REVIEW OF EFFECTS OF PAH EXPOSURES ON MARINE MAMMALS AND A SUGGESTED APPROACH FOR ASSESSING PAH LEVELS AND EFFECTS IN *SOTALIA*

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Abstract:

Historically, studies of the effects of oil and oil related contaminants (i.e., polycyclic aromatic hydrocarbons—PAHs) on marine mammals were few in number, and most concerns regarding effects of oil exposure on these animals were confined to rare, acute events where mortality was documented. However, this approach has changed since the Exxon Valdez spill in 1989. The results of work done since that event document unexpected persistence of toxic sub-surface oil, and chronic exposures, even at sub-lethal levels, have continued to affect wildlife in Prince William Sound, Alaska. An emerging appreciation has occurred that oil effects on wildlife may be substantial over the long-term, through interactions between natural environmental stressors and compromised health of exposed animals that ingest contaminated prey or forage in persistent sedimentary pools of oil. After stress or exposure to pollutants such as crude oil, mammals can respond acutely or chronically in ways that affect homeostasis of the immune or reproductive systems. Such effects may impair long-term survivorship for individuals and, if widespread, affect the status of a population. Evidence suggests that chronic and/or acute exposure to oil may lead to a range of ailments and conditions including skin irritation, conjunctivitis, hepatic and hypothalamic lesions, hepatic necrosis, cancer, and poor survival of offspring born after an acute oil exposure. Support is growing for the inclusion of a range of physiological, biochemical and histopathological evaluations of toxicity. Because the experimental use of marine mammals for optimal toxicological research is often neither desirable nor logistically feasible, little information on the effects of oil may be expected in the future from laboratory studies. This emphasizes the need for an investigative program of marine mammals in the field, which rigorously correlates known and relevant clinical indices of toxicological effects (i.e., biomarkers) with a definitive chemical analysis of hydrocarbon burdens. This type of monitoring approach should make use of stranded marine mammals influenced by both acute and chronic oil exposure.

Introduction

The level and variety of chemical contamination to which coastal and inshore environments are exposed escalates continually. Even small amounts of certain contaminants, when ingested or absorbed, may damage structure or physiological function (Fowler 1993). Most toxicological research addresses human health concerns. With respect to particular species of marine mammals, specific diagnostic toxicology expertise and supporting research are often lacking, in part, because of the diversity of form and function among the 120+ species of marine mammals (Reynolds and Rommel 1999).

Marine mammals fulfill diverse ecological roles, from primary consumers to top carnivores. Their global distribution and range of functional and morphological adaptations means that they collectively are unlikely to possess a common response or set of responses to exposure to particular levels of toxic substances in the environment (O'Hara and O'Shea 2001). In addition, marine mammals are long-lived and have relatively large amounts of body fat (necessary for energy storage, thermoregulation, etc.), placing them at significant risk for accumulating lipophilic organic contaminants. This makes marine mammals both vulnerable to and sensitive indicators of acute and chronic exposure to recalcitrant contaminants.

Significant uncertainties exist regarding effects of contaminants on marine mammals (O'Shea et al. 1999). For example, although numerous studies have documented levels of organochlorines (OCs), polychlorinated biphenyls (PCBs) and even some metals in marine mammal tissues (O'Shea 1999), research to date has rarely clarified either *biological effects* of such levels or dose responses (e.g., on immune function, reproduction, or survival) on individual animals or populations. An international workshop concluded that it is critical to examine effects of toxicants and recommended the use of biomarkers to do so (O'Shea et al, 1999).

Prohibition of dose response research with living marine mammals due to political and ethical concerns means that biological responses to lethal or sub-lethal contaminant exposures cannot be predicted well. The issue of biological effects is especially critical in some parts of the world with regard to polycyclic aromatic hydrocarbons (PAHs), chemical contaminants associated with oil pollution. Despite regional concerns about the effects of oil and gas development, toxicant studies of marine mammals to date have generally focused on body burdens of contaminants other than PAHs (O'Shea 1999).

Toxicity of PAHs

The National Research Council (NRC; 2003) estimated that over 1.3 million metric tons of oil (more than 1.3 billion liters) are released annually into the marine environment. Oil contains thousands of compounds, the most toxic being the PAHs, sixteen of which have been included in the Environmental Protection Agency's and World Health Organization's lists of priority pollutants. Correlations exist between high levels of PAHs and cytotoxic, genotoxic, immunotoxic, and carcinogenic effects on aquatic wildlife (NRCC 1983).

