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0# Diesel water-accommodated fraction induced lipid homeostasis alteration in zebrafish embryos☆

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ABSTRACT

To investigate the developmental effects and corresponding molecular mechanism of diesel in freshwater organisms, zebrafish embryos were exposed to 0# diesel water-accommodated fraction (WAF) at different concentrations. Mortality, embryonic morphological endpoints, transcriptional profile and lipid profile were evaluated after exposure. Exposure to 0# diesel WAF had no significant effect on the survival of zebrafish embryos from 1.5 to 96 hpf. However, a significant increase in mortality was observed at 144 and 196 hpf in the groups of 20 and 40 mg/L 0# diesel WAF. RNA-Seq results demonstrated that 0# diesel WAF could induce significant alterations in transcription profile at concentrations of 0.05 mg/L (the limit for petroleum hydrocarbon concentration in surface water in China) and 5 mg/L. Gene Ontology enrichment and similarity analysis indicated that lipid metabolism, lipid synthesis, biological transport, drug metabolism and homeostatic processes were the most altered biological processes after exposure to 0# diesel WAF. Further, transcription levels of genes involved in cholesterol and fatty acid synthesis were significantly inhibited by diesel WAF according to qPCR results. Lipidomics results also indicated that several lipid species (cholesterol ester, fatty acid, diglyceride and triglyceride) decreased after 0# diesel WAF exposure. These results reflect the potential risk of diesel pollution in freshwater ecosystems especially on the alteration of lipid homeostasis and enable a better understanding of the molecular pathways underlying the action of diesel WAF in zebrafish embryos.

1. Introduction

Recently (January 6, 2018) a ship collision accident occurred near the estuary of Yangtze River, resulting in large amount condensate and fuel oil into the offshore of the East China Sea. Although condensate evaporates quickly from seawater, the potential risk of remaining fuel oil towards marine and freshwater ecosystems has aroused widespread concern>.

The occurrence of oil originating from ship accidents or other anthropogenic activities (e.g., ship operation, oil drilling, pipe cracks and transport of bunker fuel) in water environments may have negative effects on aquatic ecosystems (Crone and Tolstoy, 2010; Miljeteig et al., 2013; Won et al., 2016). In a typical freshwater oil spill, approximately 4 million liters of oil were accidentally discharged into the Arroio Saldanha River in 2000, affecting an area of 2.5 km^2, including rivers and small streams in Barigui and Iguacu (Silva et al., 2009). Although these kinds of large oil spills are widely covered in the media, the main source of petroleum contamination in inland waters is thought to be ship leaks into surface water along with small, continuous leakages from underground bulk storage tanks, which reach groundwater and later rivers (Tiburtius et al., 2005). Previous studies have found that crude and fuel oil exposure can induce multiple responses (e.g., oxidative stress, reproductive toxicity, DNA damage, behavioral effects and immune response) in mammals, fish, crustaceans and phytoplankton in marine environments (Monson et al., 2011; Dubansky et al., 2013; Mager et al., 2014; Alloy et al., 2015; Ozhan et al., 2014).

Oil contamination poses considerable threats to the yield and

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quality of aquatic production as well as the personal health of consumers. In consideration of the impact of petroleum hydrocarbon pollution on aquatic organisms, the Chinese government imposed a maximum oil level in surface water of 0.05 mg/L (Ministry of Environment Protection, 2002). However, according to the environmental monitoring results obtained in major fisher areas in China, petroleum hydrocarbon contents exceeding this threshold have been detected in recent years (Ministry of Agriculture and Ministry of Environment Protection, 2014–2016). Therefore, it is necessary to investigate the toxic effects of petroleum contaminants in aquatic organisms to determine the ecological risks of oil pollution.

0# diesel is widely used as a fuel for the diesel engines of ships and vehicles and is one of the main sources of petroleum hydrocarbons in freshwater. Exposure to diesel or its derivatives can induce a variety of toxic symptoms in animals. Studies on goldfish (*Carassius auratus*) have shown that the water-soluble fraction of diesel oil can significantly induce antioxidant defenses at concentrations of 0.05 and 0.1 mg/L over 40 days of exposure (Zhang et al., 2004). Geraudie et al. (2016) reported that diesel shows neurotoxicity towards Icelandic scallops (*Chlamys islandica*) after seven days of exposure. Studies on mammals have also indicated that diesel pollutants cause inflammation, reproductive toxicity and immune response in various organisms (Kisin et al., 2015; Yanamala et al., 2013; Cole et al., 2016). Despite the reported toxicity data, the lipid homeostasis alteration and developmental effects of diesel pollutants remain uncharacterized.

