# USE OF AUTOCHTHONOUS SPECIES OF INVERTEBRATES IN AQUATIC TOXICITY TESTS

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Brazil has one of the largest reserves of fresh water in the planet. To preserve the brazilian freshwater ecosystems, it is fundamental to know the impacts caused by anthropic activities, in such a way to allow multiple uses of waters and ensure sustainable development. The use of ecosystems representative species is always recommended for those studies. CETESB (Company of Environmental Sanitation Technology), environmental control body in São Paulo State, recommends the use of autochthonous species for toxicity tests, requiring, most of times, adaptations upon optimization of cultivation in laboratory and tests conditions (CETESB, 1992). However, exotic species are often used, because data validating the use of native species are not available. The knowledge of sensitivity to reference substances is one of the criteria that validate the use of organisms in tests (FIG.1).

*Daphnia laevis, Ceriodaphnia silvestrii* and *Chironomus xanthus* are among the autochthonous species whose biology has been studied and which have been used as test-organisms. However, routine actions are required to be introduced in assay labs, such as sensitivity evaluations and establishment of control-charts.

Based on *Daphnia laevis, Ceriodaphnia silvestrii* and *Chironomus xanthus* continuos cultures in laboratories involving biology studies for methods standardization, this research shows species sensitivity based on tests with reference substances, a fundamental requirement for them to be considered eligible for toxicity evaluations when within the specific sensitivity range for a reference substance. This requirement meets what is established in quality systems for assay laboratories, according to ABNT ISO/IEC 17025 (ABNT, 2001) and Good Laboratory Practices (OECD, 1998).

Standardized cultivation procedures have been developed for *Daphnia laevis, Ceriodaphnia silvestrii* and *Chironomus xanthus* species, according to ABNT ISO/IEC 17025 (ABNT, 2001) and Good Laboratory Practices (OECD,1998), from biology studies of species based on existing literature and guidelines by ABNT (1993), CETESB (1991), ASTM (2000) and EPA (2002, 2004). *D. laevis* and *C. silvestrii* have been cultivated in natural water with hardness adjusted to 44 mg/L of $CaCO_3$. *C. xanthus* has been cultivated in distilled water with hardness adjusted to 10 mg/L of $CaCO_3$. The cultures were kept at 20$^0$C (*D. laevis*), 24$^0$C (*C. xanthus*) and 25$^0$C (*C. silvestrii*), 16 hours light, 1000 lux luminosity intensity, under mild aeration. *D. laevis* and *C. silvestrii* were fed with *Selenastrum capricornutum* alga at 1.5 x 105 cells/Ml concentration. In *C. silvestrii* cultures, a compound food has been added, prepared with fish food and ferment. *C. xanthus* was fed with TetraFin(r), fish food diluted in distilled water (100g/L). The sensitivity tests were performed with Potassium Dichromate and Sodium Chloride as reference substances, by using *D. laevis* and *C. silvestrii* youngs less than 24 hours old and *C. xanthus* in the second instar. The control chart was determined by calculating the average of 5 tests results, and two standard deviations compared to average values established were used as upper and lower limits. Reconstituted distilled water was used as dilution water for test solutions preparation, in such a way to decrease the variability in organisms responses. The requirements to consider the tests valid were: mortality lower than 10% upon control, oxygen content higher than 2 mg/L and water temperature varying ± 2ºC. Statistical analysis was performed with the computer program "LC50 Programs JSpear Test" (HAMILTON et al., 1977).

Values of CE50;48H obtained were: *D. laevis*: 0.09 mg/L of $K_2Cr_2O_7$ and 2.54 g/L of NaCl; *C. silvestrii*: 0.13 mg/L of $K_2Cr_2O_7$ and 1.50 g/L of NaCl. The value of CL50;96H for *C. xanthus* was 16,0 mg/L of $K_2Cr_2O_7$ and 4.02g/L of NaCl. Values are similar to those known for exotic species, validating the use of autochthonous species as test-organisms in studies of environmental monitoring. The precision of the tests sensibility can be quantified by the repeatability (intra-laboratory variability). Results obtained in this work show that the variation coefficients are within or very close to analytical accuracy expected for sensitivity tests. Variation coefficients from 9 to 35% are within the expected magnitude. According to EPA (1991), results between 8 and 41% are considered excellent. Intra-laboratory variation is within the expected magnitude, validating the procedures adopted.

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FIGURE 1 - Aquatic Toxicity Tests.