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Developmental toxicity of Louisiana crude oil-spiked sediment to zebrafish

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ABSTRACT

Embryonic exposures to the components of petroleum, including polycyclic aromatic hydrocarbons (PAHs), cause a characteristic suite of developmental defects and cardiotoxicity in a variety of fish species. We exposed zebrafish embryos to reference sediment mixed with laboratory weathered South Louisiana crude oil and to sediment collected from an oiled site in Barataria Bay, Louisiana in December 2010. Laboratory oiled sediment exposures caused a reproducible set of developmental malformations in zebrafish embryos including yolk sac and pericardial edema, craniofacial and spinal defects, and tissue degeneration. Dose–response studies with spiked sediment showed that total polycyclic aromatic hydrocarbons (tPAH) concentrations of 27 mg tPAH/kg (dry weight normalized to 1 percent organic carbon [1 percent OC]) caused a significant increase in defects, and concentrations above 78 mg tPAH/kg 1 percent OC caused nearly complete embryo mortality. No toxicity was observed in Barataria sediment with 2 mg tPAH/kg 1 percent OC. Laboratory aging of spiked sediment at 4 °C resulted in a nearly 10-fold decrease in sensitivity over a 40-day period. This study demonstrates oiled sediment as an exposure pathway to fish with dose-dependent effects on embryogenesis that are consistent with PAH mechanisms of developmental toxicity. The results have implications for effects on estuarine fish from oiled coastal areas during the Deepwater Horizon spill.

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1. Introduction

The 2010 Deepwater Horizon oil spill resulted in over 4 million barrels of crude oil released from the Macondo well into the Gulf of Mexico (Crone and Tolstoy, 2010). Over 1600 km of coast line were oiled including sandy beaches and coastal marshes, with moderate to heavy oiling of approximately 75 km of Louisiana salt marshes (Barron, 2012; Silliman et al., 2012; Moody et al., 2013). Although concentrations of polycyclic aromatic hydrocarbons (PAHs) significantly declined in the water column after the spill, oiled sediment impacts in many marsh habitats may remain a concern for years or decades (Silliman et al., 2012). PAHs are more persistent in sediment, which can be a sink for persistent toxic compounds and may provide a continuing source of exposure to benthic invertebrates and fish following disturbance events (e.g., dredging, flooding) as well as tidal cycling. Additionally, shoreline containment of oil by marsh vegetation may prolong oil residence at marsh edges (Silliman et al., 2012).

Short-term early life stage exposures to oil products, weathered oils, and specific polycyclic aromatic compounds have been reported to cause a suite of toxic effects in fish that includes edema, hemorrhaging, malformations, cell death, anemia, and impaired fitness (Barron et al., 2004; Incardona et al., 2005; Colavecchia et al., 2007; Billard et al., 2008; Carls and Meador, 2009; Dubansky et al., 2013). Some of these manifestations are similar to dioxin-like toxicity, whereas disruption of cardiac function and morphogenesis may be independent of the aryl hydrocarbon receptor (AhR) pathway (Incardona et al., 2005; Carls and Meador, 2009; Jung et al., 2013). Early life stage toxicity can be manifested within 24 h of exposure at low part per billion concentrations of PAHs (Jung et al., 2013). Overall, ample evidence exists indicating that early life stage exposures to single PAHs and petrogenic mixtures can alter normal fish development through both AhR and AhR-independent mechanisms and that exposure to the complex mixture of hydrocarbons in oil results in multiple mechanism of toxic action controlled by the composition of polycyclic aromatic compounds (Incardona et al., 2005; Billard et al., 2008; Jung et al., 2013).

The zebrafish (*Danio rerio*) embryo-larval development test has become increasingly used as both a rapid fish toxicity test and

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developmental effects assay because of easy maintenance of fish cultures, production of large numbers of offspring, rapid embryonic development, and transparency of embryos for assessment of developmental abnormalities. The repeatability and sensitivity of these tests have resulted in the zebrafish becoming a standard model for understanding specific toxic mechanisms and potential long-term effects of developmental toxicants (Scholz et al., 2008). Additionally, both the genome and embryology of the zebrafish have been well characterized. The initial application of the zebrafish development test targeted the 48 h embryonic development as a proxy for acute toxicity of fish in general (OECD, 2006). While embryo development endpoints have shown good correlation with fish 96 h LC50 values (Bellinger et al., 2013), extending the test to include eleutheroembryos would provide additional endpoints that would increase its utility (Fraysse et al., 2006; Embry et al., 2010). Modifications of the original test to evaluate toxicity of whole sediments expanded its applicability to more environmentally realistic exposure regimes (Hollert et al., 2003).

