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Potential Toxicity Study Outline

Study Objectives:

Shortly after the Cosco Busan oil spill, the shoreline habitat group collected bivalves from different shoreline habitat types throughout the spill zone and measured PAHs in whole body tissues. The bivalve tissues that contained Cosco Busan oil had concentrations that ranged from less than 1 mg/kg total PAH (tPAH; dry weight) to almost 200 mg/kg tPAH. Although studies of exposure and potential effects of PAHs to bivalves are available in the literature, the numbers of studies in which bivalves are exposed to oil (not individual PAHs) and in which relevant endpoints are correlated to a body burden are limited. Therefore, the intent of this investigation is to explore potential health effects (including biochemical, reproductive and metabolic) to a relevant species as a result of exposure and bioaccumulation of Cosco Busan source oil, at body burden concentrations similar to those detected during field collections post-spill.

Study Design:

Phase I (Time Critical): Field collection of mussels from throughout the spill zone to evaluate reproductive status of mussels (proportion of individuals entering into reproductive status and general reproductive stage and development) **during same season** as spill occurred. In addition, an estimation of “baseline” lysosomal stability (with tPAH body burden correlation) would be pursued by measurement of groups of subsamples from throughout the collection areas.

- Adult mussels will be collected from multiple sites from within the spill zone this winter
- Reproductive Index development for field collected mussels
- Measurement of lysosomal destabilization in hemocytes
- Measurement of PAHs in whole body mussel tissue
- Based on presence or absence of mussels at the appropriate reproductive stage, decide whether to proceed with early life stage study (see Phase III b below)

Phase II: Conduct laboratory pilot study to determine appropriate exposure to reach similar ranges of body burdens and PAH profiles as seen in 2007/8 field collected bivalve tissues from within the spill zone.

- Expose mussels to oil during acute laboratory exposure at 8 to 10 concentrations
- Measure PAHs in water and/or mussels
- Estimate PAH concentrations and profiles in mussels from water concentrations, if not directly measured
- Identify the appropriate subset of concentrations to use in the full laboratory study that will result in body burden concentrations across the range of body burden concentrations in field collected bivalves post-spill

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Phase III a: Conduct laboratory study on potential health effects (including biochemical, reproductive and metabolic) of exposure and bioaccumulation of Cosco Busan source oil to *Mytilus*, at body burden concentrations similar to those detected during 2007/8 field collections post-spill

- Obtain commercial stock of immature and adult *Mytilus*
- Expose mussels to range of concentrations of Cosco Busan oil found in the pilot study to result in similar body burden concentrations as field collected bivalves post-spill; following exposure period transfer to clean sea water
- Measure growth in immature mussels
- Measure reproductive status in adult mussels
- Measure lysosomal destabilization in adult mussels
- Measure PAHs in adult and immature mussels

Phase III b: If evidence of active gonadal ripening and/or spawning during winter season found in Phase I field collections, evaluate potential health effects of Cosco Busan oil on early life stages of *Mytilus*.

- Spawn *Mytilus* in the laboratory
- Measure viability endpoints in pre-fertilization gametes and post-fertilization embryos exposed to Cosco Busan oil in the laboratory