DAPHNIA REPRODUCTIVE BIOASSAY FOR TESTING TOXICITY OF AQUEOUS SAMPLES AND PRESENCE OF AN ENDOCRINE DISRUPTER

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. patent application Ser. No. 08/762,382, filed Dec. 6, 1996, now U.S. Pat. No. 5,932,436.

GOVERNMENT SUPPORT

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BACKGROUND OF INVENTION

In early toxicity tests of chemicals, the measured endpoint was acute toxicity. Over the past ten years, toxicity testing has expanded to include measures of subchronic toxicity including cancer, immune suppression, developmental effects and endocrine system disruption. Today, there is increasing concern about the long-term effects of anthropogenic chemicals such as polychlorinated biphenyls (PCBs), pesticides and plasticizers, among others, found in water, food, air and the materials around us. An increasing number of wildlife species are having reproductive difficulties, and there is current concern about declining sperm counts in human males.

Safe environmental concentrations of toxicants are those that allow humans and indigenous organisms in nature to complete their life cycles unimpaired. Toxicity tests that include exposure through the entire life cycle are the most useful tools for such measurements but can be too long and costly to be applied in most toxicity assessments. As a result, several short-term tests have been developed to estimate chronic toxicity, representing compromises of speed, sensitivity and cost. Most measurements of toxicity today rely on these short-term tests to indicate concentrations above which toxic effects are expected.

Daphnia are widely used in testing for aquatic toxins because of their rapid clonal reproduction, ecological importance, and sensitivity to their chemical environment. Toxicity tests using Daphnia have typically been used to detect changes in survivorship and fecundity.

Cladoceran zooplankton such as Daphnia can employ a reproductive strategy known as cyclical parthenogenesis in which one generation of sexual reproduction is interspersed with many generations of asexual reproduction. The ability to alternate life history strategy allows cladocerans such as Daphnia to achieve a high reproductive rate asexually when conditions are favorable, and to produce offspring sexually for survival when the environment becomes unsuitable. Under favorable environmental conditions, Daphnia reproduce asexually by producing eggs that hatch into female offspring that, in turn, asexually produce eggs that also hatch into female offspring, and so on. Populations can achieve high growth rates during the asexual phase as females mature in 8 to 11 days. The Daphnia population in Lake Mendota, Madison, Wis., is typically entirely female with occasional males and sexual females in late summer.

Sexual reproduction is initiated when females produce males and haploid resting eggs under certain conditions such as crowding, food scarcity, low temperatures, short photoperiod, or chemical cues emitted by predators. Males mate with sexual females to produce resting eggs that can persist in a dormant state for years, allowing the population to survive hard times. Fertilized zygotes develop into embryos that enter diapause. These embryos are contained in a durable ephippium and can remain viable for years in sediments before hatching in response to environmental cues. Production of ephippia can be essential to maintain a Daphnia population in an environment that periodically becomes inhospitable.

Bioassays that employ Daphnia are used to monitor and give a rough indication of the level of contamination in waters, and to test a specific chemical to predict the risk posed to biological communities. There are several standard assays that presently use Daphnia for measuring the toxicity of chemical substances in an aquatic sample. One such bioassay has been developed by the U.S. Environmental Protection Agency (EPA) to assess the relative toxicity of effluents and surface waters (U.S. EPA, "Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms"(3d ed.), Section 13, Daphnid, *Ceriodaphnia dubia,* Survival and Reproduction Test, Method 1002.0, Lewis et al. (eds.), Environmental Monitoring Systems Laboratory, Cincinnati, Ohio (EPA/60014-91/002, July 1994)). The EPA bioassay employs neonates (<24 hours old) of *Ceriodaphnia dubia* during a three-brood, 7-day static renewal test, with test results measured in terms of survival and reproduction.

Another bioassay that is currently used is a 21-day test provided by the American Society for Testing and Materials (ASTM) to examine the toxicity of a water sample or other material (ASTM Standards on Aquatic Toxicology and Hazard Evaluation, Standard Guide for Conducting Renewal Life-Cycle Toxicity Tests with *Daphnia magna,* Method E 1193-87, Philadelphia, PA (ASTM PCN 03-547093-16, May 1988). The ASTM Daphnia assay is labor-intensive and the organisms are grown at high food levels which involves regularly changing water to keep the organisms well fed and maximally reproducing. A single organism is placed into each of ten separate vials, the test is run for 21 days, and the endpoint measurement is the total number of surviving organisms. Under these conditions, the Daphnia produce female offspring by asexual reproduction.

A drawback of the EPA and ASTM Daphnia bioassays is that the assays only consider the effects on Daphnia during the asexual phase of their life cycle. In those bioassays, the animals are grown under conditions that optimize growth and asexual reproduction. However, projections of the species, community or ecosystem level risk posed by waterborne contaminants based on bioassays of asexual reproduction may ignore serious effects on reproductive strategy. Such bioassays can miss or make false predictions about the real effects of toxic chemicals in the environment because they do not measure toxic effects during a fundamental part of the life cycle of the model organism. All animals show variable sensitivity at different points in their development and over the course of an annual cycle. Invertebrates such as Daphnia with complex, multi-stage life histories may be especially useful sentinels for these complex sensitivities. Moreover, effects of toxins on various life stages can have entirely different consequences if exposure coincides with sub-optimal environmental factors. An assay is needed that can measure these effects at every stage of development in conditions that reflect those in nature.

Other disadvantages of the EPA and ASTM assays are that they tend to provide inconsistent and highly variable, irreproducible results within and between laboratories, do not measure the effects of contaminants on production of males, and provide no information on sex ratio or on developmental changes.