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Exxon Valdez to Deepwater Horizon: Comparable toxicity of both crude oils to fish early life stages

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ABSTRACT

The 2010 Deepwater Horizon disaster in the Gulf of Mexico was the largest oil spill in United States history. Crude oils are highly toxic to developing fish embryos, and many pelagic fish species were spawning in the northern Gulf in the months before containment of the damaged Mississippi Canyon 252 (MC252) wellhead (April–July). The largest prior U.S. spill was the 1989 grounding of the Exxon Valdez that released 11 million gallons of Alaska North Slope crude oil (ANSCO) into Prince William Sound. Numerous studies in the aftermath of the Exxon Valdez spill defined a conventional crude oil injury phenotype in fish early life stages, mediated primarily by toxicity to the developing heart. To determine whether this type of injury extends to fishes exposed to crude oil from the Deepwater Horizon – MC252 incident, we used zebrafish to compare the embryotoxicity of ANSCO alongside unweathered and weathered MC252 oil. We also developed a standardized protocol for generating dispersed oil water-accommodated fractions containing microdroplets of crude oil in the size range of those detected in subsurface plumes in the Gulf. We show here that MC252 oil and ANSCO cause similar cardiotoxicity and photo-induced toxicity in zebrafish embryos. Morphological defects and patterns of cytochrome P450 induction were largely indistinguishable and generally correlated with polycyclic aromatic compound (PAC) composition of each oil type. Analyses of embryos exposed during different developmental windows provided additional insight into mechanisms of crude oil cardiotoxicity. These findings indicate that the impacts of MC252 crude oil on fish embryos and larvae are consistent with the canonical ANSCO cardiac injury phenotype. For those marine fish species that spawned in the northern Gulf of Mexico during and after the Deepwater Horizon incident, the established literature can therefore inform the assessment of natural resource injury in the form of potential year-class losses.

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1. Introduction

Crude oils are complex mixtures that contain thousands of different chemicals. Oils from different geological sources differ primarily in the ratios of large and interrelated families of compounds (Stout and Wang, 2007). These include multiple classes of aliphatic hydrocarbons, aromatic hydrocarbons, resins, asphaltenes, and polar compounds containing nitrogen, sulfur or oxygen atoms (NSO compounds). The relative proportions of these compounds determine the general chemical characteristics of a given crude oil. For example, heavier, sulfur-rich crude oils such as Alaska North Slope crude oil (ANSCO) have proportionally more sulfur-containing aromatic heterocycles than the lighter, low-sulfur crude oils from the *Deepwater Horizon* – MC252 well and other South Louisiana formations (Wang et al., 2003). Within crude oil mixtures, toxicity has been attributed primarily to the proportional content of polycyclic aromatic compounds (PACs) and related families of heterocyclic NSO compounds (Carls and Meador, 2009). These chemicals have higher water solubility relative to aliphatic hydrocarbons and polycyclic alkanes, and they are characteristically taken up by oil-exposed organisms (Council, 2003).

Research spanning the past two decades has revealed a common form of injury among teleost embryos exposed to crude oil. Cardiotoxicity is generally the most sensitive phenotype, and this is primarily evident as fluid accumulation (edema) in the pericardial space or yolk sac. This loss of circulatory function and corresponding change in morphology has been documented for several different crude oils, including Alaska North Slope, Mesa light,

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Iranian heavy, and Bass Strait (Couillard, 2002; Incardona et al., 2005; Jung et al., 2013; Pollino and Holdway, 2002) across a range of freshwater and marine species, including mummichog (*Fundulus heteroclitus* (Couillard, 2002)), zebrafish (*Danio rerio* (Carls et al., 2008; Incardona et al., 2005)), rainbowfish (*Melanotaenia fluviatilis* (Pollino and Holdway, 2002)), pink salmon (*Oncorhynchus gorbuscha* (Marty et al., 1997b)), and Pacific herring (*Clupea pallasi* (Carls et al., 1999; Incardona et al., 2009)). Many of the gross morphological features of the crude oil cardiac toxicity syndrome can be attributed to secondary consequences of reduced circulatory function or heart failure (Incardona et al., 2004). Weathering studies previously showed that aliphatic or volatile monoaromatic compounds are not required to produce this cardiovascular syndrome (Marty et al., 1997b). In the zebrafish model, similarities in phenotype between oil-exposed embryos and embryos with a genetic loss of cardiac function led to the discovery that the most abundant classes of PACs in crude oil, the tricyclic fluorenes, dibenzothiophenes and phenanthrenes, are cardiotoxic (Incardona et al., 2004, 2005). Certain crude oils also cause additional morphological defects that are unrelated to PAC cardiotoxicity, including pectoral and caudal fin blisters in response to ANSCO exposure (Incardona et al., 2005) and tail bud defects in response to Iranian heavy crude oil exposure (Jung et al., 2013). Nevertheless, numerous dose–response studies have consistently shown that the developing fish heart is a key target organ for toxicity across crude oil types, and that cardiac effects are the most sensitive indicators of adverse impacts following both lab and field exposures (Carls et al., 1999, 2008; Hicken et al., 2011; Incardona et al., 2012a, 2012b).

