The effects of copper-cadmium mixtures on the development of zebrafish embryos

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1. Introduction

The aquatic environment is highly impacted by pollution due to the fact that it receives contaminants from a wide range of sources, including effluents, run-off of surface waters, the industry and mining as well as from natural sources. Metals have been substances of concern in the aquatic environment for some time so their effects on aquatic organisms have been well researched. However, it is also well known that substances never occur individually and in recent years an increasing effort has been put into understanding the effects of the presence of multiple contaminants on the environment [1]. Research has shown that substances interact and interactions can be either synergistic, stronger than predicted, or antagonistic, less than predicted. So far there has been little success in gaining a complete understanding of mixture interactions. Therefore the aim of this study is to increase the knowledge and understanding of a binary mixture of two commonly found metals, copper and cadmium.

Zebrafish embryos were exposed to the two metals both individually as well as in mixtures and mortality and a number of sub-lethal endpoints were assessed for a period of 120 hours post fertilization (hpf). The individual data was used to make mixture predictions based on the commonly used Independent Action (IA) model. The predicted effects were then compared to the observed effects to determine the interactions of this binary mixture.

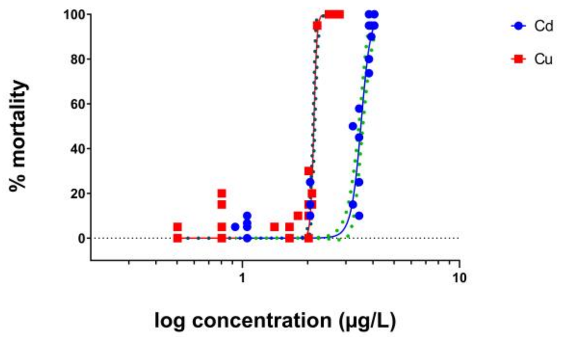
2. Materials and methods

The zebrafish embryos were exposed according to the OECD guidelines for the fish embryo toxicity test [2]. The parental fish were placed into breeding tanks the evening prior to the start of the experiment and the eggs were collected in the morning and placed into the exposure before 2 hpf. The well plates were kept at a constant temperature of 28 ºC ± 1 ºC. Developmental endpoints were evaluated every 24 hours and an extensive final scoring was performed at 120hpf. The endpoints assessed included, mortality, heart rate (24hpf), hatching, spine curvature, oedema, blood accumulation and swim bladder inflation among others. Additionally, swimming behaviour was analysed using a Zebrabox (Viewpoint).

Individual exposures for Cu ranged between 3 µg/L and 635 µg/L and for Cd exposures ranged from 5 µg/L to 11241 µg/L. In mixtures the exposures were chosen on the basis of a full factorial design ranging from 1 to 100 µg/L Cu and 0.1 to 100 µg/L Cd (Figure 1).

3. Results and discussion**Mortality**

The mortality dose-response curves obtained for Cu and Cd using zebrafish embryos were very steep and Cu was found to be the more toxic of the two metals with LC_{50} values of 135.6 µg/L and 3262 µg/L for Cu and Cd respectively (Figure 2). There is a clear increase in mortality in the embryos exposed to mixtures of the two metals when compared to the individual mortality (Figure 3).



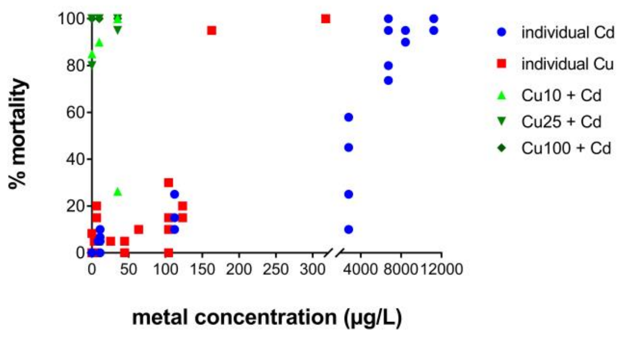


Figure 2: Dose-response curves (including error bars) for the mortality observed in embryos exposed to Cu and Cd

Figure 3: Mortality in zebrafish embryos exposure to Cu and Cd individually and in mixtures

3.2. Swim bladder inflation

One of the most sensitive sub-lethal endpoints of this study was found to be the inflation of the swim bladder. The dose-response curves for the swim bladder inflation were less steep than those recorded for the mortality and unlike in the mortality curves Cd was found to have a stronger effect, with an EC_{50} value of 34.46 µg/L for Cu and an EC_{50} value of 10.12 µg/L for Cd (Figure 4). A strong mixture interaction was also observed on the basis of swim bladder inflation.

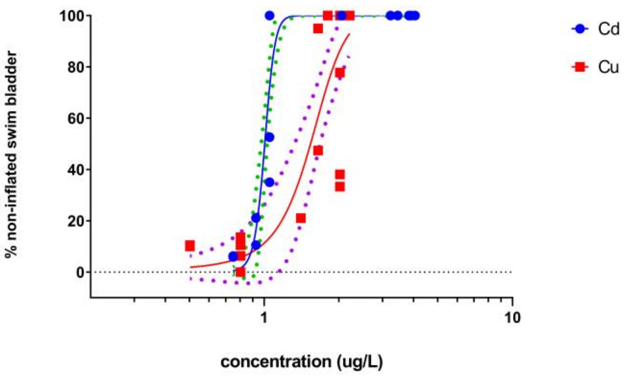


Figure 4: Dose-response curves (including error bars) for the swim bladder inflation observed in embryos exposed to Cu and Cd

4. Conclusions

The results of this study showed that Cu was more toxic than Cd on the basis of mortality, however based on the sub-lethal endpoint of swim bladder inflation Cd exposure resulted in a stronger effect. The binary Cu-Cd mixtures tested showed a strong synergistic interaction in zebrafish in reference to the IA model.

5. References

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Acknowledgement – Katherine I. Cordery is supported by the FWO Flanders.