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Sexual Differences in Reproductive Toxicity and Transcriptome Analysis to Reveal the Toxic Effects of Short-Chain Chlorinated Paraffins on Zebrafish (*Danio rerio*)

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ABSTRACT

Short Chain Chlorinated Paraffins (SCCPs) are straight-chain chlorinated hydrocarbons with carbon chain lengths between C10 and C13, which are Persistent Organic Pollutants (POPs). The health risks of SCCPs to humans and aquatic organisms are still largely unknown. Currently, the reproductive toxicity of SCCPs generally focuses on only one sex (female or male), and no comprehensive analysis comparing both female and male has been reported. The effect of SCCPs exposure on changes in gene expression in different tissues and organs remains unknown. In this study, female and male AB zebrafish were exposed to 25 μg/L, 50 μg/L, 100 μg/L, 200 μg/L and 400 μg/L SCCPs by gavage. The effect of SCCPs on the development in different tissues and organs of the offspring were assessed by the neutrophils, motor neurons and blood vessels from the hybrid progeny of the transgenic lines. In addition, we performed the RNA-seq analysis of liver, muscle and testis of adult zebrafish, including differential gene expression analysis, protein interaction prediction, enrichment analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) to fill this knowledge gap. Our study found that the effects of SCCPs on zebrafish offspring included the reduction of neutrophils (immune development suppression), blocked neuronal development (malformation of motor neuronal cell development) and thickened blood vessels. The results of RNA-seq provide some molecular evidence that SCCPs increase liver cancer risk, neurotoxicity and reduce sperm quality.

Keywords: SCCPs; Emerging contaminants; Zebrafish; Reproductive toxicity; RNA-seq

INTRODUCTION

Short Chain Chlorinated Paraffins (SCCPs) are straight-chain chlorinated hydrocarbons with carbon chain lengths between C10 and C13, which are widely used in textiles, paints, cosmetics, electronic and electrical products and fishing gear [1]. SCCPs have been officially included in the controlled list of the Stockholm Convention on Persistent Organic Pollutants in May 2017, and have been gradually banned by countries. However, SCCPs are persistent organic pollutants and will still coexist with humans for a long time [2].

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Note: Xiurong Yang and Huiyun Gan contributed equally to this work, so they chosen to have joint Corresponding authorship.

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Since SCCPs are widely distributed in the environmental matrix, human exposure to SCCPs through food, inhalation and skin contact is unavoidable [3]. At present, the health risks of SCCPs to humans and aquatic organisms are still largely unknown, but it is difficult to control experimental variables and collect samples for human exposed samples. Zebrafish (*Danio rerio*), as a model organism widely used in the field of environment and biomedicine, has up to 87% homology with the human genome, physiological and metabolic processes are similar to humans, and have similar organs to humans while also transparent and easy to observe [4].

Previous SCCPs-related studies on zebrafish were conducted on the behavior of embryo, the morphological alterations of embryo, the RNA-seq of embryo, and the metabolome analysis of embryo [5-9]. However, the advantage of transparent observability of transgenic zebrafish embryos is not reflected in these studies, and moreover, transcriptomic studies in embryo were unable to distinguish transcriptomic differences in tissues and organs. Numerous specific promoters of tissues and organs in zebrafish have being successfully cloned, such as the neutrophil-specific promoter coro1a, motorneuron-specific promoter olig2, and the vascular endothelial cell-specific promoter fli1a [10-12]. With the addition of fluorescent protein elements after these promoters, the effects of pollutants on the growth and development of zebrafish tissues and organs can be directly observed under fluorescence microscopy, and the continuous effects of pollutant exposure on offspring can be observed.

SCCPs can be in contact with the immune system through the blood stream and are potentially immune-toxic. However, studies currently reported on the immunomodulatory effects of SCCPs are limited and mostly focused on exposing contemporary immune-toxic responses [13]. SCCPs have been reported to influence glycerol-phospholipid metabolism in animals, which is particularly important for CNS biochemistry because it contains large amounts of lipids, and changes in brain phospholipid levels are associated with many neurological diseases [14]. The effect of SCCPs exposure on neuronal development in the offspring remains unknown. Vascular dysfunction and dysregulation of new vessel formation have been associated with the occurrence and progression of many diseases, including cancer, ischemic diseases, inflammation, and immune disorders, but the effect of SCCPs on vascular development has not been reported [15]. SCCPs has been reported to have reproductive toxicity, but the reproductive toxicity of SCCPs usually focuses on female or male single-sex reproductive toxicity, and to date, no comprehensive analysis of the reproductive toxicity of SCCPs has been reported [16].

