Lemna sp. Growth Inhibition Test

Lemna sp. are aquatic plants, floating on or just beneath the surface of fresh water. Its small size, simple structure, asexual reproduction, short generation time, and sensitivity towards chemicals make it very suitable for laboratory testing. Lemna gibba and Lemna minor, representative species for higher aquatic plants, have been studied extensively for use in phytotoxicity tests. They have been widely used for testing plant protection products and environmental samples. The objective of this study is to determine effects of different concentrations of a test item on the growth of Lemna sp. to derive the effective concentration (Ecx), lowest observed effect concentration (LOEC), and no observed effect concentration (NOEC).

Monocultures of Lemna gibba and Lemna minor are grown in 20X AAP medium and SIS medium, respectively. Healthy plants are inoculated with a fresh sterile medium in aseptic condition and sub-cultured at regular intervals. Positive control study is performed with 3,5-dichlorophenol, at least, twice a year, to check the sensitivity of the test system and reliability of experimental techniques.

Depending on properties of the test item, this study is designed. If the test item is stable in the test medium, study is performed in a static test design and if the test item is un-stable in the test medium, study is performed in a semi-static test design. The exponentially growing 7-10 days old Lemna gibba and Lemna minor cultures are used for the study. Each test vessel is inoculated with 9 to 12 fronds. Test flasks are maintained at controlled environmental condition, during the study period. The frond count in each flask is determined at 3, 5, and 7 days, after the start of the study. In the static test design, pH of the test media is measured from all test item concentrations and in control, at the beginning (0 day) and at the end (7 day) of the test. In a semi-static test design, pH is measured in each batch of "fresh" test solution, prior to each renewal and also in the corresponding "spent" solutions. It is impractical to measure the temperature of solutions in test vessels, while maintaining axenic conditions. Therefore, one extra test vessel is prepared for measuring the temperature of the solution, during the test. The temperature of the medium in a surrogate vessel, held under same conditions in the room, is recorded daily. Additionally, daily measurement, of the maximum and minimum temperature, is recorded. The duration of the exposure is 7 days. During the test period, all culture flasks are maintained within 22 and 26 °C, controlled at ± 2 °C. Illumination is maintained between 6500 and 10000 lux (± 15% of mean value).

At the start of the test, frond number is counted. Frond numbers, appearing normal or abnormal, are determined at the beginning of the test and on days 3, 5, and 7. Below mentioned process is followed to evaluate the increase in the dry weight over the course of the test. At the initiation of test, at least, 20 fronds in six replicates are processed, using the same drying method as to be used at the test termination, to establish the treatment and the control mean dry weight of fronds. Plants (including roots and root fragments) for a test vessel (replicate) are removed from the test solution, rinsed with distilled water, blotted to remove excess water, placed in previously dried and tarred weighing pan, to determine their dry weight.

The EC_x (e.g., EC_{50}) is determined based on the average specific growth rate (E_rC_x) and yield (E_yC_x), based on frond number and an additional measurement variable (dry weight). The desired toxicity parameters are therefore four EC_x values for each inhibition level, viz., E_rC_x (frond number), E_rC_x (dry weight), E_yC_x (frond number), and E_yC_x (dry weight). The EC_x is calculated with in-house validated computer software. The value of the No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) is determined following a suitable statistical procedure, for multi-sample comparison (e.g., Analysis of Variance, Dunnett's Test and Student's t-test), using individual replicate value, with in-house validated computer software.

Results of in-house positive control study:

End points	Lemna minor	Lemna gibba
	mg 3,5-dichlorophenol /L	
For Frond		
E_rC_{50}	2.75	10.46
E_yC_{50}	1.61	6.92
NOEC	0.30	2.70
LOEC	0.70	4.40
For Dry Weight		
E_rC_{50}	1.54	7.27
E_yC_{50}	1.16	5.44
NOEC	0.30	4.40
LOEC	0.70	7.00

References:

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About The Author

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Jigar is a senior research officer, leading a team of Ecotoxicology. He has very good experience of conducting aquatic and terrestrial studies and has been actively involved in validation of Ecotoxicity studies. He is a member of Society of Toxicology, India. He has professional experience of more than 10 years in CRO industry.