August 2, 2010

Dispersants Toxicity Testing – Phase II
Questions and Answers

Q1. What tests were conducted in Phase II of dispersant toxicity testing?

EPA conducted acute toxicity tests of 1).multiple concentrations of Louisiana Sweet Crude Oil alone, and 2.) combinations of Louisiana Sweet Crude Oil with each of the eight dispersants. The organisms tested were two Gulf of Mexico aquatic species: (1) the mysid shrimp, *Americamysis bahia*, an aquatic invertebrate, and (2) the inland silverside, *Menidia beryllina*, a small estuarine fish. These species are standard test organisms used in toxicity test for a variety of pollutants. The tests were conducted on mixtures of Louisiana Sweet Crude Oil and eight dispersant products found on the National Contingency Plan Product Schedule – Dispersit SPC 1000, Nokomis 3-F4, Nokomis 3-AA, ZI-400, SAFRON Gold, Sea Brat #4, Corexit 9500 A and JD 2000.

Q2. What did the test results show?

The results indicate that the eight dispersants tested are similar to one another based on standard toxicity tests on sensitive aquatic organisms found in the Gulf. These results confirm that the dispersant used in response to the oil spill in the Gulf, Corexit 9500A, is no more or less toxic than the other available alternatives.

Q3. Is there a difference in toxicity between oil alone and mixtures of oil and dispersants?

For all eight dispersants in both test species, the dispersants alone were less toxic than the dispersant-oil mixture. Oil alone was found to be more toxic to mysid shrimp than the eight dispersants when tested alone. Oil alone had similar toxicity to mysid shrimp as the dispersant-oil mixtures, with exception of the mixture of Nokomis 3-AA and oil, which was found to be more toxic. The oil results for small fish were inconclusive. EPA will perform additional testing of the toxicity of oil to small fish.

Q4: Why were the oil results for the small fish inconclusive?

For the highest concentration of oil tested, only 7 % of the the inland silverside, the small estuarine fish, died. To estimate the LC50 – the goal of this standard toxicity test – 50% mortality is needed. The test was conducted over a range of five concentrations and at the highest concentration only 7% mortality was achieved. The test will be repeated using a series of oil in water concentrations with results that encompass 50% mortality of the test organisms.

Q5: What tests did EPA use to assess acute toxicity to shrimp and small fish?

A: Acute toxicity tests are used to determine lethal concentrations of the test chemicals. The acute toxicity to shrimp and fish was determined using a standardized 48-hour mysid shrimp and a 96-hour small fish test to evaluate the potential toxicity of the dispersant. Established testing

procedures are specified for both the mysid shrimp and small fish. Both species live in the bays and estuaries of the northern Gulf of Mexico and are commonly used in toxicity tests. These two tests are required by EPA to list a dispersant on the **National Contingency Plan Product Schedule**. The test protocol exposes mysid shrimp or small fish to a range of dispersant concentrations and dispersant-oil mixtures or oil alone separately in the laboratory. Toxicity is determined by comparing the survival of the mysid shrimp or small fish exposed to the dispersants, dispersant-oil mixtures, or oil alone to survival of these organisms kept in clean, untreated seawater. The aquatic organisms used as test species are small mysid shrimp, *Americamysis bahia*, and a small fish, *Menidia beryllina*. Survival of the organisms exposed to multiple concentrations of the dispersants, dispersant-oil mixtures, or oil alone is determined for each species. The concentration lethal to 50 percent of the test organisms is calculated and compared between dispersants and between the toxicities of the oil-dispersant mixtures to determine the most and least toxic chemicals and combinations.

Q6. Why did EPA only test eight out of the total 14 dispersants on the National Contingency Plan Product Schedule?

EPA chose eight dispersants (Dispersit SPC 1000; Nokomis 3-F4; Nokomis 3-AA; ZI-400; SAFRONGOLD; Sea Brat #4; Corexit 9500 A; JD 2000) from the dispersants listed on the National Contingency Plan Product Schedule based on three criteria: 1) lower toxicity of the dispersant or of the dispersant when mixed with oil; 2) availability of sufficient quantities to

respond to the Gulf spill; and 3) immediate availability of samples for testing.

Phase I- Dispersant Testing- Questions and Answers

Q7. What toxicity tests were conducted to determine the least toxic dispersants?