To address these gaps, in this study, we evaluated the offspring of transgenic zebrafish strains with specific tissue organs (neutrophils, motor neurons, and vascular endothelial cells) with different concentrations of SCCPs, and explored the sexul differences in the growth toxicity of SCCPs in these offspring. RNA-seq analysis of three key organs (liver, testis, muscle) of exposed zebrafish adults was conducted to analyze the gene level of physiological progression affected by SCCPs exposure in terms of differentially expressed genes, signaling pathway enrichment and interaction protein prediction. Our study aims to provide clues for further scientific research and development of management policies to mitigate the adverse health effects of SCCPs exposure and provide a solid foundation for accurate assessment of human health risks of SCCPs.

MATERIALS AND METHODS

Fish Husbandry and Lines

Zebrafsh (*Danio rerio*) were bred and maintained according to standard procedures, all experiments listed were performed within the relevant guidelines and regulations of the China Zebrafish Resource Center (CZRC) and the experimental protocols were approved by the local Animal Care Committee of Guangxi University. We use the adult wild type AB and the transgenic *Tg (coro1a: EGFP)*, *Tg (fli1a: EGFP)* and *Tg (olig2: DsRed2)* zebrafsh strains for all experiments listed [10,12,17]. All the above zebrafsh strains were purchased from CZRC. Embryos were raised at 28°C on a 14 h light/10 h dark cycle in 90 mm2 petri dishes containing aquaria water. Before spawning, male and female adult fish (male/female ratio: 1/1) were placed separately by isolation boards in spawning boxes overnight. The next morning, turned on the lights and removed the isolation boards, spawning and fertilization started. Embryos were selected, using disposable pipettes to rinse them.

Chemicals and Reagents

SCCPs solution in Cyclohexane (C10-C13/51.5% Cl) were purchased from TMRM Quality Inspection Technology Co., Ltd. (China), CAS no. 85535-84-8. Cyclohexane (AR) were purchased from Fuyu chemical Co., Ltd. (China), CAS no. 110-82-7. Stock solution of SCCPs was dissolved in cyclohexane at a concentration of 100 μg/mL. For intragastric exposure, solutions were diluted in double distilled water with a 0.4% final cyclohexane concentration.

SCCPs Oral Gavage

All zebrafish treated with SCCPs were wild type AB strain zebrafish. The experiment was divided into 14 groups (7 groups in female AB and 7 groups in male AB) with 8 adult zebrafish in each group. They were divided into untreated control group, vehicle control group and gavage groups with different concentrations of SCCPs. The untreated control group was fed normally in the standard breeding system. The zebrafish in the vehicle control group were given 5 μl 0.4% cyclohexane by gavage once a day after anesthesia with a micro-injection tube for three weeks. Zebrafish in the SCCPs group were given 5 μl of 25 μg/L, 50 μg/L, 100 μg/L, 200 μg/L and 400 μg/L SCCPs once a day with a micro-injection tube after anesthesia, and gavage continuously for 3 weeks. During the experiment, zebrafish in all groups were fed 3 meals a day normally, cultured in an incubator at 28°C, and the breeding water was changed once a day.

Reproductive Toxicity in Embryo Evaluation

AB strain zebrafish gavage with SCCPs were copulated with the transgenic *Tg (coro1a: EGFP)*, *Tg (fli1a: EGFP)* and *Tg (olig2: DsRed2)* zebrafsh strains, respectively. The embryos of hybrid offspring of zebrafish were collected for experiments. The vascular thickness, neutrophil count and neuronal fluorescence intensity of 96 hpf (hours post fertilization) zebrafsh embryos were observed and analyzed under microscope (Leica, Solms, Germany).

The reproductive toxicity of SCCPs evaluated by the zebrafish offspring developmental phenotypes, the offspring phenotypes of male and female zebrafish in the above-mentioned groups after gavage were respectively observed and photographed.

The mortality, malformation rate (including yolk sac edema, yolk deformation, hemorrhaging, pericardial edema, spinal curvature, uninflated swim bladder and malformation of tail) of 96 hpf zebrafsh embryos were observed and analyzed under microscope (Olympus, Tokyo, Japan).

Construction of cDNA Libraries and Sequencing

At the end of this experiment, adult AB strain zebrafish were euthanized and dissected, and RNA was extracted from liver, muscle and gonad tissues of control group (n=10) and 400 μg/L SCCPs group (n=10). Total RNA from different tissues of zebrafish was extracted by trizol, and the quality of RNA was detected by agarose gel electrophoresis. RNA samples that passed quality assessment were sent to Beijing Novogene Bioinformatics Technology Co., Ltd. for cDNA library construction and sequencing.

RNA-Seq Sequencing Quality Control

After sequencing, the raw reads were subjected to data quality control and filtering using the fastp (v0.20.1) software (removing reads with adapters, uncertain base calls, and low-quality reads) to obtain clean reads [18]. The resulting clean reads were then assessed for error rates, GC content, Q20, and Q30 to ensure their high quality and suitability for subsequent bioinformatics analyses.