The water-accommodated fraction (WAF) of oil is the fraction that contains the largest amount of water-soluble hydrocarbons, and WAF exposure is considered as an alternative method to evaluate the toxic effects of oil (Mager et al., 2014; Singer et al., 2000; Temkin et al., 2016). Currently, aquatic bioassay has been an frequently approach to evaluate the impacts of hazardous chemicals (Qiao et al., 2016), and zebrafish (*Danio rerio*) embryo is one of the most widely used model for eco-toxicological investigations (Mu et al., 2018; Batel et al., 2018; Kim et al., 2018). In the present study, the mortality, developmental effects, lipid homeostasis alteration and corresponding biological pathways of 0# diesel WAF are investigated using zebrafish embryos as a model. These results reflect the potential risk of diesel pollution to freshwater animals and enable a better understanding of the molecular mechanism of diesel WAF in zebrafish embryos.

2. Materials and methods

2.1. Zebrafish maintenance and embryo collection

Adult wild-type AB zebrafish (10 months old) were obtained from China Zebrafish Resource Center (Wuhan, China). All adult zebrafish were maintained in flow-through feeding equipment (Esen Corp.) at 26 °C with a 14/10 h (light/dark) photoperiod and fed daily with live brine shrimp (*Artemia salina*). The preparation of zebrafish embryos was carried out as described in our previous work (Mu et al., 2013).

2.2. Chemicals and reagents

Commercial 0# diesel was purchased from a local fuel supplier. Standard water was prepared in the lab with the formula of iso-7346-3 (ISO, 1996, Supporting Information).

2.3. Preparation of WAF

0# diesel and standard water was mixed at a ratio of 1:10 (v:v). The mixture was stirred using a magnetic stirrer for 24 h and then allowed to stand for 3 h. The water phase was separated from the mixture as the WAF for further toxicity tests. The amount of total petroleum hydrocarbon (PH) was measured spectrometrically. The total PH content of diesel WAF prepared as described above was generally in the range of 300–500 mg/L. The levels of 16 polyaromatic hydrocarbons (PAHs) were measured chromatographically (see Supporting Information for details).

2.4. Exposure and sample collection

Experiments were performed in accordance with current Chinese legislation and were approved by the independent animal ethics committee at the Chinese Academy of Fishery Sciences.

2.5. Exposure for morphological endpoints

Test solutions of 0# diesel WAF with total PH contents of 0 (control), 0.05 (threshold for PH in surface waters), 0.1, 0.5, 1, 5 10, 20, and 40 mg/L were made using standard water. The concentrations were chosen on the basis of pre-experiment data. Embryos at cell stages 4–16 (1–1.5 h post-fertilization, hpf) were randomly transferred into test solutions in 24-well plates. Twenty wells were used in each plate, and each well contained 2 mL of exposure solution and one embryo. Each treatment was replicated three times. The exposure lasted 8 days, and the embryos were transferred into freshly dosed plates every 24 h. From 144 hpf, the exposure solution for all treatment was prepared with standard water and concentrated paramecium solution (density of 100/mL, Barton, 2007) in order to provide exogenous food resource for larvae. The external conditions during exposure, including the temperature, humidity and light cycle, were the same as in the culture environment.

2.6. Exposure for lethality test

To further confirm the reason for the rise in mortality after 96 hpf, we conducted another lethality test (Exp2). In this test, embryos were exposed to 5, 10, 20 and 40 mg/L 0# diesel WAF in 24-well plates. 20 embryos were put in a plate as a replicate. The exposure period is from 96 to 192 hpf. From 144 hpf, the exposure solution for all treatments was prepared with standard water and concentrated paramecium solution (density of 100/mL, Barton, 2007) in order to provide exogenous food resource for larvae. The exposure process and condition parameters is the same as described in exposure for morphological endpoints.

2.7. Exposure for transcriptomic analysis

Embryos at cell stages 4–16 were randomly transferred into test solutions (0.05 and 5 mg/L) in 1-L beakers. Each beaker contained 500 mL of exposure solution and about 100 embryos, and each treatment group included three beakers. At 96 hpf, 50 hatched (or decorticated) larvae from each replicate (beaker) were collected and washed twice with standard water (25 for RNA-seq and 25 for qPCR validation). The embryo samples were stored at −80 °C until analysis.

2.8. Exposure for qPCR tests

Embryos at cell stages 4–16 were randomly transferred into test solutions (0.05, 5 and 40 mg/L) in 1-L beakers. Each beaker contained 500 mL of exposure solution and 40 embryos, and each treatment group contained three beakers. At 96 hpf, 25 hatched (or decorticated) larvae from each replicate (beaker) were collected and washed twice with standard water for RNA extraction. The embryo samples were stored at −80 °C until analysis.