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STUDY TITLE: Deepwater Program: Evaluating Sublethal Effects of Exposure to Petroleum Additives on Fishes Associated with Offshore Platforms

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BACKGROUND: Recently, new technology has pushed petrochemical exploration into increasingly deeper water (>305 m) at increased risks to marine fauna. One risk is from chemical additives used to enhance deepwater production, such as ethylene glycol and methanol, which are used during the production and treatment of petroleum to prevent the formation of gas hydrates in deepwater wells and pipelines. Deepwater petroleum production requires that a number of additives, such as ethylene glycol and methanol, be transported and stored offshore in large quantities posing the risk of spills. Although these additives may not be highly toxic per se, it is important to evaluate and understand the risks to marine organisms, especially fishes that are often strongly associated with oil production facilities.

OBJECTIVES: The objectives were to provide an estimate of the 24-h LC50, to identify sublethal exposure levels from which individuals could apparently recover after short-term exposures expected in open marine systems, and to evaluate sublethal effects by

comparing changes in individual swimming performance before and after exposure to ethylene glycol, methanol and their combination.

DESCRIPTION: Juvenile Florida pompano *Trachinotus carolinus* were used in a series of controlled experiments to test the sublethal effects of 3.0% ethylene glycol (EG), 1.07% methanol (MeOH) and a combination of the two chemicals (EG + MeOH) on swimming performance of individual fish. Florida pompano swimming performance was evaluated by comparing differences in pre- and post-exposure critical swimming speeds (U_{crit}) for each individual to quantify sublethal effects. The experimental protocols included identical fasting, exposure, acclimation, and swimming experience for each group and required up to18 days for each experimental trial. An additional ethylene glycol exposure experiment was conducted on a second species, juvenile Atlantic spadefish *Chaetodipterus faber*.

SIGNIFICANT CONCLUSIONS: The results of the juvenile Florida pompano study showed that exposure to 1.07% methanol and 17 hr recovery did little to impair their swimming performance. Sublethal exposure to methanol had only a short-term effect on behavior but little effect on measured swimming performance. The lack of detectable levels of methanol in the plasma samples suggests that pompano completely metabolized methanol (probably into formaldehyde and formate products) during the ~ 20-hr swimming and recovery process. In contrast, the mean percentage (±1 SE) of ethylene glycol still present in pompano plasma samples ~ 20 h after exposure was 0.623 ± 0.04% (v/v).

The reduction in swimming performance of juvenile Florida pompano (a 13.0% reduction) exposed to ethylene glycol was substantially higher than the reduction in performance of Atlantic spadefish (a 6.9% reduction). Spadefish may be more efficient at buffering against or removing the metabolite responsible (glycolic acid) for the toxic effects of ethylene glycol.

Pompano exposed to the combination of ethylene glycol and methanol exhibited the most profound reduction in swimming performance. Fish that were exposed to the combination of ethylene glycol and methanol performed far worse than fish exposed to the single concentration of ethylene glycol. Methanol and ethylene glycol may become more toxic when fish are exposed to a combination of both chemicals. Similar joint action or additive interactions occur when toxicants have similar modes of action but act independently resulting in the toxicity of the mixture amounting to the sum of the toxicities of the individual toxicants present. Interactive action can be either synergistic (more than additive) or antagonistic (less than additive). These interactions occur when one toxicant alters the toxicity of another toxicant present. The results of the Florida pompano study showed that a synergistic interaction probably occurred after juveniles were exposed to the combination of ethylene glycol and methanol.

Future studies should concentrate on accumulating more data on the sublethal and lethal effects of ethylene glycol, methanol, and other additives on a variety of species associated with deepwater oil and gas platforms (i.e., encrusting organisms, planktonic

species, etc.). This would aid in developing a working model to better understand potential impacts on predator-prey interactions, chemical avoidance, behavior and feeding activity of fishes after exposure. Quantifying the sublethal and lethal effects of ethylene glycol and methanol at all trophic levels of the ecosystem would be a good first step in understanding the effects of deepwater oil development on fisheries in the northern Gulf of Mexico.

STUDY RESULTS: Ethylene glycol toxicity tests on juvenile Florida pompano and subsequent probit analysis identified the LC50 as 5.63% (volume per volume) at 30 practical salinity units and 25°C. Behavioral observations of treatment and control groups during 24-h exposures and 15-h recovery trials showed that 2.1% ethylene glycol was the lowest concentration at which individuals displayed lethargic behavior relative to controls after 24 h. Fish exposed to concentrations of 3.1% or less became lethargic or distressed relative to controls, but showed signs of recovery after 15 h in clean seawater. The mean (± SE) U_{crit} of juveniles (23.1±4.73 g) was evaluated before and after exposure to a 3.0% concentration. The mean U_{crit} declined significantly by 13.5% (P<0.0002) from 95.9 ±2.37 cm/s in pre-exposure trials to 83.0 ±3.45 cm/s in post-exposure trials. Exposure to ethylene glycol did not impact all individuals to the same degree, and smaller fish experienced a greater percent reduction in swim performance (%R) than did larger individuals (%R = - 53.5 + [1.7 x Body Mass], R^2 = 0.3148, df = 13, P<0.0295). We also evaluated the toxicity and sublethal effects of methanol on the swimming performance of juvenile Florida pompano. A 24-h static exposure identified the LC50 of methanol as 1.28% (volume per volume, % v/v) at 30 practical salinity units and 25°C. The mean (± SE) U_{crit} of juveniles (20.5 ± 4.59 g) was evaluated before and after exposure to a 1.07% concentration of methanol and showed that U_{crit} was significantly reduced (P < 0.0002) from 90.1 ± 1.35 cm/s to 84.2 ± 1.36 cm/s in post-exposure trials. Following exposure and a 17-h recovery period in clean seawater, the mean 6.5% decline in performance of the methanol treatment group contrasted sharply with a mean increase of 4.0% in the control group, indicating that conditioning and/or training effects were significantly surpassed by the negative sublethal effects of methanol exposure. In a combination experiment (i.e., Control, EG, MeOH and EG + MeOH) on juvenile Florida pompano swimming performance, single exposures to ethylene glycol and the combination of ethylene glycol and methanol significantly reduced U_{crit} by 13.0 and 42.0%, respectively. In this experiment, no detectable differences in U_{crit} were found for Florida pompano exposed to methanol or for the controls. Juvenile Atlantic spadefish were used to test the single effects of 3.0% ethylene glycol on swimming performance of individuals using the same protocol developed for pompano. Treatment fish experienced a 6.9% reduction in U_{crit} compared to pre-exposure swimming performance and a 17.9% reduction compared to the controls. The reduced ability of Florida pompano and Atlantic spadefish to sustain high prolonged and burst performance levels, even after a 17 hr recovery from exposure could affect their ability to avoid predators and feed effectively.

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