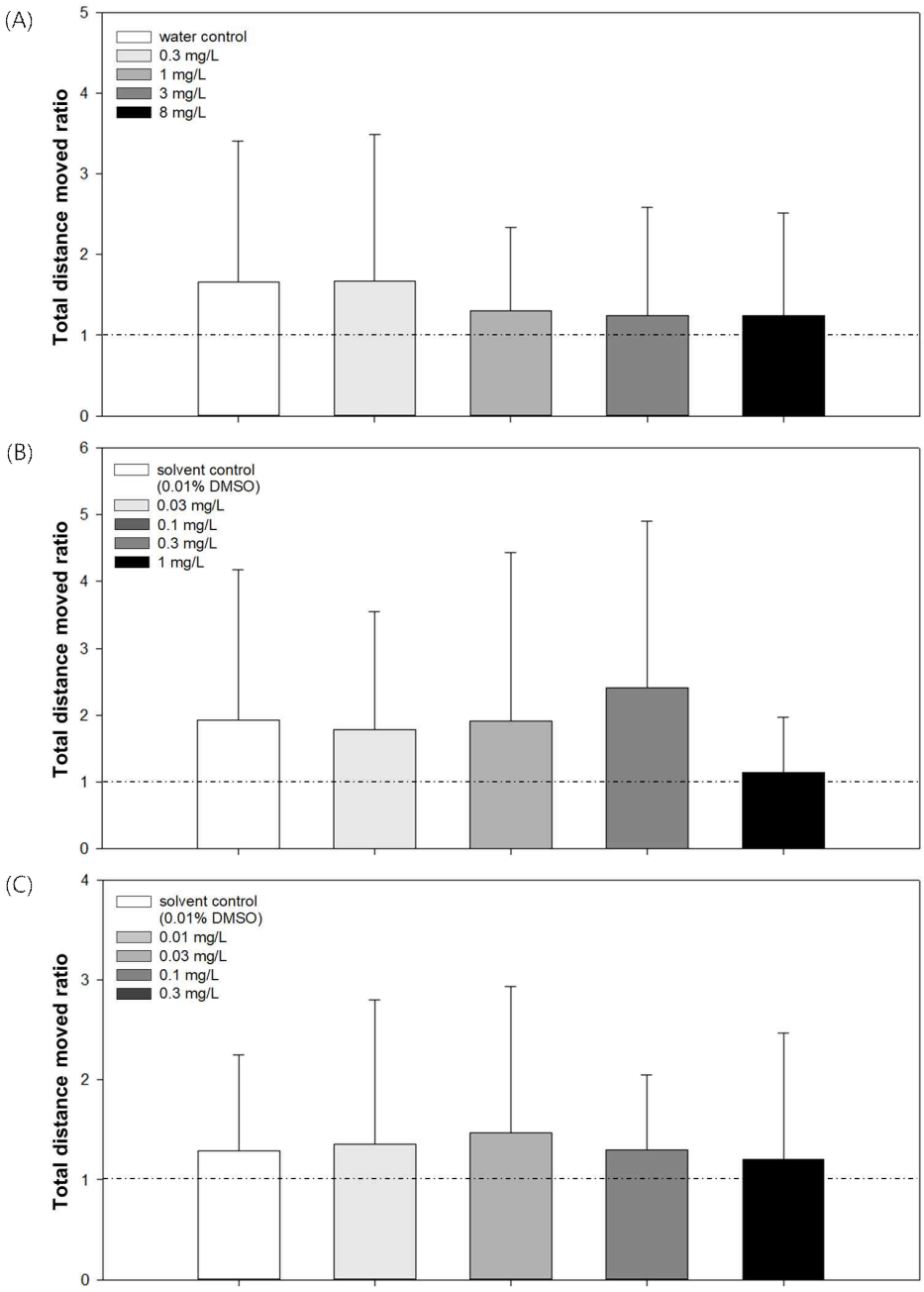


Figure 5. Effects on thigmotaxis in zebrafish larvae following exposure to MK (A), HHCB (B), or AHTN (C).

4. Discussion

4.1. Thyroid hormone disruption

The observation of decreased T4 following exposure to MK, HHCB, and AHTN, shows the thyroid disrupting potentials of the SMCs in the zebrafish (Fig. 1). Thyroid hormone effects of these chemicals have seldom been reported. Regulatory changes of related genes may, to a certain extent, explain these observations.

