

# Determining the suitability of *Ceriodaphnia rigaudii* as a toxicity test species

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## Abstract

The selection criteria for toxicity test species though well documented, does not provide a clear approach for establishing the suitability of a species for toxicity testing. This study looks at an approach used for establishing *C. rigaudii* as a suitable freshwater toxicity test species for regulatory testing in Trinidad and Tobago. A comparison was made between the life cycle, toxicological responses and sensitivity of *C. rigaudii* and *D. magna*. The results showed that *C. rigaudii* (0.45mm) was significantly smaller in size, had a shorter life cycle, matured faster and was more sensitive to toxicants than the temperate cladoceran species, *D. magna*. This study was useful in defining a stepwise approach to help establish *C. rigaudii* as an indigenous tropical toxicity test species in Trinidad.

*Keywords: ceriodaphnia rigaudii, cladoceran, Trinidad, interspecies correlation, species selection, toxicity test.*

## 1 Introduction

Toxicity tests generally follow standard protocols developed for both aquatic and terrestrial organisms. These tests are often standardized with respect to species selection, pre-test maintenance/care of organisms, age of test organisms, food, duration, ambient light conditions, temperature, and end-points. Standardised test protocols [1–4], are commonly used by various environmental protection agencies (U.S. Environmental Protection Agency, Environment Canada and Environmental Management Authority in Trinidad), forming an integral part of their environmental monitoring programs.

The choice of test organism has a major influence on the relevance, success, and interpretation of toxicity tests. No one organism may be suited for all



toxicity test protocols. Though the selection criteria for test species are well documented [5], they do not yield sufficient information about species sensitivity and offer little assistance in helping to designate a new species as being acceptable for regulatory testing. Studies investigating the sensitivity of test species have often been based on either; (1) Toxicity evaluation of single or combination of toxicants [6–8], or (2) interspecies and intertaxa correlations, which evaluated the comparative sensitivities of different species [9–17]. Though these studies identified a method of assessing the sensitivities of different species they do not show how it could be combined to evaluate whether a test species is acceptable for regulatory testing.

Toxicity tests commonly utilise a wide variety of fish, decapod and insect species. However the various species of daphnia (*Daphnia magna*, *Daphnia pluex*, *Ceriodaphnia dubia*) and mysids (*Americamysis bahia*, *Americamysis bigelowi*, *Americamysis almyra*) are often preferred. *Ceriodaphnia rigaudii*, a tropical daphnid species, and *M. insularis* have recently [18–20] been screened as potential indigenous tropical toxicity test species for Trinidad. The integration of toxicity testing into the legislative framework for the control of water pollution in Trinidad and Tobago began with the introduction of the Environmental Management Act 2000. This Act mandated the development of rules that would incorporate all of the factors necessary for the effective monitoring and control of effluent discharge. These regulations have proposed the use of biological methods for effluent monitoring, with designation of indigenous estuarine/marine species as the accepted test species. This paper looks at the approach used to assess the suitability of *C. rigaudii* as an indigenous freshwater toxicity test species for use in Trinidad and Tobago

## 2 Method

The assessment of the suitability of *C. rigaudii* as a toxicity test species, took into consideration; life cycle assessment, toxicity evaluation, interspecies comparison and sensitivity evaluation.

### 2.1 Life cycle assessment

*Ceriodaphnia rigaudii* collected from pools at Valencia in northern Trinidad, were maintained in the laboratory at 25°C; 0‰ salinity; pH 7-8; total alkalinity 40-50 mg/L; total hardness 90-100 mg/L and dissolved oxygen greater than 4 mg/L. These specimens were not been exposed to toxic materials *in situ* before testing. Cultures were fed on a diet consisting of a mixture of chlorophyll extract, yeast and fish food. A minimum of fifty individuals were sampled for morphological characterization [19]. *C. rigaudii* maturation was assessed by monitoring the moulting rate and the time required for deposition of the first eggs into the brood chamber. The reproductive capacity was assessed by determination of the number of broods and the total number of juveniles produced by each organism.



## 2.2 Toxicity characterization and sensitivity evaluation

Acute toxicity tests were conducted in sterile 24 well culture clusters (Corning Incorporated), using juveniles (<24h old neonates), obtained from in-house laboratory cultures. Juveniles were exposed to a control and ascending series of five to seven concentrations for each of six toxicants (Cadmium chloride, Potassium dichromate, Sodium dodecyl sulfate, Potassium chloride, Tritox X-100 and Copper (II) sulphate) [12, 21]. All toxicants were analar grade chemicals (99% pure) obtained from Sigma-Aldrich chemical company. Test solutions were prepared by volumetrically mixing aliquots of a stock solution with dilution water. Three replicate test concentrations were used with a minimum of five neonates in 2 mL of test solution. Interspecies correlation and 'sensitivity factor' (SF) (LC50sp1/LC50sp2) estimations of the acute toxic response of *C. rigaudii* and *D. magna* to the six chemicals were then used to compare sensitivities [12].

## 3 Results

*C. rigaudii* typically has a short life cycle and is relatively easy to culture and maintain under laboratory conditions. It has a rounded body and is about  $0.45 \pm 0.03$ mm long [19], whereas *D. magna* may attain lengths of 6-7mm [3]. The body is covered by a single folded carapace with large hexagonal reticulations and opens ventrally, giving the appearance of a bivalve [19]. The average life span of *C. rigaudii* was found to be 10-15 days, which was less than that reported for other temperate species such as *D. Magna* (40-56), *C. dubia* (30-50) and *D. pulex* (approximately 50 days) [3].

