

ALGAE TOXICITY TEST

6.3.1

1. Introduction

To determine the toxicity of Citrex liquid, an investigation using the freshwater algae *Raphidocelis subcapitata* and activated sludge was performed.

In the algae toxicity test the inhibition of growth and biomass formation is examined over a period of 72 hours under standardized conditions. The respiration inhibition test simulates an aerobic sewage treatment plant. The inhibition of the respiration is determined by the decrease in the oxygen consumption rate of the activated sludge in a concentration range of the test substance under standardized conditions.

The nitrification inhibition test is a simulation of an aerobic nitrifying treatment plant. The test is based on the conversion of ammonium to nitrate and nitrite. The decrease in ammonium-N concentration is a measure for the activity of the nitrification process. Introduction of test substance with increasing concentrations will affect this process.

In this report the methods and results are described. The raw data are included in the Annex.

2. Methods

2.1. Sample storage

Citrex liquid was stored in the dark at a temperature of 4-6 °C until use.

2.2. Algae toxicity test

The acute toxicity test with *Raphidocelis subcapitata* was performed as described in ISO 8692.

Raphidocelis subcapitata was exposed for 72 hours to several concentrations of Citrex liquid. The growth rate was determined every 24 hours by spectrophotometric analysis. The test was performed in triplicate and the controls were included in six-fold. The following range of concentrations was used: 0.10, 0.32, 1, 3.2, 10 and 32 µg/l.

The effect of Citrex liquid is expressed as the decrease in growth rate or production of biomass as compared to the control after 72 hours of incubation.

2.3. Respiration inhibition test

The respiration inhibition test was performed as described in NEN-EN-ISO 8192 method B guideline.

Activated sludge was exposed to several mixtures of a biodegradable substrate with an increasing concentration of Citrex liquid. After an incubation period of 30 minutes, the oxygen consumption rates were measured using an oxygen electrode. The percentage of inhibition of the oxygen consumption is estimated by comparison with a control mixture containing no test material. The test was performed singular. The following range of test concentrations was used: 1, 10, 100, 1000, 3200, 10,000 mg/l.

RESPIRATION INHIBITION TEST

6.3.2

3. Results

3.1. Algae toxicity test

Two algae toxicity tests were performed.

The first test was performed with triplicate vessels per concentration. The following range of test concentrations was used: 0.32, 1.0, 3.2, 10, 32 and 100 µg/l.

This test was invalid due to an indistinct dosis-effect relation and a high level of variance between the measured data. Therefore the test was repeated with a higher number of duplicates per concentration. The second test was performed in triplicate using the following range of test concentrations: 0.1, 0.32, 1.0, 3.2, 10 and 32 µg/l.

The same indistinct effects occurred in this test, to a somewhat lesser extent. As both tests were valid according to the guideline and AquaSense internal quality criteria it is unlikely that the odd results were caused by incorrect performance of the test.

Above 10 µg/l, the variation between the replicates was high and the dosis-effect relation was indistinct.

The final results of the algae toxicity test were based upon the second test.

Table 3.1 Results of the (second) algae toxicity test

	control	0.1 µg/l	0.32 µg/l	1.0 µg/l	3.2 µg/l	10 µg/l	32 µg/l	NOEC µg/l	EC ₅₀ µg/l
inhibition based upon growth rate(%)		3	4	3	2	2	13	10	>32
Inhibition based upon biomass formation (%)		1	10	7	6	1	36	10	>32

As shown in Table 3.1 significant toxic effects were apparent above 10 µg/l. Due to the low toxicity at the tested concentration range a reliable EC₅₀ could not be calculated.

In the first test, Citrex liquid caused, at a concentration of 100

3.2. Respiration inhibition test

A substantial respiration inhibition was measured at concentrations above 1000 mg/l (Table 3.2 and Figure 3.1). Adjustment of pH was carried out at the test substance-substrate mixtures of 3200 and 10,000 mg/l. After adjustment of the pH, a change in color was visible. Since inhibition was only measured at these concentrations, it is not clear whether the measured inhibition is affected by the change in the pH of the test solutions.

The EC_{50} of Citrex liquid is determined at 3300 mg/l.

Table 3.2 Results of the respiration inhibition test with Citrex liquid.

	control	1 mg/l	10 mg/l	100 mg/l	1000 mg/l	3200 mg/l	10000 mg/l
Oxygen consumption rate (mg O ₂ ·l ⁻¹ ·h ⁻¹)	93.12	94.20	96.20	100.08	86.25	44.78	13.80
Inhibition (%)		-1.2	-3.3	-7.5	7.4	52	85

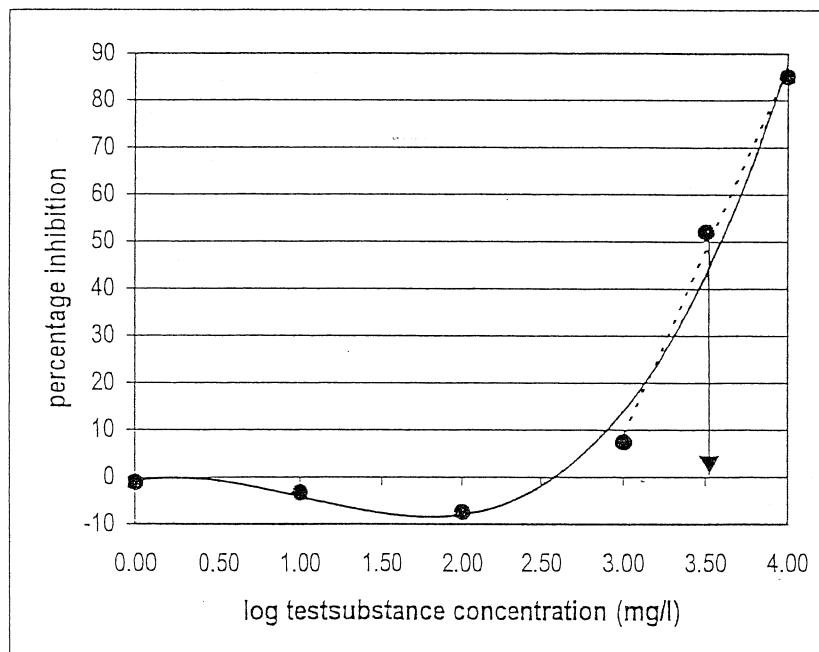


Figure 3.1 Respiration inhibition curve of Citrex liquid.