Differential Gene Expression and Enrichment Analysis

First, the zebrafish reference genome file and annotation file were downloaded from the Ensembl website (GRCz11 version). The clean reads were aligned to the reference genome using the Hisat2 (v2.2.1) software [19]. The resulting SAM files were converted to sorted BAM files using samtools (v1.1.4) software [20]. The HTseq-count (v0.6.1p1) software was used to calculate the count numbers for each gene. Differential expression analysis was performed using the edgeR (v1.34.0) package, with the control group as the denominator [21,22]. Differentially expressed genes were selected based on criteria of FDR<0.05 and |log2 FoldChange| ≥ 1. The clusterProfiler (v4.0.5) R package was used for GO and KEGG enrichment analysis of differentially expressed genes [23]. Utilizing the cytoHubba plugin within the Cytoscape software, we identified the top ten hub genes and visualized them for further analysis [24].

Statistical Analysis

The data were statistically analyzed in Microsoft Excel 2016 and Prism 6 (GraphPad, San Diego, CA, USA), and the processed data were presented as the mean ± Standard Error of the Mean (SEM). The statistical analysis was performed using a repeated-measured ANOVA with Fisher's least significant difference to compare the behavioral endpoints (locomotion, path angle, and two-fish interaction) between the treatment and the control. The significance threshold was set at $p<0.05$ for all experiments.

RESULTS

SCCPs Exposure Resulted in More Significant Immuno-developmental Suppression in the Offspring of Female Zebrafish

Workflow of this study is shown in **Figure 1**, adult AB strain zebrafish of both genders were given different concentrations of SCCPs by gavage (5-6 zebrafish per group), and then mated with the transgenic strain mentioned in materials and methods to produce offspring, randomly selected samples under a fluorescence microscope to observe the development of neutrophils, motor neurons and blood vessels. At the endpoint of the experimental, RNA extraction and RNA-seq analysis were performed on zebrafish liver, muscle, and gonads from the untreated control group and 400 μg/L SCCPs gavage group.

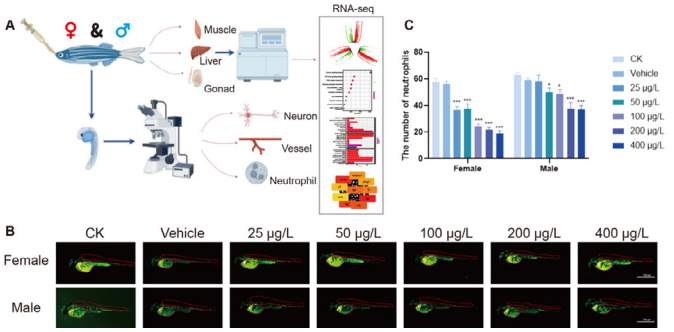

Figure 1: Work flow and the assessment of the inflammatory risk to offspring of SCCPs, (A) The work flow of this study, (B) The neutrophil fluorescence of the offspring of the transgenic zebrafish strain *Tg (coro1a: EGFP)* mated with different concentrations SCCPs gavage zebrafish, the neutrophil count region within the red dotted line. Scale bar=750 μm, (C) The number of Neutrophils of the offspring of the transgenic zebrafish strain *Tg (coro1a: EGFP)* mated with different concentrations SCCPs gavage zebrafish, significance *p<0.05, ***p<0.001. CK represents the untreated control group and vehicle represents the vehicle control group (0.4% cyclohexane)

The immune system is essential for human health and is a target of many invading microbes [25]. In order to explore the effects of SCCPs exposure on the immune system development of zebrafish progenies, we observed and counted neutrophils in the offspring of zebrafish mating *Tg (coro1a: EGFP)* in 14 experimental groups, the statistical areas were the yolk sac and dorsal region within the red dashed line in the above figure, and all groups of zebrafish neutrophils were found throughout the body.

Further counting neutrophils in the region, we found that the offspring of the 25 μg/L SCCPs gavage female group had a significant immunosuppression phenotype with a significant reduction in the number of neutrophils ($p<0.001$), and that neutrophils decreased more significantly with higher concentrations of SCCPs. For the offspring with male gavage, the 50 μg/L SCCPs group began to show a distinct immune development suppression phenotype, and the reduction in neutrophils was also exacerbated with increasing concentrations of SCCPs. Accordingly, it is suggested that the immune system development of the offspring of females would produce a more sensitive response after SCCPs exposure.

