Vitamins as Confounding Factors when Measuring PAHs by FACS

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Abstract
Crude oil contamination has commonly been tracked by measuring polycyclic aromatic hydrocarbons (PAHs) in fish bile and tissues using fluorescence. Research on the planktivorous fish species, menhaden, raised questions about other types of fluorescent compounds measured in these assays—particularly vitamins A and E, which are obtained from phytoplankton. Menhaden are fluorescent compounds, and it is possible that these vitamins are confounding factors when trying to measure PAHs using fluorescence. In order to evaluate this scanning fluorescence spectroscopy was used to detect PAH and vitamin standards alone and when combined with fish oil. Fish oil was obtained from an “over the counter brand” (Nature’s Bounty), a prescription brand (Lovaza), a commercial product (menhaden especially important to the commercial fisheries of the Gulf and Atlantic coasts. It polycyclic aromatic hydrocarbons (PAHs). Menhaden is a marine teleost fish that is from the British Petroleum’s DeepWater Horizon Oil Rig on April 20, 2010 has affect fisheries, with high potential of affecting human health. The release of crude oil appears to lower vitamins. Results indicated that vitamins in fish may be confounding factors when detecting PAHs using fluorescence technologies.

Introduction
Cardiovascular disease has been a popular topic for the past few years. Research has shown that cardiovascular disease is one of the most lethal diseases, and is very common in the United States (Go, et al 2012). An effective way to prevent cardiovascular disease is by taking fish oil, which is rich in omega-3 fatty acid, and appears to lower cholesterol.

Crude oil contamination is an ongoing environmental issue that is likely to affect fisheries, with high potential of affecting human health. The release of crude oil from the British Petroleum’s DeepWater Horizon Oil Rig on April 20, 2010 has caused concern for the Gulf of Mexico’s fisheries, in particular the impact of polycyclic aromatic hydrocarbons (PAHs). Menhaden is a marine teleost fish that is especially important to the commercial fisheries of the Gulf and Atlantic coasts. It is an oily, prey species used in the bait and reduction industries as well as for making fish oil. Due to their oily nature, menhaden will likely accumulate crude oil contaminants. Contaminated omega-3 from menhaden fish oil, may expose humans to PAHs. Some PAHs are known carcinogens. For this project, fish oil was obtained from an “over the counter brand” (Nature’s Bounty), a prescription brand (Lovaza), a commercial product (menhaden from DayBrook Industries) and wild menhaden collected in the fall 2010 from the Delaware Bay, NJ (MVNJ) and from Barataria Bay, LA (GILA). GILA fish were collected September 30th, 3 months after the DWH pipeline was capped. Menhaden oil was tested for vitamins A and E. Because it was anticipated that at first order consumers of phytoplankton, menhaden would bioaccumulate high levels of these vitamins. Since vitamins A and E contain an aromatic ring, they were likely to fluoresce under test conditions making them confounding factors when trying to measure PAHs using fluorescence. Analysis of fluorescent aromatic compounds (FACs) is commonly used to monitor PAHs following crude oil spills (Krötschel, et al 2010).

Materials and Methods:

Fish oil was obtained from an “over the counter brand”: Nature’s Bounty (lot DB), Lovaza (a prescription brand provided by Dr. John Sowa, Seton Hall University) Daybrook (DB) (commercial product provided from Daybrook Industries), GILA (wild menhaden from the Barataria Bay, LA in 2010) and MVNJ (wild menhaden collected from the Delaware Bay, NJ in 2010). Menhaden were collected by NJ Fish and Wildlife and LA Wildlife and Fisheries.

Methods:

Fish oil preparation:

The head and tail of the fish were cut off, and the fish filleted and de-boned.

The fillets were cut into smaller pieces and pounded into meal using a glass tube inside a round bottom centrifuge tube. The meal was centrifuged in a round bottom tube for six hours at 10,000 rpm. Following centrifugation, two top layers could be seen, one oil and one aqueous. The bottom of the tube was punctured to separate the two layers.

Vitamin Standards/PAHs Standard Analysis

to extract PAHs, the oil was thawed and mixed by vortexing. In a 1.5 ml microcentrifuge tube, 50 µl of fish oil (0.15 mg of 75% ethanol (EtOH) were combined. The mixture was vortexed continuously for 1 minute and then the oil was separated from the EtOH by centrifugation for 20 minutes at 13,000 rpm. The oil went to the bottom of the tube. One milliliters of the EtOH was removed and placed in a quartz cuvette. Samples were analyzed for fluorescent compounds using two settings on a SpectraMax M5/M5 scanning fluorometer. The first setting involved holding the emission wavelength (Em) at 350 nm and scanning for excitation wavelengths (Ex) from 200 to 350 nm. This setting was best for aromatic hydrocarbons with one or two aromatic rings such as vitamin E (1 ring) and naphthol (2 rings). The second setting involved holding the Em at 450 nm and scanning for Ex from 250 to 450 nm. This setting was best for polycyclic aromatic hydrocarbons with 3, 4 and 5 rings such as hydroxypyrene and for vitamin A even though it has 1 aromatic ring. Figure 7.

Discussion/Conclusion

Based on our data using FACS, vitamins can be a confounding factor when detecting PAHs. Vitamin A and E peaks were detected in wild fish oil and commercial fish oil products. However, commercial fish oil products also contained a major peak at Em450/Ex550. This peak was found in many 4-6 rings PAH standards but not vitamins. Results indicated that vitamins in fish may be confounding factors when detecting PAHs using fluorescence technologies.

Table 1. Fluorescent intensities (RFU) for vitamin and PAH standards at fixed excitation (Ex) and emission (Em) wavelengths. Standards were extracted into 48% or 75% EtOH. Fixed wavelengths were based on literature values used for biomonitoring PAHs in bile (Kreitsberg et al, 2010). Gray boxes indicate the fixed wavelengths used to monitor the naphthalene-like compounds. The combination of HPY and HNP generated one major peak, similar to that seen in all menhaden fish oils. For PAHs fluoresce most strongly at Em350. For vitamin standards: Vitamin A modestly alters their individual spectra with a wavelength of 320-330 nm. This setting was best for aromatic hydrocarbons with one or two aromatic rings such as vitamin A. Vit A peak appeared at 320-330 nm. This peak was best for polycyclic aromatic hydrocarbons with 3, 4 and 5 rings such as hydroxypyrene and for vitamin A even though it has 1 aromatic ring. Figure 7.

References


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