

# Cyanobacteria Growth Inhibition Test

## Objective

Assessment of inhibitory effect of the test item on the growth of Cyanobacteria, *Anabaena flos-aquae*.

## Introduction

Primary producer performs the most crucial role in the overall functioning of the aquatic ecosystem (food chain) and contributes to the essential biomass of the aquatic environment. Presence of chemicals in a different region of an aquatic ecosystem adversely affects the aquatic food chain. Chemicals create selective pressure on the sensitivity of the aquatic organism. Therefore, toxic effects on the primary producer have to be assessed for plant protection products, biocides, industrial chemicals, and medicinal products for human and veterinary use that may get into an aquatic environment. We offer growth inhibition test on primary producer [Alga (*Pseudokirchneriella subcapitata*), Cyanobacteria (*Anabaena flos-aquae*) and *Lemna sp.* (*Lemna gibba* and *Lemna minor*)]. Please refer our newsletter 4 (April 2019) for *Lemna sp.* growth inhibition test. In this newsletter, we are focusing on Cyanobacteria.

Cyanobacteria is small unicellular bacteria, inhabiting in an aquatic environment and capable of performing photosynthesis. Cyanobacteria, formerly known as blue-green algae, are oxygenic photosynthetic prokaryotes. They are widely spread in marine, freshwater and terrestrial environments. Due to their well-known property of nitrogen fixation, they have been referred to as a source of bio-fertiliser. Considering the higher sensitivity of cyanobacteria compared to that of green algae for antimicrobial agents, cyanobacteria are often recommended for growth inhibition tests. Data generated by growth inhibition test on cyanobacteria following regulatory guidelines for eco-toxicological risk assessment of pesticides is always used along with other information on toxicity tests on non-target plants.



*Anabaena flos-aquae* of Strain N° ATCC 29413, a semi-thermophilic, filamentous, heterocyst-forming cyanobacterium has served as a model organism. It is a multicellular freshwater blue-green alga used as a test system, since, its growth rate can be measured in hours. This relatively short-term test can assess the effects over several generations. *A. flos-aquae* is convenient to culture and has been recommended for use by the OECD, OCSPP, and other regulatory guidelines. The results allow the assessment of the initial effects on the organism from short-term exposures and indicate the effect of the test on cyanobacterial populations. A culture of *A. flos-aquae* is obtained from the American Type Culture Collection, Manassas, VA 20108, USA, which is used as the study's stock culture. The stock culture is multiplied by regular subculture (at least once per week) at the Section - Ecotoxicology, the Department of Chemistry, Jai Research Foundation. Positive control study is performed with potassium dichromate, at least, twice a year, to check the test system's sensitivity and reliability of experimental techniques.



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## Test Design

The impact of the test item on the growth of nitrogen - fixing cyanobacteria is studied at JRF. The test is performed under static condition. Test media is analysed for the test item concentration and stability to monitor the concentration of test solution at 0 and 72 h during the main study. On the result of cell count at every 24-hour interval, the EC<sub>x</sub> values are determined. The statistical procedure determines NOEC and LOEC for multi-sample comparison. Data of biomass, specific growth rate, and yield are subjected to Bartlett's test to meet the homogeneity of variance before conducting Analysis of Variance (ANOVA), Dunnett's Test and Students T-test through in-house developed, validated computer software. The EC<sub>10</sub>, EC<sub>20</sub>, and EC<sub>50</sub> are calculated using the Probit analysis method (Finney, 1971).

## Results of in-house positive control study

END POINTS	Concentration of Potassium Dichromate (mg/L)	95% Confidence Interval (mg/L)	
		Lower Limit	Upper Limit
E <sub>b</sub> C <sub>50</sub>	5.08	4.51	5.72
E <sub>r</sub> C <sub>50</sub>	11.47	9.79	13.44
E <sub>y</sub> C <sub>50</sub>	4.99	4.44	5.61
NOEC for growth rate, biomass, and yield	1.90	-	-
LOEC for growth rate, biomass, and yield	4.10	-	-

## References

1. OECD, 2002: The Organisation for Economic Co-operation and Development (OECD) Guidelines for the Testing of Chemicals, OECD 201, OECD, freshwater Algae and Cyanobacteria Growth Inhibition Test, Adopted by the council on July 2002
2. Singh, P. D., 2018: "Cyanobacteria (*Anabaena flos-aquae*), Growth Inhibition Test with Potassium Dichromate", JRF Study Number : 533-3-07-21893, April 20, 2019, Jai Research Foundation, Valvada, Gujarat, India, un-published report of JRF.
3. U.S. EPA, 2012: United States Environmental Protection Agency (EPA), Office of Chemical Safety and Pollution Prevention (7101) (OCSPP 850.4550) Ecological Effects test Guidelines, "Cyanobacteria (*Anabaena flos-aquae*) Toxicity" (EPA 712-C-005), January 2012.
4. Finney, D.J., 1971: Probit Analysis, 3rd Edition, the Cambridge, University Press, p. 333.





#### About the Author:

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She is a specialised Study Director for the aquatic and terrestrial studies. She has vibrant experience in handling difficult test items. She is research-oriented and actively involved in the validation of Ecotoxicity studies. At a very young age, she is the co-author of the paper, published in the peer-reviewed journal. She has an experience of more than three years in the CRO industry.



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