The Offspring of Zebrafish Exposed to SCCPs Showed Significant Neurodevelopmental Abnormalities

SCCPs were reported to induce astrocyte activation and neurons death in the gavage mice [26]. We wondered whether the neural development of zebrafish offspring was similarly affected. Therefore, the progenies of zebrafish from these 14 groups mentioned above mating with transgenic strain *Tg (olig2: DsRed2)* were subjected to fluorescence detection of motor neurons, and the fluorescence intensity of neurons in the three segments after

the cloaca of zebrafish was calculated. As shown in **Figure 2**, in both the female and male gavage groups, the fluorescence trend decreased from 100 μg/L SCCPs concentration, including not only neuronal fluorescence, but also the neuronal axons shown an abnormally shortened phenotype in the 200 μg/L SCCPs and 400 μg/L SCCPs groups. Fluorescence intensity statistics were further conducted, the fluorescence intensity of 200 μg/L SCCPs in females group was significantly decreased compared with the CK group ($p<0.01$), and the corresponding male group was also significantly different compared with the control group, but it was not significant than the female gavage group ($p<0.05$). This suggests that high exposure of SCCPs (>200 μg/L SCCPs) resulted in abnormal neuron development in offspring, and for females, the abnormal effect of offspring neurodevelopment is more obvious.

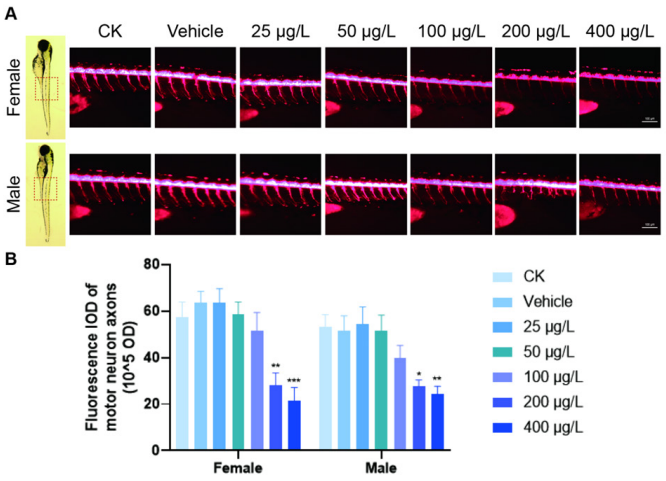

Figure 2: The neuronal development damage to offspring of SCCPs, (A) Motor neuronal fluorescence of the offspring of transgenic zebrafish strain *Tg (olig2: DsRed2)* mated with different concentrations SCCPs gavage zebrafish, Scale bar=100 μm, (B) Statistics of the motor neuronal fluorescence intensity of transgenic zebrafish strain *Tg (olig2: DsRed2)* mated with different concentrations SCCPs gavage zebrafish, neurons in the 3 segments after the cloaca of zebrafish was calculated (10^5 OD),significance *p<0.05,**p<0.01,***p<0.001. CK represents the untreated control group and vehicle represents the vehicle control group (0.4% cyclohexane)

High-concentration of SCCPs Exposure may Increase Cardiovascular Disease Risk

The cytochrome P450 metabolic transformation of SCCPs may lead to a range of adverse effects, including carcinogenicity and more severe damage to the cardiovascular system [27]. Whether there are these risks in zebrafish remains to be investigated. Therefore, we selected the offspring of gavage AB mated with *Tg (fli1a: EGFP)* strain, measured the blood vessel thickness of these offspring. Thickness of the internode blood vessels in the three segments after the cloaca of zebrafish was calculated (μm). As shown in **Figure 3**, no significant developmental abnormalities were observed in the zebrafish internodal vessels in each group. Further measurement and statistical analysis showed that the vascular thickness of zebrafish in the 200 μg/L SCCPs and 400 μg/L SCCPs groups was significantly thickened in both the female and male gavage groups. This suggests that high concentrations of SCCPs (>200 μg/L) exposure may leads to abnormal vascular development in offspring and increase the risk of cardiovascular disease.

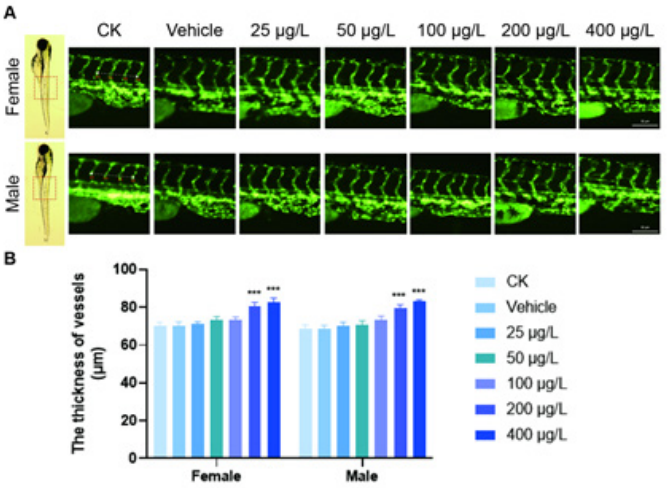

Figure 3: SCCPs stimulate vascular thickening in the offspring of gavage zebrafish, (A) Vascular fluorescence of the offspring of transgenic zebrafish strain *Tg (fli1a: EGFP)* mated with different concentrations SCCPs gavage zebrafish, Scale bar=50 μm. (B) Statistics of the vascular fluorescence intensity of transgenic zebrafish strain *Tg (fli1a: EGFP)* mated with different concentrations SCCPs gavage zebrafish, the average thickness value of 3 vessels after the cloaca (red line indicates vessel thickness), significance ***p<0.001. CK represents the untreated control group and vehicle represents the vehicle control group (0.4% cyclohexane)

