Reproducibility of median lethal (LC50) and effective concentration (EC50) endpoints with zinc as toxicant (mg/L) in *Nothobranchius guentheri*[a]

TABLE 2

Reference test	LC50	EC50
1 (Cd)	7.7 (6.2–9.6)	6.0 (4.7–7.8)
2 (Cu)	9.1 (7.5–11.1)	6.4 (4.9–8.4)
3 (unionized NH_3)	15.1 (13.6–16.8)	8.0 (5.8–11.0)[b]
4 (l–octanol)	10.8 (8.4–13.7)	7.8 (5.7–10.6)
5 (phenol)	13.7 (10.8–17.5)	8.0 (5.8–14.4)[b]
6 (PCP)	14.1 (12.2–16.4)	8.2 (6.0–11.2)
7 (TNT)[c]	10.2 (7.9–1 3.2)	7.6 (6.7–8.6)
8 (malathion)	11.7 (9.0–15.2)	6.8 (5.2–9.0)
9 (2,4–D)[d]	7.1 (5.2–9.9)	6.0 (4.7–7.7)
10 (SDS)	9.0 (7.9–10.2)	8.2 (6.7–10.2)
11 (30–d storage)	16.9 (12.5–22.8)	8.0 (6.1–10.5)
12 (60–d storage)	9.8 (7.0–13.9)[b]	7.5 (6.0–9.2)
13 (90–d storage)	9.0 (7.9–10.2)	4.6 (4.0–5.3)

[a]Trimmed Spearman–Karber method [14] was used unless noted otherwise. Fiducial limits are given in parentheses.

[b]Binomial method [20] was used.

[c]TNT = 2,4,6–trinitrotoluene.

[d]2,4–D = 2,4–dichlorophenoxyacetic acid.

Example 4

Comparison of Rapid Annual Killifish Test vs. Standard Fathead Minnow Test

Test Substance	*N. guenthert*[a]		*P. promelas*[b]
(mg/L)	24–HR LC50	24–HR EC50	96–hr LC50
ZINC	8.6 (7.49–9.88)	5.75 (4.91–6.73)	2.65 [0.87–4.7, N = 4]
CADMIUM	4.2 (3.66–4.82)	0.73 (0.64–0.84)	2.02 [0.08–6.0, N = 61
COPPER	0.039 (0.033–0045)	0.033 (0.028–0.039)	0.48 [0.25–2.1, N = 8]
1-OCTANOL	22.8 (20.28–25.62)	11.5 (10.35–12.8)	13.5 [12.6–14.4, N = 6]
PHENOL	56.7 (45.04–71.42)	44.6 (36.71–54.29)	30.3 [24.9–34.3, N = 8]
PCP	0.25 (0.22–0.27)	0.17 (0.14–0.20)	0.23 [0.02–0.32, N = 7]
AMMONIA	1.15 (0.98–1.35)	0.57 (0.53–0.62)	1.59 [1.04–1.59, N = 3]
TNT	6.33 (5.41–7.4)	3.34[c] (2.80–3.89)	3.1 [2.6–3.7, N = 3]
MALATHION	6.94 (5.84–8.25)	2.91 (2.61–3.24)	11.8 [8.7–12.5, N = 4]
SDS	39.5 (37.0–42.2)	31.11[c] (26.82–36.74)	8.0 [6.2–9.6, N = 4]

[a]Trimmed Sperarman–Karber method for LC50 and EC50 (Fiducial Limits shown in parenthesis)
[b]Data from Toussaint et al., 1995; values represent a mean LC50 (LC50 range and number of references given in brackets)
[c]Moving average for calculation of EC50 value

EC50 and LC50 results of the killifish test were compared to the fathead minnow standard EPA test results. Several compounds having various modes of toxicity including heavy metals, biocides, narcotics and common effluent constituents were evaluated. Analysis of the data revealed the rapid killifish test to be similar in sensitivity to the standard EPA test. There are several advantages to the killifish test: a 24 hour exposure duration, utilization of standard laboratory equipment, and no requirements for continuous culture on order to have fish available for toxicity screening.

Conclusions: Killifish test was similar in sensitivity to standard fathead minnow test. Killifish EC50's were more sensitive indicators of toxicity than LC50's for all compounds tested. Embryo batch variability in response to zinc exposure was low. EC50's were less variable than LC50's. Long-term storage of embryos did not affect LC50 sensitivity. Overall control mortality was low, <10%.

Although the present invention has been described in terms of a particular preferred embodiments, it is not limited to those embodiments. Alternative embodiments, examples, and modifications which would still be encompassed by the invention may be made by those skilled in the art, particularly in light of the foregoing teachings.

The invention claimed is:

1. A method for testing toxicity in a fluid test sample comprising storing a species in a diapause state in a storage medium, said storage medium maintains said species in a diapause state and controls moisture content and separates the individual species, exposing said species to a hatching medium, allowing the diapause species to form hatchlings, and exposing the hatchlings to toxicity test materials.

2. The method of claim **1** wherein the diapause species is an annual fish species.

3. The method of claim **2** wherein the annual fish species is *Nothobranchius guentheri.*

4. A method for testing the toxicity of a fluid test material comprising exposing test material to hatchlings from a species that includes a diapause state, said hatchlings being stored and maintained in a diapause state in a suitable storage medium prior to becoming hatchlings, and determining the effect of the test material on the hatchlings.

5. The method of claim **1**, wherein said storage medium includes a solid component and a liquid component.

6. The method of claim **5**, where said storage medium has a moisture content between about 50% and 80% by weight.

7. The method of claim **6**, wherein said storage medium has a moisture content of about 75% water by weight.

8. The method of claim **5**, wherein said storage medium solid component comprises filter paper.

9. The method of claim **5**, wherein said storage medium solid component comprises peat moss.

10. The method of clam **9**, further comprising the step of grinding said peat moss to a particulate rating that will pass through a 0.6 sieve.

11. The method of claim **5**, further comprising the step of filtering said storage medium to remove particles that exceed a predetermined minimun embryo size.

12. The method of claim **11**, further comprising the step of straining said storage medium to separate said species in a diapause state from said storage medium.

13. The method of claim **1**, further comprising the step of sterilizing said storage medium before use.

14. The method of claim **1**, further comprising the step of boiling or autoclaving said storage medium before use.

15. The method of claim **1**, wherein the step of exposing said species to a hatching medium comprises saturating said species with culture water.