

THE ROLE OF CHRONIC TOXICITY TESTING IN A RIVER BASIN MANAGEMENT PROGRAM

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ABSTRACT

The EC funded Ecoriver demonstration program (LIFE02/ENV/P/000416) is being implemented in River Trancão Basin, a heavily industrialised and densely populated area. The program aims to demonstrate the importance of ecotoxicology for wastewater management and in planning and controlling industrial and municipal wastewater network systems. The Direct Toxicity Assessment approach can be of added value when faced with complex effluents. Industrial effluents may contain many chemicals which cannot be quantified and for which interactive effects may be significant. Acute and chronic approaches can, in this sense, be used to protect the receiving waters. In the study a set of 20 wastewater samples from different industrial sectors (Chemical, Metal, Printing, Repair and Surface treatment) were fully characterised at chemical and ecotoxicological levels. Bacterial, algal, invertebrate and plant tests were used to assess acute effects while the *Daphnia magna* reproduction test was used to assess chronic toxicity. In the *Daphnia* chronic test the number of living offspring per living parent and time to first brood were used as endpoints for the determination of NOEC/LOEC and EC₅₀ values. The results will be reported and the added value of chronic assessment in relation to acute toxicity and chemical assessment will be discussed. The potential role of *Daphnia* reproduction test in better characterizing the toxic hazards of wastewater discharges will be considered.

INTRODUCTION

The Trancão River Basin (Portugal) is heavily populated and has severe pollution problems. As part of an overall programme to address these issues a demonstration programme evaluating the ecotoxicity of municipal and industrial wastewaters has been conducted.

When there are issues with complex effluents for which many chemicals cannot be quantified and / or interactive effects are likely to be significant or the receiving water is impaired and the causes cannot be identified, the Direct Toxicity Assessment (DTA) approach can provide added value. A DTA based approach uses bioassays with different test species in the assessment of effluents ecotoxicity. In this programme samples were fully characterised at chemical and ecotoxicological levels using bacteria, algae, invertebrates and aquatic plants. *Daphnia magna* reproduction test was used to assess chronic toxicity.

The present work aims to discuss the chronic data and point out the role of chronic assays in river basin management.

MATERIALS AND METHODS

SAMPLING:

A set of 20 wastewater samples from different industrial sectors, namely: Chemical (C), Metal (M), Printing (P), Repair (R) and Surface treatment (ST) were analysed.

Composite or point samples were collected depending on the process, in two sampling campaigns: 2003 and 2004. Samples were kept frozen till analysis.

ORGANISM

Test organism: *Daphnia magna* from laboratory cultures.

BIOASSAYS

• *Daphnia* acute test (ISO 6341, 1996). Exposure time: 48 h. Endpoint: EC₅₀ (%v/v).

• *Daphnia* chronic test (ISO 10706, 2000). Exposure time: 21 d. Endpoints: NOEC, LOEC, EC₅₀-21d (% v/v).

Concentrations were chosen according to previous information from acute test.

For each sample 5 concentrations and 5 replicates *per* concentration were used. Daphnids were checked and fed daily and media changed every other day. The number of living offspring per living parent and time to first brood were registered.

A non-parametric test, Mann-Whitney, was used to assess whether there are significant differences between each sample concentration and the control.

RESULTS AND DISCUSSION

Effluent samples presented medium to high organic load, except for one sample from a Metal industry. Some samples did not comply with existing chemical specific permit limits also for iron, zinc, cyanide, nitrate or total suspended solids.

The acute toxicity data from the *Daphnia magna* immobilisation test showed marked differences between industries. In the group of 20 samples, nine were classified as acutely toxic or very acutely toxic according to Tonkes *et al* (1999). The *D. magna* immobilisation test showed a strong correlation ($p < 0.05$) with another crustacean acute toxicity test - *Thamnocephalus platyurus*. According to Sloof index (Sloof, 1983) from all species used in the acute tests, the most sensitive was *Vibrio fischeri*, followed by *D. magna*.

Where no or limited toxicity was measured in the acute *Daphnia* test, an absence of effects on juvenile production, relative to the controls, was evident in all of the test concentrations of the longer-term reproduction test. In situations where there was demonstrable toxicity in the acute *Daphnia* test, effects on juvenile production were also seen in the reproduction test (Figure 1). The acute to chronic ratio (48h EC₅₀ / 21d NOEC) for these samples showed values between 2 and 16.

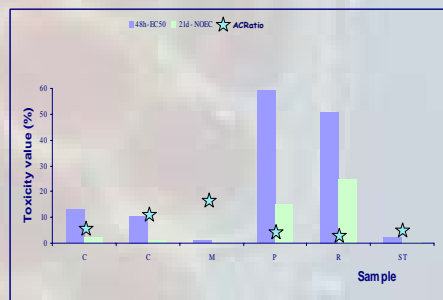


Figure 1. Acute and chronic toxicity values for *D. magna* exposed to different wastewater samples (2004 sampling campaign) and acute/chronic ratio.

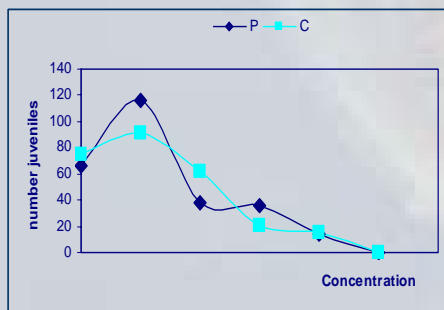


Figure 2. Examples of the bell shaped response obtained for two samples from the Chemical and Printing sectors.

For certain samples the number of juveniles *per* surviving adult showed a pattern which was not typical of the normal concentration-response curve. The *D. magna* reproduction tests showed increasing numbers of juveniles at low exposure concentrations (relative to levels in the controls) up to a plateau before the numbers declined at higher exposure concentrations (Figure 2). This bell shaped response is probably the result of the competition between two factors:

- The presence of increasing levels of nutrients and/or food items with increasing sample concentration
- The presence of increasing levels of contaminants with increasing sample concentration

At the lower exposure concentrations the enhanced effects of the nutrients and/or food items on juvenile production appear to outweigh the effects of the contaminants present and result in a stimulatory response. At the higher exposure concentrations the effects of the contaminants appear to outweigh the effects of the nutrients and/or food items. This finding highlights the potential problems of directly applying test methods designed for evaluating the toxicity of pure substances to the assessment of effluent toxicity.

CONCLUSIONS

- For the Trancão River Basin the application of a Direct Toxicity Assessment approach to waste waters has represented a valuable means for identifying toxic discharges to the system and the likely magnitude of their effects on the receiving water system;
- In this study the assessment of the chronic effects of some wastewaters was complicated by the non-sigmoidal concentration-response curves obtained. Instead, bell shaped curves were found indicating hormesis at lower exposure concentrations. These responses may be related to the presence of high organic loads in the samples in addition to chemical contaminants;
- As a result in certain instances there may be issues associated with the use of the *Daphnia* reproduction test for characterising the sub-lethal toxic effects of wastewater discharges.