Reproductive Toxicity of SCCPs and RNA-Seq Analysis of Testis

According to the results of the offspring of transgenic zebrafish, SCCPs exposure produced immune, neural, and vascular effects on the zebrafish offspring, so we observed the malformation phenotype of the offspring of gavage zebrafish. As shown in **Figure 4**, zebrafish with 100 μg/L SCCPs gavage began to show minor deformities, such as tail shortened or slightly curved, while zebrafish with 200 μg/L SCCPs gavage showed obvious tail curvature. The offspring of 400 μg/L SCCPs gavage zebrafish had the most severe malformation, and even developed a strong deformity of 180° folded and pericardial edema. Other malformation phenotypes are detailed in **Figure 5**.

To further explore the altered zebrafish gene expression caused by SCCPs, we treated the parental untreated CK group and the 400 μg/L SCCPs gavage group AB strain zebrafish by euthanasia sacrifice, dissected three solid organs and tissues, namely, gonad, muscle and liver, and extracted RNA and performed RNA-seq analysis. Unsuccessful extraction of sufficient female ovary RNA in this experiment, considering that the RNA extraction may be affected due to excessive ovarian damage by SCCPs. Therefore, we analyzed the RNA-seq results from successfully extracted testis tissues. In total, we screened 7344 significantly differentially expressed genes in 400 μg/L SCCPs gavage zebrafish testis, of which 5169 were upregulated and 2175 were downregulated. In the testis, the top 10 hub genes include the serpind1, ahsg1, f2, fga, plg, fgg, vtnb, c9, ambp, fgb. For the enrichment analysis, significantly enriched to 12 signaling pathways in testis, including ECM-receptor interaction, Pyrimidine metabolism, Tyrosine metabolism, Glycerophospholipid metabolism, Drug metabolism-other enzymes. The 37 GO entries were significantly enriched in testis, mainly includes external encapsulating structure, extracellular matrix, collagen-containing extracellular matrix, peptidase inhibitor activity, endopeptidase inhibitor activity. Related signalling pathways

such as ECM-receptor interaction, Pyrimidine metabolism, Tyrosine metabolism, Glycero-phospholipid metabolism may be responsive to SCCPs treatment. These results are consistent with the phenotypic results of the offspring, all acting as important pathways affecting the development of the immune system and nervous system.

