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Endocrine disruption and differential gene expression in sentinel fish on St. Lawrence Island, Alaska: Health implications for indigenous residents☆

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A B S T R A C T

People living a subsistence lifestyle in the Arctic are highly exposed to persistent organic pollutants, including polychlorinated biphenyls (PCBs). Formerly Used Defense (FUD) sites are point sources of PCB pollution; the Arctic contains thousands of FUD sites, many co-located with indigenous villages. We investigated PCB profiles and biological effects in freshwater fish (Alaska blackfish [*Dallia pectoralis*] and ninespine stickleback [*Pungitius pungitius*]) living upstream and downstream of the Northeast Cape FUD site on St. Lawrence Island in the Bering Sea. Despite extensive site remediation, fish remained contaminated with PCBs. Vitellogenin concentrations in males indicated exposure to estrogenic contaminants, and some fish were hypothyroid. Downstream fish showed altered DNA methylation in gonads and altered gene expression related to DNA replication, response to DNA damage, and cell signaling. This study demonstrates that, even after site remediation, contaminants from Cold War FUD sites in remote regions of the Arctic remain a potential health threat to local residents – in this case, Yupik people who had no influence over site selection and use by the United States military.

1. Introduction

Arctic Indigenous Peoples are among the world's most highly exposed populations to certain persistent organic pollutants (POPs) (Ayotte et al., 1996, 1997; Blais, 2005; Dewailly et al., 1989, 1999; Walker et al., 2003). The Arctic acts as a “cold trap” and is a hemispheric sink for certain POPs that are transported from lower latitudes through atmospheric global distillation (Blais, 2005; Wania and Mackay, 1993, 1996). Once POPs enter the Arctic, their deterioration slows due to low temperatures and low intensity sunlight, which makes them available for long-term incorporation into biological systems (Bard, 1999; Wania and Mackay, 1993). POPs bioaccumulate and biomagnify in the lipid-rich arctic food webs, some to toxic levels (Bard, 1999; Wania and Mackay, 1993). Arctic Indigenous Peoples often subsist on high trophic level, long-lived animals, and thus may ingest levels of POPs that lead to adverse health outcomes, such as thyroid disorders, developmental disorders, and certain cancers (Ayotte et al., 1995; Wania and Mackay, 1993). These findings prompted the inclusion of concern about exposure to POPs by Arctic Indigenous Peoples in the Stockholm Convention on Persistent Organic Pollutants, which bans or restricts some of the world's most toxic POPs, including polychlorinated biphenyls (PCBs) (UNEP, 2001).

PCBs disrupt several endocrine pathways, including the thyroid and sex steroid axes, and, depending upon the particular congener,

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display estrogenic or anti-estrogenic effects (Brown et al., 2014b; Cooke et al., 2001; Letcher et al., 2010; Schell et al., 2008, 2014). PCB exposure in humans induces numerous adverse effects, including low birth weight, slow growth, reduced immunocompetence, cancer, reproductive impairment, neurological impairment, and reduced intelligence (Ayotte et al., 2003; Boucher et al., 2009; Carpenter, 2006; Dallaire et al., 2006, 2014; Lauby-Secretan et al., 2013; Schantz et al., 2003; Weisglas-Kuperus, 1998; WHO, 2016).

Global distillation is not the only source of POPs in the Arctic. Many locally contaminated sites also contribute POPs to the arctic environment with implications for the health of people and wildlife (Brown et al., 2014a). For example, Alaska has approximately 600 formerly used defense (FUD) sites dating from World War II and the Cold War, many of which are located adjacent to Alaska Native villages in remote parts of the state (Scrudato et al., 2012; von Hippel et al., 2016). When the U.S. military abandoned these sites at the end of the Cold War, a legacy of contaminants, including PCBs, remained. Given that the village residents did not elect to use chemicals at the FUD sites or benefit from them, and yet likely suffer the consequences of exposure, these sites present cases of environmental injustice (Rechtschaffen et al., 2009).

A multitude of factors determines the environmental fate of PCBs (Gioia et al., 2013; Lammel and Stemmler, 2012; Macdonald et al., 2005; Scheringer, 2009; Wania and Mackay, 1993). Global distillation results in a shift to a greater mass fraction of lightly-to intermediately-chlorinated PCBs with higher latitude because heavier PCBs are less volatile and condense out of the atmosphere closer to their emission source (Iwata et al., 1993; Scheringer, 2009; Wania, 2003; Wania and Mackay, 1993, 1996). This latitudinal fractionation enriches arctic environments with less chlorinated PCBs (Iwata et al., 1994; Wania and Mackay, 1993). For example, atmospheric deposition results in *tri*-chlorinated congeners predominating in surface sediments of the Bering Sea (contributing 47–92% of total PCBs), followed by *penta*-chlorinated and *hexa*-chlorinated congeners (Hong et al., 2012). Conversely, PCBs derived from point sources such as FUD sites are more likely to be heavier congeners (Muir et al., 2000). Differentiating contamination due to atmospheric deposition vs. local sources is important for site characterization and remediation. For example, PCB contamination due strictly to global distillation could not be ameliorated via removal of materials from a FUD site. On the other hand, detection of PCB contamination from a FUD site could justify the initiation or extension of site clean-up.