Three distinct developmental phases were identified for *C. rigaudii*; eggs, juveniles, adults. *C. rigaudii* reached sexual maturity within 2 days when the first brood sac appears and the first eggs appear. However, *D. magna* is reported to attain sexual maturity between 6-10 days while *C. dubia* takes 4-10 days [3]. Female of *C. rigaudii* generally produces an average of 2 juveniles in the first brood, which are released approximately 2 days after the eggs first appear. During subsequent broods adults produce an average of 4 juveniles per brood. A single female may have about 4-5 broods during its life span, averaging 1 brood every 2 days, and generating about 10-16 juveniles. However, Stross and Kangas [22] reported that *D. magna* typically averaged about 4.8 juveniles for the first brood and 14 per brood for subsequent broods.

Presently, though toxicity data is available for cladoceran species (*C. reticulata*, *C. dubia*, *D. magna* and *D. pulex*) from temperate regions, the body of data available for tropical species is limited [12, 18, 20, 21]. The 48h LC50 values for *C. rigaudii* ranged from 0.002 mg/L (potassium dichromate) to 21.1 mg/L (potassium chloride) whereas those for *D. magna* ranged from 0.3 mg/L for copper sulphate to 418.87 mg/L (Table 1) for potassium chloride. The LC50 values for *C. rigaudii* were significantly less ( $P < 0.05$ ) than *D. magna* for six of the compounds tested. The interspecies correlation also showed a low positive correlation ( $R^2 = 0.5$ ), suggesting that the sensitivities of both species were not very similar for the compounds tested. The sensitivity factors of *C. rigaudii* and



*D. magna* for the toxicants, ranged between 0.01 and 12.3 [12]. These values all suggested that *C. rigaudii* was more sensitive than *D. magna* for of the compounds tested (Table 1).

Table 1: Mean 48h LC50 values for *Daphnia magna* and *Ceriodaphnia rigaudii* [12].

Toxicants		Mean LC50 (mg/L) and Standard Deviation (SD)			Sensitivity Factor (C. rigaudii/D. magna)
		<i>Ceriodaphnia rigaudii</i>	<i>Daphnia Magna</i>	<i>D. magna</i> (literature values)	
Copper sulphate	Mean	<b>0.34</b>	<b>0.28</b>	0.041mg/L [15]; 0.01mg/L, [4]; 0.038mg/L [23]; 0.0495mg/L [24]	<b>1.3</b>
	SD	0.006	0.04		
Potassium chloride	Mean	<b>21.11</b>	<b>418.87</b>	83mg/L, 166mg/L, 93mg/L, 149mg/L [4]	<b>0.05</b>
	SD	3.1	32.2		
Sodium dodecyl sulphate	Mean	<b>20.87</b>	<b>1.8</b>		<b>12.3</b>
	SD	2.9	0.35		
Cadmium chloride	Mean	<b>0.2</b>	<b>0.52</b>	0.178mg/L [15]; 0.01mg/L, 0.166mg/L, 0.065mg/L [4]	<b>0.4</b>
	SD	0.08	0.13		
Potassium dichromate	Mean	<b>0.002</b>	<b>0.25</b>	0.112mg/L [15]	<b>0.01</b>
	SD	0.0006	0.05		
Triton X100	Mean	<b>5.85</b>	<b>11.2</b>		<b>0.53</b>
	SD	0.31	0.69		
Zinc Chloride	Mean	<b>1.09</b>	<b>1.8</b>	0.1mg/L, 0.334mg/L, [5]; 0.068- 0.798mg/L [25]; 0.84mg/L [26]	<b>0.62</b>
	SD	0.09	0.35		

#### 4 Discussion

Toxicology tries to identify the effects of chemicals on humans or the environment and relies on the use of appropriate test species. The choice of test organism has a major influence on the relevance, success and interpretation of toxicity tests while no one organism is best suited for all test protocols. Though



the selection criteria for species used in toxicity testing have been well documented [5] very little is available on an approach that may be followed to help establish a species as being appropriate for testing.

The approach introduced in this study has thus far been successful used to establish two indigenous tropical species, *C. rigaudii* and *M. insularis* as potential test species, suitable for use in regulatory toxicity test in Trinidad and Tobago. It involved identification and life cycle characterization, toxicological assessment using standard chemicals [21] and subsequent evaluation of its sensitivity with respect to *D. magna*, an established test species. The results of these tests suggest that *C. rigaudii* was significantly smaller in size, has a shorter life cycle and matures faster than temperate species such as *C. dubia*, and *D. magna*. Many of these temperate species are typically 3mm in length as adults, where as the size of *C. rigaudii* is approximately 0.45 mm at maturity. Average life spans are usually 30-40 days and attain sexual maturity in about 3-5 days for *C. Dubia* and 6-10 days for *D. magna*, whereas *C. rigaudii* has a life span of about 10-15 days and reaches sexual maturity within 2 days. Comparative sensitivity evaluation also suggested that *C. rigaudii* was more sensitive than *D. magna* to the toxicants tested. It is probable that the difference in relative sensitivities may be due to temperature preferenda and that toxicity data generated with *D. magna* may not be appropriate for Trinidad.

Previously, sensitivity studies for temperate species reported separately on tests with specific compounds or comparative sensitivities between species. However these methods were never combined to show how they could be used to establish a species as being suitable for use in regulatory testing. The approach presented in this paper showed to be a useful, logical series of steps that helped evaluate the suitability of *C. rigaudii* standard test species for regulatory purposes, in Trinidad and Tobago.

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