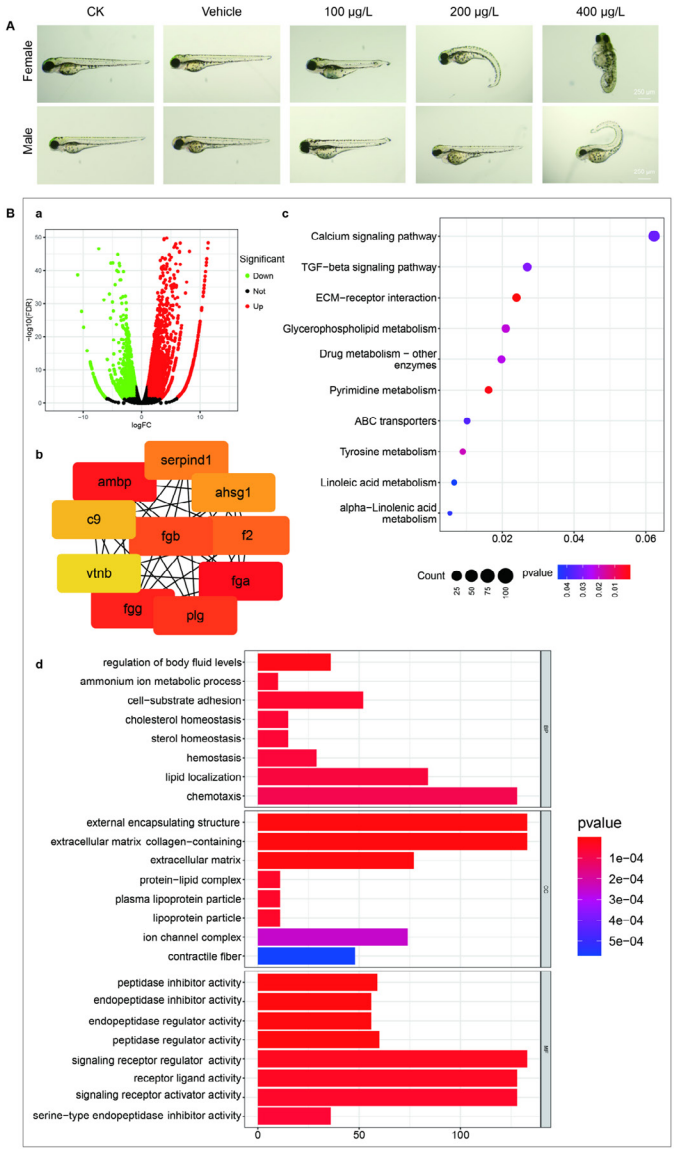

Figure 4: Toxic effects of SCCPs on the development of zebrafish offspring as well as the RNA-Seq analysis of the parental gonads (A) Developmental phenotypes of gavage offspring with different concentrations of SCCPs, Scale bar=250 μm. (B) Transcriptome analysis of testis tissues in 400 μg/L group SCCPs gavage zebrafish versus CK group zebrafish, a) Analysis of differentially expressed genes in 400 μg/L SCCPs zebrafish versus control testis, X axis represents differential fold values after log2 conversion, Y axis indicates significance after-log10-conversion, red represents upregulated differentially expressed genes, green represents down-regulated differentially expressed genes, and black indicates genes with no significant difference, b) Protein-Protein Interaction Analysis, c) KEGG enrichment analysis of differentially expressed genes, d) Bar graph of GO enrichment analysis of differentially expressed genes. CK represents the untreated control group and vehicle represents the vehicle control group (0.4% cyclohexane)

RNA-Seq Analysis of Liver and Muscle

Previous research reported the liver damage and behavioral effects of SCCPs in animals. To explore the genetic alterations responsible for these effects, we analyzed RNA-seq results in muscle tissue, which is closely related to exercise. And the key metabolic organ, liver (**Figure 6**).

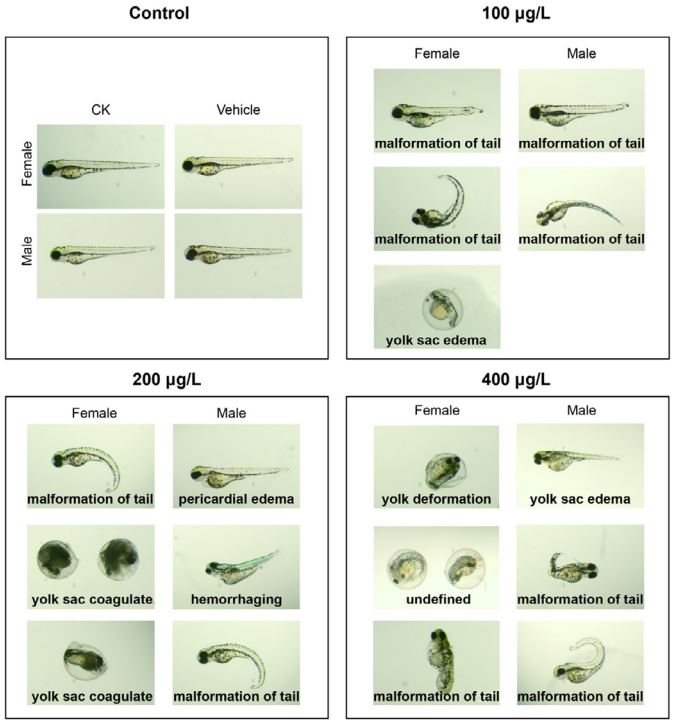

Figure 5: Types of malformation in offspring of zebrafish with SCCPs gavage*Malformation include malformation of tail, yolk sac edema, yolk sac coagulate (dead), pericardial edema, hemorrhaging, yolk deformation and some of undefined types