The same results of decreased T4 were shown for all three substances, but the mechanisms appear to be different. Up-regulation of *crhβ* observed in zebrafish larvae was common following the exposure to MK and AHTN, but the successive transcriptional changes were different by chemical (Fig. 2). Corticotropin releasing hormone (CRH) and thyroid stimulating hormone (TSH) are regulators of the hypothalamic - pituitary - thyroid (HPT) axis (Yu et al., 2013). Up-regulation of *crh* could result in up-regulation of *tsh* in zebrafish larvae, and hence stimulate the synthesis of thyroid hormones. However, the response of *tshβ* gene was not in line with the *crhβ* signal. Moreover, the down-stream genes in thyroid gland, e.g., *nis* or *tg*, showed different pattern of alteration following the exposure to MK and HHCB (Fig. 2). MK exposure led to significant down-regulation of *nis* and *tg*, while HHCB exposure resulted in no meaningful changes in both genes. It appears that following MK or HHCB exposure, signaling of *crhβ* gene was not responded by key genes in pituitary and thyroid gland which are responsible for synthesis of thyroid hormones in the larval fish. Exact mechanisms underlying this observation should further be investigated.

Down-regulation of *ugt1ab* gene was common for both MK and

HHCB exposure, and should be interpreted as a compensatory effort to cope with decreased T4. UGT (uridine diphosphate (UDP)-glucuronosyltransferases) is involved in metabolism of thyroid hormones, e.g, T4 conjugation pathway, and plays an important role in TH homeostasis (Hood and Klaassen, 2000). Previous studies also reported the negative association between T4 levels and *ugt1ab* expression (Chen et al., 2012; Yu et al., 2010; Zhai et al., 2014). This was also confirmed through a previous study reported for zebrafish following BPS or triphenyltin (TPT) exposure (Yao et al., 2020; Zhang et al., 2017). Down-regulation of *ttr* gene should therefore be interpreted as a compensatory effort of the larval fish to increase the concentration of free form of T4. The *ttr* gene is a coding gene for transthyretin which acts as a major transporter of thyroid hormones (Zhang et al., 2021), and hence influence the circulatory levels of free form of thyroid hormones.

For AHTN, different pattern of transcriptional changes is observed. Up-regulation of *crhβ* gene was coincided with seemingly increasing pattern of *tshβ* and up-regulation of *nis* gene. TSH is the primary regulator of *nis* and *tg* genes expression in thyroid glands (Kogai and Brent, 2012). Decreased level of T4 following AHTN exposure can be due to the up-regulation of *ugt1ab* gene. Up-regulation of *dio2* can also be interpreted as a compensatory effort of the larval fish to increase the circulatory level of T3 by increasing deiodination of T4.

Up-regulation of *krueppel-like factor 9* (*klf9*) gene was observed following HHCB exposure, suggesting potential effects on neurodevelopment (Fig. 2C). The *klf9* gene is a T3-inducible gene which plays a key role in neuronal morphogenesis (Denver and

Williamson, 2009; Gilbert et al., 2016). Because of limited amount of specimen, T3 could not be measured in the present study, and hence this observation of up-regulated *klf9* gene should be further confirmed in future investigations.

Thyroid hormone is crucial for development, and because of the roles of thyroid hormones in the early life stage, thyroid disrupting chemicals are more important in the larval fish (Jia et al., 2016). Our observations confirm that exposure to SMCs had a toxic effect on thyroid hormone in the early-life stage of zebrafish. However, information is insufficient to explain the mechanism underlying these findings and warrant further studies.

4.2. Changes in neuronal genes and behavior

Following the exposure, transcription of several neuronal genes were influenced, but the alteration pattern was different by chemical. Significant down-regulation of *myelin basic protein* (*mbp*) or *gap43* genes were observed following the exposure to MK and HHCB, suggesting neurotoxicity potentials of both chemicals. But in AHTN, such alterations were not seen (Fig. 3).

The *mbp* is an important gene in neurodevelopment and synaptic transmission (Pullaguri et al., 2020). Following exposure to MK, *c-fos* gene which has been known as a marker of neuronal activity (Chung, 2015) was also down-regulated. HHCB exposure led to down-regulation of *gap43* gene. The growth associated protein 43 (gap43) encoded by this gene is a crucial component of neuron, playing a critical role in neurite formation. Hence the down-regulations of these genes (Fig. 3A, B) may be interpreted as indicating potential adverse effects of both SMCs in neuronal development in the developing fish larvae.

Interestingly, following the exposure to AHTN, *gap43* gene was up-regulated (Fig. 3C), suggesting different neurotoxic effects by this chemical.

