

## Substance in crude oil harms fish hearts, could affect humans as well

January 31 2017, by Taylor Kubota

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Research on tunas in the lab of Stanford Professor Barbara Block identified the substance in crude oil that causes dysfunction in heart cells. This is a Pacific bluefin tuna in the Open Sea exhibit at the Monterey Bay Aquarium. Credit: Monterey Bay Aquarium

Research from Stanford University's Hopkins Marine Station has identified a substance in oil that's to blame for the cardiotoxicity seen in fish exposed to crude oil spills. More than a hazard for marine life exposed to oil, the contaminant this team identified is abundant in air pollution and could pose a global threat to human health.

The pollutant at the center of this finding, phenanthrene, is a type of polycyclic aromatic hydrocarbon (PAH). Due to widespread use of petroleum, PAHs are also found in land-based stormwater runoff, contaminated soil from defunct industrial sites and air pollution. PAHs have been investigated as cancer-causing chemicals for nearly a hundred years but other potential health effects have been given far less attention. The environmental health risks of phenanthrene, in particular, have received secondary consideration to other PAHs more strongly implicated in the development of cancer.

"By carefully isolating heart cells from tunas, Olympians of the sea, and using electrophysiological and confocal microscopy techniques, we recorded ionic currents and found exactly where phenanthrene blocks the heart excitation-contraction coupling pathway, which is the link between the on-off switch, or excitation, and the contraction that powers every heart beat," said Barbara Block, professor in marine sciences. Block was senior author of the paper, published in the Jan. 31 issue of *Nature Scientific Reports*.

## **Wide-ranging potential for disruption of hearts**

The experiments described in the paper provide direct evidence of how phenanthrene impacts the heart, showing how it causes both irregular heartbeat (arrhythmia) and weaker contractions of heart cells. Similar results were found in the heart cells of all three species tested: bluefin and yellowfin tunas and mackerel. At the cellular level, these athletic fish with remarkable aerobic poise are similar to higher vertebrates,

which include mammals and birds. This suggests that the cardio-toxicants may also act upon the hearts of higher vertebrates, as all of these animals have similar methods of regulating the activation of heart cells.

"The mechanism which alters cardiac function in fish and the protein that phenanthrene targets – the ion channel responsible for potassium movement from the cell – is also present in humans," said Fabien Brette, a research associate at Stanford University at the time of the study and co-lead author of the current paper. "What we measured on fish cardiac cells can occur on human cardiac cells and this could mean risk of sudden death."

Urban air pollution, laden with PAHs, has been implicated in cardiac distress. The current study points the finger at phenanthrene, which could enter the bloodstream through respiratory pathways such as breathing.

## **Exxon Valdez and Deepwater Horizon**

Current trends in this field were influenced by research that followed the Exxon Valdez disaster in 1989. The tanker ran aground in Alaska's Prince William Sound and spilled almost 11 million gallons of [crude oil](#) in an otherwise pristine ecosystem. In light of evidence that wild fish exposed to this oil spill exhibited developmental abnormalities, scientists began to look more closely at PAHs. These chemicals were linked to a range of cardiac defects in pink salmon and Pacific herring, species that spawned in oiled coastal habitats in the aftermath of the spill.

"From the results of the Exxon Valdez assessment effort, and from subsequent laboratory studies using the zebrafish experimental model, we hypothesized that the heart cell excitability pathway is a key pharmacological target for PAHs in crude oil," said Nat Scholz, the

leader of the NOAA Fisheries Ecotoxicology Program at the Northwest Fisheries Science Center and a co-author of this study. "However, this remained conjecture for more than a decade, as we didn't have the right expertise and equipment at our facility to test this directly."

In 2010, the Deepwater Horizon disaster resulted in one of the largest [oil spills](#) in history, spilling 4 million barrels of crude oil into the Gulf of Mexico in spring when multiple species of fish spawn, including the Atlantic bluefin tuna. Following this spill, Stanford released research, published in *Science*, showing that crude oil disrupted the heart excitation-contraction coupling pathway in tunas, specifically blocking a critical potassium ion channel.

