

Comparison of two marine test species: *Acartia tonsa* and *Tisbe battagliai* in Acute Toxicity Tests acc. to ISO 14669

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Introduction

Toxic pollutants enter coastal areas through water and sediment and therefore often accumulate in the sediment section. As the marine meiobenthic fauna is of major ecological significance in coastal and estuarine biosystems, determination of the impact of pollutants on marine copepods is very important. The ISO guideline 14669 (1999) describes a method for assessing acute toxicity data with three representative benthic species of wide geographic distribution. During the past two years, numerous acute toxicity tests with *Acartia tonsa* and *Tisbe battagliai* were performed with selected environmental samples as well as for an extensive set of reference chemicals in a close cooperation with the Bundesanstalt für Gewässerkunde in Koblenz, Germany. The results are presented with regard to test design and handling of organisms as well as to compare the sensitivity of both species.

Methods



Acartia tonsa (own breeding, origin: Danish Hydraulic Institute, DHI); corresponding test replicates below



Acute Toxicity Test acc. to ISO 14669:

- 48 h, static test design
- 16 h/8 h light/dark photoperiod, 20 ± 2 °C
- Measurements after 24 and 48 h
- Test endpoint: LC₅₀ (48 h)
- Test medium: M7 + artificial seawater

Both test species were simultaneously exposed to pore waters of two environmental sediment samples and to the following reference chemicals:

- 3,5 DCP
- K₂Cr₂O₇
- CuSO₄

For evaluation of the sediment samples, resulting G-values (highest dilution factor without any significant effect) were determined for each sample and test species.

Evaluation of the reference chemicals was done via comparison of the resulting LC₅₀-values after 48 hours.



Tisbe battagliai (origin: Guernsey Sea Farms); corresponding test replicates below



Results

Test organism	3,5 DCP	K ₂ Cr ₂ O ₇	CuSO ₄
LC50-value (48 h) [mg/L]			
<i>Acartia tonsa</i>	0.346	> 2.5	0.694
<i>Tisbe battagliai</i>	0.256	> 2.5	0.656
Test organism	Sediment Sample 1	Sediment Sample 2	
G-value			
<i>Acartia tonsa</i>	16	8	
<i>Tisbe battagliai</i>	32	16	

The toxicity data for the three reference chemicals 3,5-DCP, K₂Cr₂O₇ and CuSO₄ were of similar magnitude for both test species. For K₂Cr₂O₇ LC₅₀-values above 2.5 were determined for both *A. tonsa* and for *T. battagliai*.

Both environmental sediment samples showed distinct effects on the test species. Thereby the effects on *T. battagliai* were more pronounced represented by higher G-values (= higher toxicity).

During implementation and test conduction, some methodical differences were observed dependent on the test species used (see table below).

Comparison of acute toxicity study design

<i>Acartia tonsa</i>	<i>Tisbe battagliai</i>
- Easy separation of test organisms without microscope (1 – 1.2 mm size)	- Only microscopic evaluations possible
- High reproduction during breeding, enabling numerous parallel studies	- Reproduction during breeding rather low, comparable higher effort for provision of food
- High test volume necessary	- Low test volume (multiwell plates), low space requirements, high replication
- Difficult assessments due to rapid movement of organisms	- Easy handling during assessments due to slow movement of test organisms

Conclusions

A. tonsa and *T. battagliai* showed comparable sensitivities towards the tested reference chemicals. The remarkably low sensitivity towards K₂Cr₂O₇ might be caused by chelating components of the artificial marine test medium.

In contrast to these almost similar results, the effects of both sediment samples were considerably more pronounced in *T. battagliai* than in *A. tonsa*.

This finding clearly supports the assumption that the sensitivity of an organism towards certain reference chemicals can not be transferred to its general sensitivity towards environmental samples containing a multitude of possible pollutants.

Although test procedures for *A. tonsa* and *T. battagliai* are defined by the same guideline, the different test organisms require slightly deviating techniques for breeding and handling as well as for testing.