Following exposure to SMCs, behavior of the fish as measured by the total distance moved (mm) was significantly altered, and thigmotaxis showed a changing trend (Fig. 4 and 5). Hypoactivity can be interpreted as a reduced capacity to find food and escape from predators (Paiva et al., 2020; Vannuci-Silva et al., 2019), which may eventually lead to ecological mortality. On the other hand, thigmotaxis, as indicated by the ratio of total distance moved

between the peripheral and the central area of the well, showed decreasing pattern although statistical signficance was not detected. Thigmotaxis or wall hugging, as measure by the tendency to remain close to the wall (peripheral), is considered as an indicator of anxiety (Peng et al., 2016; Schnörr et al., 2012). Ecological implication of decreased thigmotaxis remains to be further investigated.

The neurological effects of SMC seem to be different by chemical. The present observation shows that MK and HHCB have greater neurological and behavioral toxicity potentials than AHTN exposure.

5. Conclusion

MK, HHCB, and AHTN are frequently used SMCs that are also found in water environment. Following a 5 day exposure in zebrafish larvae, MK and HHCB decreased thyroid hormone, and altered transcription of related genes. In addition, several key genes related to neurodevelopment were down-regulated. Moreover, decrease of movement and thigmotaxis were observed, suggesting neurobehavioral effects of these chemicals. Exposure to AHTN, in contrast, showed different patterns of thyroid and neuronal genes, even though thyroid hormones showed the same decreasing trend. AHTN exposure showed decreased movement but showed no effects on thigmotaxis, suggesting different effects on behavior.

Since the environmental levels are several orders of magnitude lower than the experimental concentrations, interpretation of this observation should be cautioned. Considering the importance of thyroid hormone regulation and behavior, ecological implication of this finding warrants further investigation.

6. References

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국문 초록

생애초기발달단계의 제브라피쉬(*Danio rerio*)를 이용한 합성머스크류(MK, HHCB, AHTN)의 갑상선 및 신경독성 영향 연구

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합성머스크류는 천연향료의 대체 목적으로 향수, 화장품, 비누, 섬유유연제와 같은 생활화학제품 및 위생용품 등 광범위한 분야에서 사용되고 있다. 합성머스크류는 일반적으로 친지질성 물질이기 때문에 환경 중 잔류성과 체내 축적 가능성이 있어 주의가 필요하다. 하지만, 현재 합성머스크류에 대한 내분비 및 신경독성 관련 지식은 부족하다.

제브라피쉬 배아(수정 후 4시간 미만)를 5일간 musk ketone(MK), 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta[g]-benzopyran(HHCB), 6-Acetyl-1,1,2,4,4,7-hexamethyltetralin(AHTN)에 노출시킨 후 갑상선 호르몬을 측정하고, 갑상선 및 신경관련 유전자 수준 변화를 살펴보았고 Daniovision® Ethovision XT를 이용하여 물고기의 행동변화를 관찰하였다.

합성머스크류 노출은 제브라피쉬의 갑상선호르몬, 신경발달 및 행동에 유의한 변화를 초래하였다. MK와 HHCB에 노출된 후 T4농도는 감소하

였다. 갑상선 관련 유전자 중 *crhβ*는 유의하게 증가하였으나 뇌하수체와 갑상선의 조절유전자는 이에 상응하지 않았다. 이 두가지 물질 노출에 의해 관찰된 *ugt1ab*의 전사 감소가 갑상선호르몬의 저하를 설명하는 것으로 추측된다. 또한, 신경발달 관련 유전자인 *mbp*, *gap43*, 또는 *c-fos* 등의 전사가 유의하게 감소하였다. 행동학적 변화에서도 농도의존적으로 이동거리가 감소하는 패턴을 보였다. AHTN에 노출된 치어도 T4가 감소하는 경향성이 나타났다. 그러나 유전자 발현의 변화를 살펴본 결과 갑상선호르몬 교란의 기전과 신경발달에 초래하는 영향의 방향이 다른 물질과 상이한 것으로 관찰되었다. 제브라피쉬 치어 노출 결과 MK, HHCB는 갑상선 및 신경발달에 영향을 미치나, AHTN은 상대적으로 갑상선에만 영향을 미치는 것을 확인하였다.

합성머스크류에 노출된 생애초기발달단계의 제브라피쉬는 갑상선 호르몬 조절 및 신경발달 독성을 일으킬 수 있음을 확인하였다. 하지만, 고농도에서 단시간 노출로 인한 독성영향을 확인하였기에, 환경 중 농도에서의 장기노출로 인한 독성연구가 필요하다.

MK: musk ketone; HHCB (Galaxolide®): 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta-(g)-2-benzopyran; AHTN(Tonalide®): 7-acetyl-1,1,3,4,4,6-hexamethyltetralin

주요어: 합성머스크류, 갑상선호르몬, 신경발달, 행동관찰, 제브라피쉬

학번: 2019-29555