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Geologically distinct crude oils cause a common cardiotoxicity syndrome in developing zebrafish

Jee-Hyun Jung [a], Corinne E. Hicken [b], Daryle Boyd [c], Bernadita F. Anulacion [c], Mark G. Carls [d], Won Joon Shim [a], John P. Incardona [c,*]

[a] Oil and POPs Research Group, Korea Institute of Ocean Science & Technology, 391 Jangmok-ri Jangmok-Myon, Geoje 656-830, Republic of Korea
[b] University of Alaska–Fairbanks Juneau Center, UAF Fisheries Division, 17101 Point Lena Loop Rd., Juneau, AK 99801, United States
[c] Environmental Conservation Division, Northwest Fisheries Science Center, National Oceanic and Atmospheric Administration, 2725 Montlake Blvd. E, Seattle, WA 98112, United States
[d] Auke Bay Laboratories, Alaska Fisheries Science Center, National Oceanic and Atmospheric Administration, 17109 Point Lena Loop Rd., Juneau, AK 99801, United States

HIGHLIGHTS

- ▶ Crude oils from distinct geological sources produce an overlapping cardiotoxicity syndrome in developing zebrafish embryos.
- ▶ The patterns of AHR activation in cardiac tissue depended on the degree of weathering of the oils.
- ▶ Mechanisms of cardiotoxicity of petrogenic PAH mixtures likely shift with changes in mixture composition due to weathering.
- ▶ Data collected on intensively studied crude oils such as ANSCO can help predict the impacts of other oil spills.

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ABSTRACT

Crude oils from different geological formations vary in composition, yet most crude oils contain a polycyclic aromatic hydrocarbon (PAH) fraction that would be expected to produce cardiotoxic effects in developing fish. To determine whether different crude oils or PAH compositions produce common or distinct effects, we used zebrafish embryos to directly compare two crude oils at different states of weathering. Iranian heavy crude oil (IHCO) spilled in the Yellow Sea following the 2007 Hebei Spirit accident was compared to the intensively studied Alaska North Slope crude oil (ANSCO) using two different exposure methods, water-accommodated fractions containing dispersed oil microdroplets and oiled gravel effluent. Overall, both crude oils produced a largely overlapping suite of defects, marked by the well-known effects of PAH exposure on cardiac function. Specific cardiotoxicity phenotypes were nearly identical between the two oils, including impacts on ventricular contractility and looping of the cardiac chambers. However, with increased weathering, tissue-specific patterns of aryl hydrocarbon receptor (AHR) activation in the heart changed, with myocardial AHR activation evident when alkyl-PAHs dominated the mixture. Our findings suggest that mechanisms of cardiotoxicity may shift from a predominantly AHR-independent mode during early weathering to a multiple pathway or synergistic mode with prolonged weathering and increased proportions of dissolved alkyl-PAHs. Despite continued need for comparisons of crude oils from different sources, the results here indicate that the body of knowledge already acquired from studies of ANSCO is directly relevant to understanding the impacts of other crude oil spills on the early life history stages of fish.

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1. Introduction

Systematic comparison of toxic responses to different crude oils could help guide and expedite assessment of recent and future spills. Crude oils from different origins have distinct chemical compositions (Stout and Wang, 2007), hence potentially differing toxicity. A few studies compared the general toxicity (e.g. LC50s) of different types of crude oils and refinery products in aquatic

Abbreviations: AHR, aryl hydrocarbon receptor; ANSCO, Alaska North Slope crude oil; BTEX, benzene, toluene, ethylbenzene, xylene; CYP1A, cytochrome P4501A; HEWAF, high-energy water accommodated fraction; hpf, hours post fertilization; IHCO, Iranian heavy crude oil; OGE, oiled gravel effluent; PAHs, polycyclic aromatic hydrocarbons.

* Corresponding author. Tel.: +1 206 860 3347.
E-mail address: john.incardona@noaa.gov (J.P. Incardona).

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species (Anderson et al., 1974; Neff et al., 2000). The most extensive information on the toxicity of crude oil to developing fish is derived from studies on Alaska North Slope crude oil (ANSCO), following the 1989 Exxon Valdez oil spill (Peterson, 2001). Those studies focused primarily on pink salmon (*Oncorhynchus gorbuscha*) and Pacific herring (*Clupea pallasi*), species impacted by the spill. More recent studies in our laboratory utilized the zebrafish model to dissect mechanisms of developmental toxicity associated with ANSCO and polycyclic aromatic hydrocarbons (PAHs) derived from crude oil and other pyrogenic sources (Incardona et al., 2004, 2005, 2006; Hicken et al., 2011; Incardona et al., 2011). Only a few other studies have examined the effects of geologically distinct crude oils on developing fish, and have focused on different species. This includes the effects of Mesa light and Bass Strait crude oils on mummichog (*Fundulus heteroclitus*) and rainbowfish (*Melanotaenia fluviatilis*), respectively (Couillard, 2002; Pollino and Holdway, 2002). While all of the aforementioned studies point to a common cardiovascular syndrome induced in developing teleost embryos following exposure to crude oil, there have been no systematic comparisons of different oil types using cardiotoxic endpoints, and in a species that is as tractable for mechanistic insight as zebrafish. Our studies in zebrafish indicate that functional and morphological impacts on the developing heart are among the most sensitive indicators of exposure to ANSCO (Carls et al., 2008; Carls and Meador, 2009; Incardona et al., 2009; Hicken et al., 2011). Identification of common mechanisms among other crude oils would be useful in terms of assessing oil spill impacts on the most sensitive and informative endpoints. Insights from past research on ANSCO could help guide and expedite the assessment of more recent oil spills, including the Deepwater Horizon incident, if the cardiotoxic effects of different crude oils to fish early life history stages are similar.

