provided greater resolving power (statistical power) allowing the determination that dieldrin reduces sex ratio above about 30 ppb, a value lower than previously suspected of being biologically significant to Daphnia.

A decrease in sex ratio can occur via several basic mechanisms during early development, including:

1) Higher male mortality, with female production remaining the same. However, it was observed that the total number of offspring was not affected by dieldrin exposure.

2) Increase in female production, with an increase in total fecundity. However, there was no evidence that exposure to dieldrin increased fecundity.

3) A change in embryonic developmental rate, e.g., faster female maturation or slower male maturation induced by dieldrin exposure. However, male and female neonates are released at the same time from the adult's brood chamber.

4) Suppression of feeding rate or food quality which would also reduce total fecundity.

5) Seasonality which would not affect sex ratio in a 6-day bioassay.

6) A shift in the developmental process that results in fewer males and more females, with no change in fecundity.

The results of the bioassay supports the interpretation that dieldrin is an exogenous agent that interferes with the action of natural hormones in the Daphnia embryo that are responsible for reproduction development, i.e., dieldrin shows endocrine disruption activity in Daphnia. This endocrine disruption activity has two implications, one for aquatic ecology and one for human health.

Aquatic Ecology. Chemicals that change Daphnia development or reproduction are clearly of ecological concern. Daphnia is an ecologically important algae-consumer and fish-food in lakes all over the world. In particular, a decrease in the number of males has the potential of reducing Daphnia's ecological success over many generations, because sexual production is thought essential for preparing a population for changes in the environment. Any chemical that interferes with normal Daphnia ecology will also have indirect effects on water quality and fish production.

Evidence indicates that Daphnia reproduction has changed during the last century (Dodson et al., *Envir. Health Perspectives* 103 (Special Suppl. No. 4) 7–11 (1996). In Lake Mendota, Wisconsin, Daphnia, which produces up to 50% males in the late 1800s, currently produces less than 5% males. One possibility for this change is the introduction of endocrine disrupters in to the environment beginning in the 1940s.

There is interest in synergistic effects of chemical mixtures and environmental factors because mixtures of chemicals are the norm in aquatic habitats. In the case of dieldrin and endosulfan, there was no evidence for greater-than-additive (synergistic) or enhanced effect of the mixture of these two pesticides. The lack of synergism in Daphnia is consistent with results of other studies of these two chemicals at the molecular level (Ramamoorthy et al., *Endocrinology* 138:1520–1527 (1997).

Human Health. Dieldrin, a chemical known to be an endocrine disrupter in vertebrates, also affects development and reproduction in Daphnia. The result shows that the present Daphnia bioassay can be used as a rapid screen to detect chemicals of potential concern for human health. Daphnia can be useful as a whole-animal invertebrate "canary down the mine shaft" that can provide a useful screen for endocrine disruption for both environmental and human health.

What is claimed is:

1. A bioassay for testing a sample for the presence of a chemical substance that interferes with endocrine function in an animal, comprising:

maintaining a test sample and a control medium under conditions to induce sexual reproduction in Daphnia, the test sample and control each containing an effective number of adult, oviparous Daphnia of a single clone for crowding; and

comparing endpoints that indicate a deviation from normal Daphnia sexual reproduction in the test sample and control to determine the presence or absence of the chemical substance;

whereby the presence of an endocrine disrupter substance is indicated by a variance between the test sample and the control of the sex ratio, the ratio of males:total offspring the number of resting eggs, the number of neonates with a morphological abnormality the number of neonates with a behavioral abnormality the nutritional status of the offspring, or a combination thereof.

2. The bioassay according to claim **1**, wherein the endpoints include survivorship of adults and neonates, fecundity, and at least one of the following endpoints: number of male offspring, sex ratio of males:total offspring (neonates), number of resting eggs, number of offspring having a morphological abnormality, number of offspring having a behavioral abnormality, and nutritional status of offspring.

3. The bioassay according to claim **1**, wherein the sample and the control are maintained at a temperature of about 17–25° C., and a light:dark photoperiod of about 6–9 hours light to about 18-15 hours dark.

4. The bioassay according to claim **1**, further comprising changing the sample and the control on about day 3–4 of the assay period, and discarding the neonate Daphnia from the sample and the control.

5. The bioassay according to claim **2**, wherein the endpoints are measured in multigenerations of the Daphnia clone.

6. The bioassay according to claim **1**, further comprising: an initial step of determining the sublethal concentration of the test sample.

7. The bioassay according to claim **6**, wherein the sublethal concentration of the test sample is determined by maintaining a control medium and a series of aqueous dilutions of the test sample under growth conditions to induce sexual reproduction in Daphnia, each of the dilutions and the control containing an effective number of adult, oviparous Daphnia of a single clone for crowding;

comparing fecundity and survivorship in the dilutions and the control to determine the dilution having the highest concentration of the test sample at which survivorship and fecundity are maintained at about the same level as the control; and

using said dilution of the test sample in the assay.

8. The bioassay according to claim **1**, wherein the Daphnia clone produces about 5–70% males of the total offspring under the control conditions.

9. The bioassay according to claim **2**, wherein the morphological abnormality is identified by reduced or absent terminal setae on the second antennae, a forward curved tail spine, reduced swimming ability, or a combination thereof.

10. The bioassay according to claim **2**, wherein the behavioral abnormality is identified by abnormal swimming or abnormal motility.

11. The bioassay according to claim **1**, further comprising: conducting an assay to identify the chemical substance in the test sample.