A: EPA conducted several toxicity tests to provide independent scientific information about these eight dispersants. Three types of testing results on the dispersants alone are available from Phase I of the testing:
1) Potential endocrine activity: Some of the dispersants include chemicals called nonylphenol ethoxylates (NPE). NPE breaks down in the environment to nonylphenol (NP) which is a substance that could potentially cause endocrine disruption.
2) Degree each is toxic to living cells – cytotoxicity: EPA used *in vitro* assays to test the degree to which these eight dispersants are toxic to various types of mammalian cells and the potential for each dispersant to exhibit endocrine activity.
3) Acute toxicity to shrimp and small fish -Acute toxicity tests are used to determine lethal concentrations of the test chemicals.

The companies who manufacture the different types of oil spill dispersants already tested both the toxicity and the effectiveness of each of these dispersants and submitted results to EPA for listing their product on the National Contingency Plan Product Schedule. Although these industry-submitted test results provide guidance, the tests were conducted on the dispersants by different laboratories and on the dispersants mixed with No. 2 fuel oil which is not the type of oil in the Gulf. EPA wanted to

conduct its own toxicity tests in one laboratory under EPA oversight for better comparative analysis and to test the dispersants mixed with the oil from the Gulf.

Q8: What tests did EPA use to assess potential endocrine activity?

A: Some of the dispersants include chemicals called nonylphenol ethoxylates (NPE). NPE breaks down in the environment to nonylphenol (NP) which is a substance that could potentially cause endocrine disruption. Endocrine disruption can lead to defects in fetal development or can impair reproductive health in humans and aquatic species. The degree to which the eight types of oil spill dispersants are toxic to various types of cells is one good measure for estimating how much of the dispersant it would take to cause cell death. The more dispersant it takes to cause cell death, the less toxic the dispersant. Estrogen and androgen receptors are proteins in the body that interact with the hormones estrogen and testosterone and respectively control development and function of the female (estrogen) and male (androgen) reproductive organs.

Q9: What tests did EPA use to assess the degree each dispersant is toxic to living cells (cytotoxicity)?

A: In vitro assays are fast, often automated chemical screening tests that assess the potential for a chemical to affect specific biological processes that could impact human health and the environment.

Q10: What are the dispersant test results for potential endocrine activity, cytotoxicity and acute toxicity to shrimp and small fish?

A: While the dispersant products alone – not mixed with oil – have roughly the same effects, JD-2000 and Corexit 9500 proved to be the least toxic to small fish, and JD-2000 and SAF-RON GOLD were the least toxic to the mysid shrimp.

None of the eight dispersants tested displayed biologically significant endocrine disrupting activity, with the exception of a weak response for two of the dispersants (Nokomis 3-F4 and ZI-400) in one of the tests. This estrogenic result is likely not of biological significance. Cell death (degree the dispersant is toxic to living cells) was observed in some tests at concentrations above 10 parts per million. The endocrine and the cytotoxicity screening were conducted at dispersant concentrations from 0.001 parts per million up to 10,000 parts per million.
None of the dispersants triggered cell death at the likely concentrations of dispersants expected in the Gulf.

Toxicity Testing - Question & Answers

Q11: Why are toxicity tests used?

A: Toxicity tests are used to determine the potential adverse effects of chemicals on humans and other organisms. Acute toxicity tests are used on different species to determine lethal concentrations. Chronic toxicity tests can be used to determine sublethal chemical concentrations such as those adversely

affecting reproduction, growth and developmental processes as a result of long-term (chronic) exposure. In vitro assays are used to screen a large quantity of chemicals using many tests to prioritize which chemicals have the potential to be the most toxic.

Q12: What are "In Vitro Assay" screenings and why are they used?

A: In vitro assays are fast, often automated chemical screening tests that do not use live animals to assess the potential of a chemical to affect specific biological processes that may impact human health and the environment. On average, it would take a researcher eight hours a day, five days a week, for 12 years to do these assays. With computer we can do these tests and get results in three days. These types of tests are used regularly in the pharmaceutical industry to study the effects of drugs and medications and are also used to assess environmental chemicals. Traditional chemical toxicity testing (typically animal tests in the lab) are time consuming and expensive. In vitro assay screening provides fast, often automated screening results for assessing the potential of a chemical to affect human health and the environment. This type of screening can be used to prioritize which chemicals need further toxicity testing using animals. Animal tests on one chemical can costs millions of dollars. In comparison, numerous in vitro tests can be run on a chemical for about $20,000.