The Hebei Spirit oil spill occurred 7 December 2007 off the coast of South Korea, and was recorded as one of the largest tanker spills of recent years, comparable to the Prestige oil spill in 2002 and Tasman Spirit in 2003 (ITOPF, 2008). Three different kinds of crude oil (United Arab Emirates Upper Zakum, Kuwait export crude and Iranian heavy crude) were spilled. High waves (up to four meters) and northwesterly winds (10–14 m s^{-1}) for several days after the spill led to widespread distribution and stranding of oil on the coastline of Taean County (Kim et al., 2010), a region rich in marine and coastal resources. Many marine species were found dead on rocky shores and beaches and more than 8571 ha of land-based fish aquaculture facilities were directly affected by the crude oil. Hatching success rate in rockfish and flatfish declined to less than 50% in land-based aquaculture facilities using water from spill zones (National Fisheries Research and Development Institute, Republic of Korea, unpublished data). This spill provided the opportunity to directly compare a Middle Eastern crude oil to ANSCO.

Previous studies on ANSCO demonstrated that toxicity to fish embryos increased (per unit mass of total PAHs) as weathering changed the composition of dissolved polycyclic aromatic hydrocarbons (PAHs) toward an enrichment of the tricyclic fluorenes, dibenzothiophenes, and phenanthrenes (Carls et al., 1999; Heintz et al., 1999). Later studies comparing the toxicity of individual parent (non-alkylated) tricyclic PAHs to the toxicity of ANSCO using the zebrafish model showed that the oil-derived PAH mixture (with composition dominated by parent PAHs) produce defects in embryonic cardiac function that were not dependent on activation of aryl hydrocarbon receptors (AHRs) (Incardona et al., 2004, 2005). The AHR family comprises ligand-activated transcription factors that regulate the xenobiotic metabolic response to PAHs (i.e. AHR-mediated), primarily by activating the synthesis of cytochrome P4501A (CYP1A) (Nebert et al., 2004). Individual tricyclic PAHs and lightly weathered ANSCO were both cardiotoxic to zebrafish embryos without activation of AHR in myocardial cells (i.e. AHR-independent), as measured by induction of CYP1A. In assays of single compounds, dibenzothiophene and phenanthrene appeared equipotent, while fluorene produced less severe impacts on cardiac function (Incardona et al., 2004). In contrast, some higher molecular weight PAHs (e.g. benz[a]anthracene) and retene, a C4-alkyl-phenanthrene, caused "dioxin-like" cardiotoxicity that is dependent on activation of the zebrafish AHR2 isoform and is associated with myocardial CYP1A induction (Incardona et al., 2006; Scott et al., 2011). The toxicity of different crude oils may thus depend on an interplay of these (and possibly other) mechanisms, and it is unclear whether simple additivity models for PAH toxicity (French-McCay, 2002; McGrath and Di Toro, 2009) or more complex models combining multiple modes of action (e.g. Barron et al., 2004) are relevant for predicting oil toxicity to fish early life history stages. This question is important for understanding oil spill impacts because different crude oils may contain different ratios of these families of tricyclic compounds. Also, the composition of dissolved PAHs shifts during weathering from dominance by parent compounds to dominance by alkylated compounds suggesting the possibility of a corresponding shift from AHR-independent to AHR-mediated cardiotoxicity.

To both identify the potential for long-term impacts of the Hebei Spirit spill, determine what assays would be best applied in more logistically difficult studies of marine fish, and expand our understanding of basic toxicity mechanisms of different crude oils, we used zebrafish embryos to directly compare the phenotypes associated with exposure to Iranian heavy crude oil (IHCO) from the Hebei Spirit hold to ANSCO. We used two very different methods to produce water with dissolved oil components: high-energy water-accommodated fractions (HEWAFs) and oiled-gravel generator columns (oiled gravel effluent, OGE). HEWAF preparations are designed to mimic the conditions of physical dispersion of oil droplets in an open ocean oil spill under high energy wave conditions, while OGEs are designed to mimic the slow time-release of dissolved PAHs during prolonged weathering of an oiled shoreline. The goals of these studies were to determine (1) if novel phenotypes were associated with exposure to IHCO, (2) whether the effects of IHCO on heart development were similar to ANSCO; (3) how cardiotoxicity might shift with changing PAH composition during weathering; and (4) whether the different crude oils might cause different tissue-specific patterns of CYP1A induction. These studies were not designed to determine thresholds for toxicity or EC_{50}s, and the focus was on the physiological (i.e. cardiac) responses of individual animals.

2. Materials and methods

2.1. Zebrafish exposure

A zebrafish breeding colony (wild type AB) was maintained using routine procedures (Linbo, 2009). Fertilized eggs were collected in water adjusted to a conductivity of approximately 1500–1600 μS cm^{-1}, pH 7.5–8 with Instant Ocean salts ("system water"). Fish were maintained and treated according to an IACUC-approved protocol and anesthetized with ~1 mM MS-222. HEWAFs were prepared by manual shaking in separatory funnels as previously described (Carls et al., 2008; Hatlen et al., 2010). The ANSCO was partially weathered artificially by slow heating until volume was reduced by 20% (Marty et al., 1997). For HEWAF preparations, IHCO and ANSCO were diluted 1:10000 into 100 mL system water (100 ppm oil mass load) and 1:20000 into 1 L system water (50 ppm oil mass load). Gravel was coated with IHCO at a loading of 6 g oil kg^{-1} gravel by manually shaking in an uncoated stainless steel paint can, followed by air drying in a thin layer for 24 h prior to loading into a column. ANSCO oiled gravel (6 g kg^{-1}) was similarly prepared for a prior study, and had been weathered with zebrafish