We used the zebrafish embryo-larval development test to evaluate the early life stage toxicity of lightly weathered South Louisiana crude (SLC) oiled whole sediment. Previous work has determined the effects of water accommodated fractions of a nearly identical oil (Macondo well oil) and provided a model for the molecular mechanisms of crude oil mediated malformations and impaired function in zebrafish (De Soysa et al., 2012; Incardona et al., 2013). In this study, we determine dose–response relationships and median effect concentrations for developmental abnormalities and mortality from environmentally relevant concentrations of oiled sediment. This study demonstrated oiled sediment as an exposure pathway to fish embryos that can affect embryogenesis consistent with PAH mechanisms of developmental toxicity.

2. Methods

2.1. Sediment collection and dosing

Sediment was collected from one heavily oiled location following the DWH spill (Barataria Bay; December 2, 2010), and two non-oiled Northwest Florida reference sites in Choctawhatchee Bay (December 14, 2010) and Escambia Bay (June 19, 2012). The Barataria Bay sediment was collected from a shallow marsh site in Bay Jimmy and was composed primarily of silt and clay materials (surface water: pH=8.3, dissolved oxygen=10.8 mg/L, salinity=10.9 ppt, temperature=15.8 °C). Reference sediment locations were selected for gross similarity to the physical characteristics of Barataria Bay (Supplemental Information). Superficial sediments (< 10 cm) were collected within the intertidal zone at each site using stainless steel scoops, then homogenized, partitioned into glass containers, and frozen and stored at −20 °C until used.

Sediment profiles for each field location were determined using grain size distributions using a sieve analysis (ASTM, 2009). Duplicate 50 g subsamples of each sediment batch were placed in pre-weighed aluminum tins. The first set of samples was dried at 100 °C for 24 h, removed, allowed to cool to room temperature and weighed for percent water. The second set of samples was dried at 35 °C for three days, cooled to room temperature, weighed and broken back into individual grains using a mortar and pestle. The separated sediment was placed in pre-weighed sediment sieves stacked on top of each other in descending order: 500 μm, 125 μm, 62 μm, 32 μm, catch pan. The sediment was separated using an industrial test sieve shaker. Sieves were re-weighed and percent grain size calculated. Total organic carbon was determined using an analysis of PC/PN with a Carlo Erba Elantach Flash EA. Organic content was determined using an optional digestion step prior to analysis. Inorganic carbon was below detection limits for both reference sediments and minimal for the Barataria sediment.

Reference sediment samples were double rinsed with standard dilution water (ISO 7346-3; salinity=0; pH=7.6–7.9; Hollert et al., 2003) prior to spiking to remove salinity and ammonia in the interstitial water. Reference sediments were spiked with laboratory-weathered SLC oil (U.S. EPA Reference Oil Lot# WP 681; obtained from RT Corporation, Laramie WY). Prior to spiking, the crude oil was artificially weathered by rigorous bubbling with nitrogen to an approximate 18 percent reduction in mass through loss of small alkanes and naphthalenes. Reference sediments were spiked at nominal concentrations of 1900, 3800, 7500, 15,000, 30,000 mg oil/kg wet sediment in single concentration batches. To facilitate mixing, one half of the sediment was first spiked with oil followed by the addition of the second half of the sediment. Each batch was mixed in stainless steel containers using a pneumatic paint can shaker for a total of 20 min. A 475 mL sample of the spiked sediment was frozen at −20 °C for chemical analyses. Sediments were refrigerated at 4 °C prior to testing. Barataria Bay sediment was used without manipulation. Although Barataria Bay sediment was collected from 10.9 ppt salinity surface water, 2 mL of dilution water was added to 1 g of the sediment in the test chamber (see Section 2.3), resulting in a test chamber salinity of < 3 ppt.

