

ORD Critical Review of Rico-Martinez et al. (2013) Synergistic toxicity of Macondo crude oil and dispersant Corexit 9500A to the *Brachionus plicatilis* species complex (Rotifera). *Environ Poll* 173:5-10.

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ORD scientists reviewed the Rico-Martinez et al. (2013) article for technical adequacy and determined that apparent flaws in the report cause the results to be highly uncertain. Chief concerns are detailed below and include uncertainties in methods and results, lack of analytical verification, and over speculation. Overall, the results of this toxicity study with rotifers should not be broadly extrapolated to impacts of dispersed oil in the Gulf of Mexico.

1) Overview of Rico-Martinez et al. (2013)

Rico-Martinez et al. (2013) conducted acute toxicity and short-term reproduction tests with five strains of rotifers exposed to multiple concentrations of oil alone, dispersant alone, three levels of dispersant mixed with oil (dispersant to oil ratios of: 1:10; 1:50; 1:130), and a minor component of dispersant, propylene glycol. The toxicity tests utilized static exposures to water samples prepared by mixing these test materials using a modification of standard methods. The authors reported that mixtures of oil and dispersant (1:10, 1:50) were substantially more toxic than oil or dispersant alone based on nominal (rather than measured) concentrations. The authors concluded that the toxicity of chemically dispersed oil in the Deepwater Horizon (DWH) spill may have been substantially underestimated.

2) ORD Technical Concerns

a) Use of Non-standard Oil Mixing Regimes

The authors stated that standard methods were used in mixing water, oil and dispersant, but the reported methods indicate that non-standard methods were used. Oil was mixed with water for 8 hours, rather than the standard 18 hours mixing time; settling time is not specified or even mentioned. It appears that no settling of oil mixtures occurred prior to testing, in contrast to standard methods that require a 6 hour settling period. Inadequate mixing time limits the dissolution of toxic components of oil into the test water, particularly for oil-only exposures (i.e., underestimate the toxicity of oil alone). Inadequate settling time introduces an unstable mixture of particulate oil, particularly for chemically dispersed oil under conditions of similar limited mixing energy (i.e., overestimates toxicity of oil+dispersant exposures). Therefore, the authors' comparisons of toxicity results from their oil-only to oil+dispersant tests may not be valid.

b) Lack of Analytical Verification and Confounding Results

The study is based on nominal (unmeasured) concentrations, and the actual exposure concentrations are unknown. Comparisons of toxicity results from oil-only to oil+dispersant mixtures is erroneously based on these nominal concentrations, rather than measured concentrations of oil. The change in the intrinsic toxicity of the oil in the presence of dispersants can not be known without a comparison of water concentrations of oil. There is a large body of literature showing that dispersants substantially increase the water concentrations of oil, and the same amount of water may contain substantially more

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oil following dispersant mixing. It is much more likely that the dispersant increased the bioavailability of the free product oil, rather than increasing the intrinsic toxicity of the oil as the authors suggest.

c) Uncertain control survival and statistical analyses

It is unclear if a key quality control requirement for toxicity testing was met. Typically a minimum of 80% survival of control (no oil or dispersant exposure) is required for an acceptable toxicity test. Additionally, it is unclear if the statistical analyses of the acute toxicity results were corrected for control mortality, which is a basic requirement of toxicity data analysis.

d) Other concerns

The methods do not meet the scientific standard of providing sufficient detail to repeat the results. The methods are insufficiently detailed to determine the source of the oil, the volumes of water and oil mixed, the holding conditions of the test organisms, and the statistical treatment of the data. Multiple aspects of the results appear to be inconsistent or erroneously reported. For example, Table 4 references Table 1 for determining toxicity increases, but Table 1 only contains information on the characteristics of the rotifer strains. Table 2 appears to contain the data in question.