Responses of organisms to petroleum hydrocarbons can be manifested at four levels of biological organization: (1) biochemical, cellular, and tissular; (2) organismal; (3) population; and (4) community. Impairment of behavioral, developmental, and physiological processes may occur at PAH concentrations significantly lower than those that cause death. Chronic effects may impair reproductive performance, immune function, or even survival for individuals and, if widespread, affect the status of a population and community (NRC 2003). Evidence suggests that chronic and/or acute exposure to oil may lead to a range of ailments and conditions including skin irritation, conjunctivitis, hepatic and hypothalamic lesions, hepatic necrosis, cancer, and poor survival of offspring (Engelhardt 1982, Martineau et al. 1994, Loughlin et al. 1996, Peterson et al. 2003).

Marine mammals may be affected by oil through several pathways. As air-breathing organisms that obtain much or all of their food from beneath the water's surface, marine mammals must frequently pass through the air-water interface. When oil is present, pelagic animals may become physically oiled and inhale volatilized oil. Marine mammals using the intertidal zone for foraging and resting may be exposed to even greater levels of physical oiling and inhalation (Geraci 1990). Marine mammals may also ingest prey that has oil or its metabolites in their tissues.

Threats of Petroleum Pollution to *Sotalia* Habitat

Oil pollution is a threat throughout most of the range of *Sotalia*. However, certain areas pose especially great threats. Venezuela and Brazil are the largest oil producers in South America, producing approximately 2.5 and 1.3 million barrels per day (bbl/d), respectively, in 2004.Venezuela ranks ninth in world oil production and fifth in oil exportation with expectations for increased production in the future (EIA 2005). Both countries have extensive domestic oil pipeline systems, providing transportation from production centers to refineries and coastal export terminals. Additionally, in 2004, the crude oil refining capacity (mainly located in coastal areas) of these two countries was 3.18 million barrels per day (bbl/d), and plans exist to build additional refineries. Yet, fossil fuel production, refinement, exportation and in some cases, importation have contributed significantly to the degradation of these countries' natural environments (EIA 2005).

Over the years, oil spills have occurred frequently in both Venezuela and Brazil, causing severe environmental damage. Oil spill incidents in Venezuela, such as a 2001 crude oil spill into the Catatumbo and Tarra Rivers (6.88 million liters) and a 1997 spill of crude oil (4.0 million liters) into Lake Maracaibo are significant, not unusual, and are probably less damaging than the chronic input of oil from these areas. Brazil also suffers from these acute and chronic insults to coastal areas. Some of the worst spills in Brazil have taken place in Guanabara Bay (1.3 million liters crude oil), Barigui River (1.8 million liters crude oil) and Paranagua (429,000 liters fuel oil and 5.1 million liters of methanol) (EIA 2005, EPA 2006).

Due to the severity and frequency of these oil spill insults to the environment, health of these areas, including key habitat for *Sotalia* may be in decline. Although the threat of oil pollution is greatest in Brazilian and Venezuelan waters, it also exists in most *Sotalia* habitat.

Plan for Assessing Effects of Oil on *Sotalia*

Contaminant research can be expensive, and as is the case for all science, careful thought needs to go into experimental design, sample size, and other factors prior to initiating studies. Determination of PAH parent compounds and their substituted homologs by combined gas chromatography-mass spectrometry is important as this provides optimal assessment of body burden concentrations and can be useful in helping to "fingerprint" sources of oil contamination.

Sotalia tissue samples can be obtained from both stranded animals and biopsies of free-ranging animals. Samples from stranded dolphins must be collected within hours of death to permit credible biochemical analyses. In addition to body burden concentrations, analyses exist for evaluating mixed function oxidase system activity and other biomarkers of exposure and effect. These analyses include, for example, ethoxy-, pentoxy-, and benzyloxy-resorufin-*O*-deethylase, (EROD, PROD and BROD), benzo(a)pyrene monooxygenase (BPMO), DNA damage and DNA adducts (Fossi and Marsili 1997).

The following analyses and corresponding tissues or matrices are suggested for both free-ranging and stranded tissue collections:

<u>For free-ranging specimens:</u>

- Body burden concentrations of PAHs- blubber/skin biopsy
- Mixed function oxidase activity and DNA adducts and damage- skin and blood
- Clinical chemistry diagnostic markers-blood

For stranded specimens:

- Body burden concentrations of PAHs- blubber
- Mixed function oxidase activity, DNA adducts and DNA damage, and other biomarkers of exposure- blood, liver, brain, kidney

In addition, it will be useful to attempt to correlate what is found in the dolphins with levels of PAHs in the environment. To do so, we recommend sampling and analysis of sediments, which are repositories for hydrophobic contaminants such as PAHs. As such, sediment associated PAHs are bioavailable for resuspension into the overlying waters inhabited by *Sotalia* and its prey, potentially resulting in food chain accumulation.

With a carefully designed approach and adequate sample sizes, it becomes possible to (a) create baselines against which to assess future impacts, (b) determine areas where PAHs may be having critical effects on individuals and populations, and (c) direct mitigation efforts in optimal ways.

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