2.2. Zebrafish culturing and spawning

Adult zebrafish were maintained in 135 L tanks under flow-through conditions using filtered, dechlorinated city water. Water was maintained at a temperature of 26 ± 1 °C, alkalinity of 55 mg/L $CaCO_3$, hardness of 80 mg/L $CaCO_3$, and a pH between 7.2 and 7.9. The photoperiod was 14 L:10D and fish were fed once a day with TetraMin dry flakes (Tetra Sales, Blacksburg, VA, USA) and twice a day with live *Artemia* nauplii to stimulate optimal egg production. Breeding was initiated by placing four males and two females in 12 L spawning chambers 5–7 days prior to test initiation. Chambers had 3.05 mm mesh opening bottoms to allow egg passage onto fine mesh (450 μm) egg collection trays. Simulated day and night phases consisted of 14:10 light:dark photoperiod with artificial light. Because spawning occurs within 30 min of lights turning on in the culture system, eggs were collected within the first 60 min of light exposure by removing the spawning chamber and egg collection screens and transferring eggs to a petri dish containing ISO water. Eggs were sorted to segregate viable embryos at the 4–16 cell stage for use in the tests (< 2 h post fertilization; hpf).

2.3. Zebrafish embryo-larval development tests

Five whole-sediment tests were conducted to assess the impacts of sediment aging on effects concentrations and measure dose–response of zebrafish embryos exposed to sediment spiked with weathered SLC oil. The first three tests were 48 h exposures that were initiated with sediment aged 10, 21, and 38 days after spiking, respectively. The fourth and fifth tests were 96 h exposures initiated within three days of spiking sediment to evaluate developmental abnormalities and sensitivity of zebrafish to SLC oil-spiked sediment.

All tests were initiated by exposing < 2 hpf zebrafish embryos to each of the test concentrations, ISO water control, and a reference (unspiked) sediment control (Hollert et al., 2003). The first four tests also included exposure to the field-collected Barataria Bay sediment. Exposures were conducted with 1 embryo per well in 12-well polystyrene culture plates pre-soaked in zebrafish culture water. Each sediment treatment well contained 1 g sediment and 2 mL ISO water. The ISO water control treatments contained 3 mL water. Sediment was loaded into well plates approximately 24 to 48 h prior to the addition of embryos to allow settling of sediment. The first four tests contained 27 embryos per treatment. The fifth test had a total of 30 embryos per treatment because it did not include the Barataria Bay treatment. Treatments were randomly placed throughout six well plates by column to minimize cross-contamination and incubated at 26 °C on orbital shakers. The shaker speeds were calibrated to 100 rpm to minimize sediment disturbance while providing circulation to maintain dissolved oxygen (Strecker et al., 2011). Dissolved oxygen was measured in the water and sediment control treatments at approximately 4 and 96 hpf using the NeoFox Phase Measurement Systems with a fiber optic oxygen sensor (Ocean Optics AL300).

Embryos were observed every 24 h for 48 and 96 h for tests 1–3 and 4–5, respectively, using dissecting scopes (Nikon SMZ-10A and Nikon SMZ-2B). Because visual detection of abnormalities in fish embryos can be subjective, all observations were made by the same observers trained in zebrafish embryo development. Quality assurance and consistency of observations was assured through double blind observations of embryos, which showed consistent scoring among technicians. Embryos with developmental abnormalities were transferred to polystyrene 96-h well plates and photographed using a Nikon Cool Pix (E 4500) camera mounted to an inverted microscope. Endpoints that indicate lethality for embryos prior to hatching were coagulation of developing embryo, lack of somite formation, lack of heartbeat, and non-detachment of the tail (Embry et al., 2010). Sublethal endpoints assessed were classified as (Kimmel et al., 1995): developmental delays indicated by unresorbed yolk or swollen yolk sac, small embryos, or lack of pigmentation typical of the stage; cardiovascular defects (tube heart, hemorrhaging); edema (pericardial, yolk sac); musculoskeletal defects (tail malformation, length of tail, lordosis, scoliosis); and craniofacial defects (underdeveloped eyes or ears, shortened jaw). Defects were initially scored as specific as possible (e.g., underdeveloped eyes) and later placed into general groups (e.g., craniofacial defects) for statistical analyses.

2.4. Chemical analysis

An aliquot was extracted from reference sediment samples and analyzed for total petroleum hydrocarbons (TPH), petroleum related analytes, heavy metals,