Due largely to the scale of the 1989 *Exxon Valdez* oil spill disaster in Alaska's Prince William Sound, ANSCO has been by far the most intensively assessed crude oil in subsequent years, with numerous studies documenting both acute and long-term effects of embryonic exposure in fish (Carls et al., 1999, 2008; Heintz, 2007; Heintz et al., 1999; Hicken et al., 2011; Incardona et al., 2005, 2009; Marty et al., 1997a, 1997b). The more recent and much larger *Deepwater Horizon* spill in the northern Gulf of Mexico exposed embryos and larvae from a wide diversity of fish species to Mississippi Canyon (MC252) crude oil at varying states of weathering. To address whether MC252 produces canonical cardiac injury in fish early life stages, we used the zebrafish model to directly compare the toxicity of ANSCO alongside several types of unweathered and weathered MC252 oil.

We also developed a standardized protocol for producing oiled water-accommodated fractions (WAFs) that more closely emulate the dispersion of oil droplets under high pressure, as occurred during the *Deepwater Horizon* incident. Previous studies have largely reproduced environmentally relevant exposures to surface spills from ships and other sources. Standardized protocols exist for preparation of low-energy WAFs that model a surface spill; e.g., the method developed through the Chemical Response to Oil Spills: Ecological Effects Research Forum (CROSERF (Singer et al., 2000)). However, these procedures do not entrain oil droplets into the water column, and they produce a dissolved hydrocarbon profile dominated by monoaromatics and 2-ring PACs (e.g., naphthalenes). For example, the CROSERF method using Mesa light crude oil shifted the ratio of alkyl-naphthalenes to alkyl-phenanthrenes from approximately 8:1 in source oil to 60:1 in the WAF (Couillard et al., 2005). We and others have previously demonstrated that high-energy (HE)WAF protocols designed specifically to physically disperse small droplets of crude oil result in more weathered patterns dominated by 3-ring PACs (e.g., 2:1 ratio of alkyl-naphthalenes to alkyl-phenanthrenes) (Barron et al., 2003; Incardona et al., 2005). However, these methods used specialized equipment and procedures that are not readily available or standardized. We therefore established a simple method that generates weathered-pattern HEWAFs from dispersed oil droplets with high reproducibility using commercially available equipment. This method allowed for a systematic assessment of the toxicity of MC252 crude oil following dispersion into droplets under high energy, as occurred in the Gulf of Mexico during release of oil from the damaged wellhead or remixing of oil accumulated at the sea surface during high energy conditions.

2. Materials and methods

2.1. Oil samples

MC252 samples were collected by NOAA personnel in cooperation with BP during the active spill phase and transferred under chain of custody to NOAA scientists at the Northwest Fisheries Science Center (Seattle, WA). These included three different weathered samples from surface skimmers (MC252 skim 1, 2, and 3 oils) and unweathered (source) oil obtained from the riser pipe (MC252 riser oil, sample 072610-03) prior to capping of the well. Skim 1 (sample NSR570-A-1) was collected 12 May 2010 from a skimmer working offshore that had just returned to port. Skim 2 (sample CTC02404-02) was collected 29 July 2010 from a barge holding mixed oil offloaded from a number of different skimmers. Skim 3 (sample GU2888-A0719-OE701) was collected from a skimming transect completed 19 July 2010 by the U.S. Coast Guard Cutter *Juniper*. MC252 riser oil (sample 072610-W-A) was artificially weathered by heating with gentle mixing to 90–105 °C until the mass was reduced by 33–38%. ANSCO was from a previously studied stock that was weathered similarly, except heated to 60 °C until the mass was reduced by 20%. This weathering process nearly eliminated monoaromatic compounds and yielded a modest reduction of naphthalenes in the PAC fraction (Carls et al., 2008; Hatlen et al., 2010; Hicken et al., 2011; Incardona et al., 2005).

2.2. Preparation of water accommodated fractions (HEWAFs)

Two methods were used to prepare high energy HEWAFs. Small-volume (<1 L) HEWAFs were prepared by manual shaking in 500-mL or 1-L glass separatory funnels as described previously (Carls et al., 2008; Hatlen et al., 2010). Larger volume HEWAFs were prepared in a 3-speed commercial food blender with a 1-gal (3.8-L) stainless steel container (Waring CB15, Waring Commercial, Torrington, CT). A blender protocol was developed to produce a distribution of oil droplets/particles that was microscopically similar to the manual method. For each preparation, the stainless steel container was cleaned with acetone and dichloromethane, the rubber lid was lined with dichloromethane-rinsed heavy-duty aluminum foil and the container was filled with 2 L of the artificial fresh water used to maintain a zebrafish husbandry facility at the Northwest Fisheries Science Center (system water). DWH-MC252 riser oil was measured and delivered with a positive displacement pipette onto the surface of the system water in the blender container. The surface-collected skim samples were each a highly viscous mousse and were delivered by weight. A target mass was weighed onto a Teflon sheet and loaded onto the water surface by scraping. Delivered mass was calculated by re-weighing the Teflon sheet. Subsequent HEWAFs with surface samples were made by delivering oil volumetrically with a positive displacement pipette following brief (<5 min) heating at 60 °C to increase fluidity.

Water and oil were blended on the lowest speed (30 s for MC252 riser oil and 2 min for the more viscous surface slick samples) and then 1 L was poured into a 1-L separatory funnel. 200–400 mL were transferred to brown glass bottles and stabilized with 20 mL dichloromethane for analysis of PACs (prefilter sample). After sitting for 1 h to allow surface slick formation, the HEWAF was drained from the separatory funnel (with retention of the slick and ~100 mL