The most expensive clean-up of an Alaskan FUD site to date occurred at Northeast Cape on St. Lawrence Island (SLI; Fig. S1). SLI is the largest island in the Bering Sea and has a population of ca. 1600 Siberian Yupik Alaska Natives who live a mostly subsistence lifestyle in two extant villages: Gambell and Savoonga. Northeast Cape is the site of a former Yupik village that the residents of Gambell and Savoonga would like to reestablish; it is actively used for subsistence activities (USATSDR, 2015). The U.S. Air Force acquired the lands at Northeast Cape in 1952 under Public Land Order 790 and established an Aircraft Control and Warning Station (AC&WS) in 1957 (ADEC, 2013b; USBLM, 2015). The Northeast Cape facility operated until 1972 as a surveillance station, first as an AC&WS, then as a White Alice (Alaska Integrated Communications and Electronics) site to detect and provide early warning of enemy aircraft and missile attacks during the Cold War (ADEC, 2013b). At least 30 contaminated locations occur in a 19 km^2 area (ADEC, 2013b). Contamination of soils, surface waters, groundwater, and biota at Northeast Cape derives from fuel spills and releases of PCBs, pesticides, solvents, and metals (ADEC, 2013b; Byrne et al., 2015; Scrudato et al., 2012). Between 1985 and 2014, $120 million was spent on remediation of the FUD site at Northeast Cape, including the removal of structures, storage tanks and drums, and various contaminated wastes, soil and sediments (ADEC, 2013a, b; USACE, 2012). In 2014, the U.S. Army Corps of Engineers determined that sufficient cleanup occurred to cease active remediation activities.

SLI residents have concentrations of PCBs in their blood serum that are about six times higher, on average, than found in people who live in other U.S. states (Carpenter et al., 2005). Additionally, residents of Savoonga who also use Northeast Cape for subsistence activities have higher levels of PCBs in their blood serum than SLI residents who are not associated with Northeast Cape (Carpenter et al., 2005). Together, these findings suggest that atmospheric deposition of PCBs in the Arctic coupled with biomagnification into subsistence foods results in elevated PCBs in SLI residents (Welfinger-Smith et al., 2011), while some residents experience added exposure from military contamination at Northeast Cape (Carpenter et al., 2005).

From the slopes of the Kinipaghulghat Mountains above Northeast Cape, the Suqitughneq River flows north through the coastal tundra and the FUD site into the Bering Sea (Fig. S1). Freshwater habitats accumulate contaminants from their watershed (Luoma and Rainbow, 2008; Sumpter and Jobling, 1995) and fish have excellent utility as sentinels of the aquatic environment (Tierney et al., 2014). Therefore, at the request of SLI residents, we examined congener-specific profiles of PCBs and biological responses in fish collected from the Suqitughneq River to determine: 1) if remaining contamination is due primarily to the FUD site or to atmospheric deposition, 2) if biologically relevant levels of PCBs remain in fish at the conclusion of site remediation, and 3) if health findings in fish have implications for the health of the Yupik people on SLI. We examined two native fish species that commonly inhabit contaminated and clean freshwater sites on SLI: Alaska blackfish (*Dallia pectoralis*) and ninespine stickleback (*Pungitius pungitius*; hereafter 'stickleback'). We previously demonstrated that concentrations of numerous POPs in stickleback tissues closely mirror concentrations in the blood serum of SLI residents, indicating that stickleback are an effective sentinel species on the island (Byrne et al., 2015, 2017).

2. Materials & methods

2.1. Fish collection and processing

Ninespine stickleback and Alaska blackfish were collected in June 2012 and June–July 2013 from the Suqitughneq River at Northeast Cape (Fig. S1), the Tapisaggak River at Northeast Cape (a nearby reference site located in a separate drainage; stickleback only), and Troutman Lake in the village of Gambell (stickleback only). Fish were sampled from the different sites on the same days and were processed with the same protocols. Fish were collected using unbaited 0.32 cm and 0.64 cm wire-mesh minnow traps and therefore represent the sizes that cannot escape through the mesh. Fish were euthanized with an overdose of MS-222 fish anesthetic.

For gene expression studies, in the field we dissected off the head of the fish, cutting directly posterior of the pectoral fins, and isolated the trunk by removing the caudal portion of the fish posterior to the anus, preserving head and trunk immediately in RNAlater, which had access to internal tissues in the trunk from both cut ends. The caudal peduncle and caudal fin were preserved in 95% undenatured ethanol in the field. Other fish were held on ice in the field and then stored at −80 °C until analysis of concentration of PCBs, thyroxine (T_4), and vitellogenin (VTG). Fin clips from many fish were preserved in 95% undenatured ethanol for DNA extraction. All research protocols were approved by the University of Alaska Anchorage Institutional Animal Care and Use Committee (IACUC #159870-20 and 439949-1) and the University of Oregon