

INTRODUCTION

Acute fish toxicity tests have been used in many countries for effluent risk assessment and/or ecotoxicological monitoring. Within it, a relevant number of countries have used OECD 203 as a standard method - or some national standard protocol with minor differences like Brazilian NBR ISO 15088. These protocols stand the usage of juvenile/adults fish within a specific length (2 ± 1 cm head to tail), even though this metric may not be directly associated organism's age. Freiry *et al.* (2014) have shown a decrease in sensitivity to chemicals along the fish lifespan, resulting in a higher sensitivity of larvae compared to adults. Ecotoxicological literature recommends that toxicity tests should be performed within the most sensitive stage of development, enhancing its protective power for environmental extrapolations. Nevertheless, in many cases, this premise may not be reached (ie. OECD 203).



Fig. 1 : *Danio rerio* - larval stage

METHODOLOGY

Number of tests	28
Reference solution	Potassium Chloride (KCl)
Volume of test solution	250 ml
Dilution water	Deionized, reconstituted to 47 mg/L CaCO ₃
Number of dilutions	5, plus control
Number of replicates per dilution	2
Number of organisms per replicate	5
Organism test	<i>Danio rerio</i>
Origen of the organisms	Laboratory of Ecotoxicology - UFRGS
Age	2 to 24 days post hatching
Exposure Time	48 hours
Solution Renew	Static
Temperature	25 ° C ± 2 ° C
Photoperiod	16:8 (light:dark)
Feeding	<i>Paramecium sp. ad libitum</i> until 2 hours prior testing
Observed effect	Lethality
Expression of the result	LC50
Statistical Analysis	Trimmed Spearman-Kärber 1.5 (Hamilton, <i>et al.</i> , 1977)

OBJECTIVE

The objective of this research is precisely determining the optimal age for acute fish test using zebrafish larvae (*D. rerio*) as a model.

RESULTS AND DISCUSSION

KCl Sensitivity

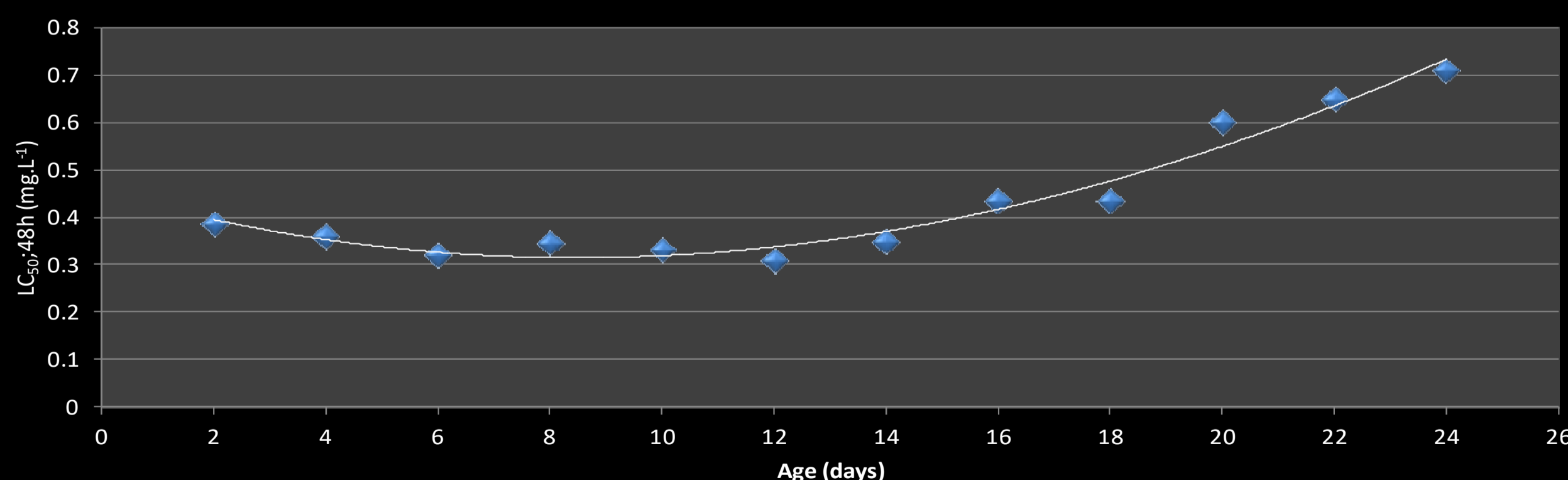


Fig. 2 Potassium Chloride (KCl) sensitivity curve based on 28 acute toxicity tests with *D. rerio* age from 2 to 24 days old.

Based on the initial results it is not possible to confirm differences in sensitivity between the 2nd and 14th day of life tested. However, an inflection point appears on the day 9, indicating loss in sensitivity after this day. Later tested ages showed considerably less sensitive to the chemical tested when compared to the earlier days (LC₅₀:24th day was double than LC₅₀ between 6-10th day). An association can be done between the sensitivity behavior of *D. rerio* until the 14th day and what is proposed by US-EPA 2000.0 using *Pimephales promelas* within the same range of age.

For ecotoxicological proposes this parallel seems to indicate that for fish early life stages post hatching time is more relevant than the species itself when determining sensitivity thresholds in decision-making. These are preliminary results and further studies using different reference solutions are being conducted.