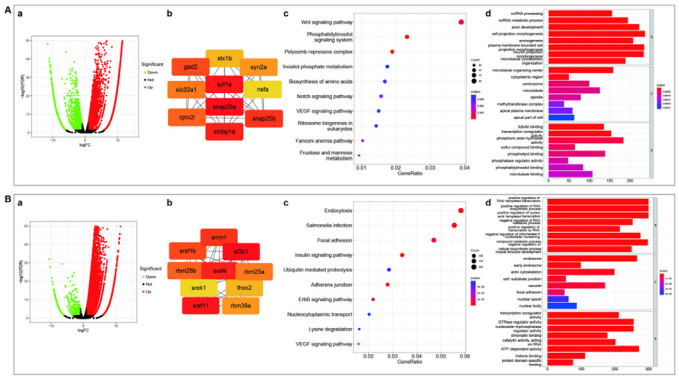

Figure 6: RNA-Seq analysis of liver and muscle tissue of SCCPs gavage zebrafish, (A) Transcriptome analysis of liver tissues in 400 μg/L group SCCPs gavage zebrafish vs CK group zebrafish, a) Analysis of differentially expressed genes in 400 μg/L SCCPs zebrafish versus control liver, X axis represents differential fold values after log2 conversion, Y axis indicates significance after-log10-conversion, red represents upregulated differentially expressed genes, green represents down-regulated differentially expressed genes, and black indicates genes with no significant difference, b) Protein-Protein Interaction Analysis, c) KEGG enrichment analysis of differentially expressed genes, d) Bar graph of GO enrichment analysis of differentially expressed genes, (B) Transcriptome analysis of muscle tissues in 400 μg/L group SCCPs gavage zebrafish vs CK group zebrafish, a) Analysis of differentially expressed genes in 400 μg/L SCCPs zebrafish versus control muscle, X axis represents differential fold values after log2 conversion, Y axis indicates significance after-log10-conversion, red represents upregulated differentially expressed genes, green represents down-regulated differentially expressed genes, and black indicates genes with no significant difference, b) Protein-Protein Interaction Analysis, c) KEGG enrichment analysis of differentially expressed genes, d) Bar graph of GO enrichment analysis of differentially expressed genes

In total, we screened up to 12165 significantly differentially

expressed genes in 400 μg/L SCCPs gavage zebrafish muscle, with 10560 upregulated and 1605 downregulated. In muscle, the potential genes affected by SCCPs include srrm1, sf3b1, rbm25a, thoc2, and rbm39a. Enrichment analysis showed that 44 signaling pathways were significantly enriched in muscle, including Insulin signaling pathway, Endocytosis, ErbB signaling pathway, Adherens junction, and Salmonella infection. In muscle, significantly enriched to 592 GO entries, including transcription coregulator activity, GTPase regulator activity, nucleoside-triphosphatase regulator activity.

In the liver, a total of 9736 significantly differentially expressed genes were screened, of which 6928 were upregulated and 280 were downregulated. In the liver, the genes affected by SCCPs may include gad2, stx1b, syn2a, nsfa, snap25b, and stxbp1a. There are 24 KEGG signaling pathways enriched in liver, such as Polycomb repressive complex, Phosphatidylinositol signaling system, Wnt signaling pathway, Notch signaling pathway. 80 GO items enriched in the liver, including ncRNA processing, microtubule organizing center, ncRNA metabolic process, axon development.

DISCUSSION

Sexual Differences in the Reproductive Toxicity of SCCPs

SCCPs have been repeatedly reported to have reproductive toxicity, but in other species, such as human, rats, mice and livestock, it is difficult to conduct comprehensive high and rapid evaluation of reproductive toxicity [16,28]. Zebrafish have a highly homologous genome and similar tissues and organs to human, which is a great advantage for environmental health risk assessment [29]. SCCPs, a large and complex family of chlorinated n-alkanes with environmental persistence, remote migration potential, and high toxicity to aquatic organisms, are ubiquitous in aquatic ecosystems and can accumulate in aquatic organisms, representing a worldwide hazardous pollutant [30]. The total concentration of chlorparaffin in natural surface water generally ranges from 4 to 1700 ng/L, SCCPs concentration in marine organisms in the Arctic marine food web range from 38.3 ng.g-1 to 687 ng.g-1 lipid weight [31]. In this study, we gavage zebrafish with SCCPs continuously for 3 weeks, and the offspring of the lowest concentration group showed a significant immune development suppression phenotype, indicating that zebrafish are very sensitive to SCCPs in the environment. When exploring SCCPs reproductive toxicity, we found that the offspring produced by SCCPs female gavage were usually more significantly hindered than the offspring produced by males, so we believe that SCCPs was more strongly toxic to female zebrafish. This suggests that in humans, women should more guard against the adverse effects of SCCPs.

In this study, we only explored the immune, neurological and vascular development effects of the first generation offspring of SCCPs exposed, and we are still largely unknown about the reproductive toxicity after the second generation offspring. In addition to SCCPs, there are many new pollutants that coexist with humans for a long time. The results of this study provide a new idea for later studies, which can optimize the exposure scheme, track more generations, and elaborate the reproductive toxicity of pollutants more systematically.

Altered Gene Expression Caused by SCCPs

Gene expression analysis using RNA-seq allows rapid quantification of known expressed genes and discovery of new transcripts, and although mRNA expression does not necessarily fully translate into corresponding changes in its protein levels, it is still valuable for inferring changes after chemical treatment [32]. In previous zebrafish studies related to SCCPs, the transcriptome of embryo overall level was sequenced, but no transcriptome analysis of adult fish tissue was reported. In our study, three key organ and tissue samples were selected for RNA-seq analysis, which supplemented this knowledge gap.