Now, by using three distinct cardiac electrophysiology approaches on several species of closely related fish, in both atrial and ventricular cells, the same researchers have provided direct evidence that phenanthrene is sufficient to disrupt the normal rhythm and cyclical contraction of isolated heart cells.

## **How the damage happens**

Bluefin tunas are capable of trans-oceanic journeys and the Atlantic species grows to enormous size, exceeding 1,400 lbs. They are challenging to study as few are successfully maintained in captivity.

For over 20 years the Stanford researchers have held bluefin and yellowfin tunas in collaboration with the Monterey Bay Aquarium at a unique facility called the Tuna Research and Conservation Center. Here the tunas swim in captive tanks and provide the capacity for conducting physiological research.

Each beat of a heart is dependent upon the exquisite timing of ion channels opening and closing to pace the cardiac rhythmicity. Once

individual heart cells were isolated from tunas, the researchers used confocal microscopy and patch clamping – a technique enabling electrical recordings of the cardiac ion currents – at Stanford University's facilities at the Hopkins Marine Station to test how the different PAHs affected the strength and the rhythm of the cells' contractions. The researchers showed that phenanthrene disrupts cardiac excitation-coupling, the process linking excitability to muscle contraction, at three key levels.

Using [confocal microscopy](#), the researchers measured changes in calcium ions in individual living muscle cells as an indicator of contractile strength. Patch clamping electrophysiology further revealed the flow of ions through channels that control the normal pace and rhythm of the fish heartbeat.

After exposure to phenanthrene, calcium ion movement into and out of the [heart cells](#) was disrupted and reduced. This exposure also led to irregularities in two major ionic currents, including a significant disruption of the outward flow of potassium ions – a finding consistent with early experiments using whole and chemically complex crude oil.

Importantly, the experiments demonstrated that exposures to low concentrations of individual PAHs, commonly found in the total PAH mixture of crude oil, indicate that the three-ring benzene PAHs (phenanthrene) have distinct physiological impacts. They disrupt the physiology of the heart cell more severely compared to PAHs composed of two and four rings.

The adverse changes in heart muscle cell function manifested within seconds, indicating that exposure to phenanthrene causes disruption immediately and is more than a chronic issue.

"In the short term, cardiac dysfunction in these fish can affect really

metabolically demanding activities like swimming or reproduction or foraging. In the long term, if you have heart function compromised, it could be fatal," said Holly Shiels, associate professor of life sciences at the University of Manchester and co-lead author on this study.

## **From fish to humans**

Further study into the ill effects of phenanthrene is needed and could lead to substantial improvements in our capacity to help fish affected by oil spills and other occurrences of PAH exposures. This could include novel tools to detect this cardiotoxic chemical and new approaches to assessing the impacts of oil spills on fish and other wildlife.

The researchers also suggest that atmospheric phenanthrene deserves more attention for the possible impact it could have on the cardiovascular health of people.

"This paper shows that phenanthrene has the properties of many drugs that cause abnormal heart rhythms as serious and potentially life-threatening side effects," said John Incardona, a developmental biologist with the NOAA Fisheries Ecotoxicology Program in Seattle. "This means that people in urbanized areas with high traffic density are potentially breathing something that has the same properties."

Better understanding of the connection between phenanthrene and human health could potentially lead to insights regarding which aspects of fossil-fuel burning are most harmful and, from there, new options for reducing this harm, such as selecting for crude oils that release less phenanthrene.

**More information:** Fabien Brette et al. A Novel Cardiotoxic Mechanism for a Pervasive Global Pollutant, *Scientific Reports* (2017).  
[DOI: 10.1038/srep41476](https://doi.org/10.1038/srep41476)

Provided by Stanford University

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