Differential expression analysis in this study, 9,736, 7,344, and 12,156 differential genes were identified in the liver, testis, and muscle of zebrafish, respectively. The results of the enrichment analysis showed that the Polycomb inhibitory complex, Wnt signaling pathway, Fanconi anemia pathway and Notch signaling pathway showed differential gene expression in liver tissue after 400 ng/μL SCCPs treatment. The Wnt signaling pathway is important for cell proliferation, differentiation, and tumorigenesis, and the core function of Wnt signaling is to regulate the phosphorylation and subsequent degradation of cytosolic β-catenin levels, which determines the activation of Wnt response genes [33]. Notch signaling is essential in Endothelial Cells (EC), and Notch signaling is activated by the inducible EC-specific expression of the Notch intracellular domain, and endothelial Notch activation disrupts hepatic homeostasis [34]. The enrichment of these liver cancer-related pathways suggests that SCCPs can disrupt zebrafish liver homeostasis and may induce liver cancer.

Potential genes affected in muscle including srrm1, sf3b1, rbm25a, thoc2, rbm39a, and ect. The sf3b1 gene, which encodes the zebrafish splicing factor SF3B1, is dysregulated, leading to defects on hematopoietic stem cells and also inducing cellular senescence, while also affecting neural crest development [35]. Dysregulation of these genes may be an important cause of the behavioral disorder in zebrafish. In the enrichment analysis of muscle tissue, we also noticed that the ErbB signaling pathway was significantly activated. NRG1/ErbB signaling controls the crosstalk between macrophages and neural crest-derived cells during zebrafish fin regeneration, and activation of this pathway suggests a response to relevant regenerative mechanisms in SCCPs exposed zebrafish organisms after injury [36].

Unfortunately, zebrafish ovarian RNA extraction after SCCPs exposure is difficult, and we failed to perform ovarian RNA-Seq. But in testis, we found some key information. The calcium signaling pathway in testis was upregulated after SCCPs exposure, and it was reported to be involved in sperm activation in multiple species, suggesting that activation signal transduction in zebrafish testis is significantly affected [37]. The candidate genes affected in testis include serpind1, ahsg1, f2, fga, plg, fgg, and etc. Among them, the serine protease inhibitor gene serpind1 is upregulated in response to stimulated infection and responds to inflammation [38]. However, the expression change of fibrinogen gene fgg suggested that SCCPs will stimulate the fibrosis of zebrafish testis, and thus affect the fertility quality of zebrafish [39].

Application Prospect of Zebrafish in the Detection of Emerging Contaminants

Although SCCPs has been banned. However, it is still difficult to detect when the SCCPs concentration is below the instrument detection limit, through this study, we can see the zebrafish in water environment pollutants detection highly sensitive and operable, and in the future we can develop specific detection of transgenic zebrafish, combined with artificial intelligence, to create a simulated zebrafish biological sensor for more quickly detect SCCPs or other pollutants in water. Currently, there are AI-driven phenotyping of zebrafish psychotropic responses and other related studies of tracking and visualization [40]. With the continuous development of biological monitoring technologies and means for the water enviroment, high-precision water quality monitoring of zebrafish will be possible, which will help us quickly detect water quality and assist in the formulation of corresponding environmental protection policies. A zebrafish model for emerging contaminants detection was constructed to provide practical and efficient methods for detecting and monitoring SCCPs in contaminated water. This innovative approach facilitates application to various environmental monitoring, enabling rapid and accurate assessment of SCCPs pollution levels. Identification of the molecular mechanisms and potential biomarkers of SCCPs toxicity in zebrafish also contributes to further expansion of the field of environmental toxicology and deepen our understanding of the adverse effects of SCCPs on aquatic organisms and ecosystems.

These findings contribute to drive knowledge advances in the field of environmental science and provide innovative solutions for SCCPs detection and monitoring. The combination of zebrafish modeling, toxicity assays, and transcriptome analysis represents a novel and deeply explored approach to address the challenges posed by SCCPs contaminated water.

CONCLUSION

Overall, this study shows that SCCPs have significant reproductive toxicity to zebrafish and are more toxic to females than to males. By evaluating the immune system development, neuronal development and vascular development of the offspring of three transgenic zebrafish strains, it was found that the effects of SCCPs include the reduction of neutrophils (immune development suppression), blocked neuronal development (malformation of motor neuron cell development) and blood vessel thickening. Moreover, RNA-seq analysis of liver, muscle and testis of SCCPs gavage contemporary adult fish predicts some potential possibilities that SCCPs increased liver cancer risk, neurotoxicity and reduced sperm quality. Taken together, this study provides insights for assessing the reproductive toxicity and health risks of SCCPs and provides valuable data for the detection and regulation of emerging pollutants in the environment.

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COMPETING INTERESTS STATEMENT

The authors have not disclosed any competing interests.

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