A 3R compliant testing strategy to predict chronic fish toxicity

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1. Introduction

To ensure an ecologically relevant basis for environmental quality standards, whole-organism vertebrate tests are considered most suitable. Currently, the fish early life-stage (FELS) test (OECD 210), which involves an exposure up to one - two months, is used to estimate chronic toxicity of regulated chemicals. Several shortcomings to the FELS test can be identified, the most important being the use of high numbers of animals, low throughput and the lack of mechanistic information. We recently started the CEFIC LRI-ECO20-UA project to develop an alternative testing strategy for prediction of chronic fish toxicity. The goal of the project is to use *in vivo* alternative, *in vitro* and *in silico* tests to predict chronic toxicity in a 3R compliant way.

Since the zebrafish is not considered a test animal according to EU regulation (2012/707/EU) up to the age of 120hpf (hours post fertilization), we put forward the 120 hpf ZFET (zebrafish embryo toxicity) test as an ideal *in vivo* alternative test system. We present a conceptual workflow combining *in vivo* alternative and *in silico* techniques to enable chronic toxicity prediction.

It has become clear that chronic toxicity prediction cannot be generalized over a large range of different working mechanisms. We propose an alernative testing strategy based on the adverse outcome pathway (AOP) framework [1], delineating the sequence of key events at increasing levels of biological organization resulting in an ecologically relevant adverse outcome at a high level, in order to increase the mechanistic input in fish toxicity assessment and increase the biological relevance of applied testing strategies. This strategy relies on a classification of compounds according to their predominant AOP. The project presently focuses on four AOPs as case studies and will put forward AOP-specific methods for chronic toxicity prediction. The following three elements will be considered for each AOP.

2. Chronic QSAR prediction

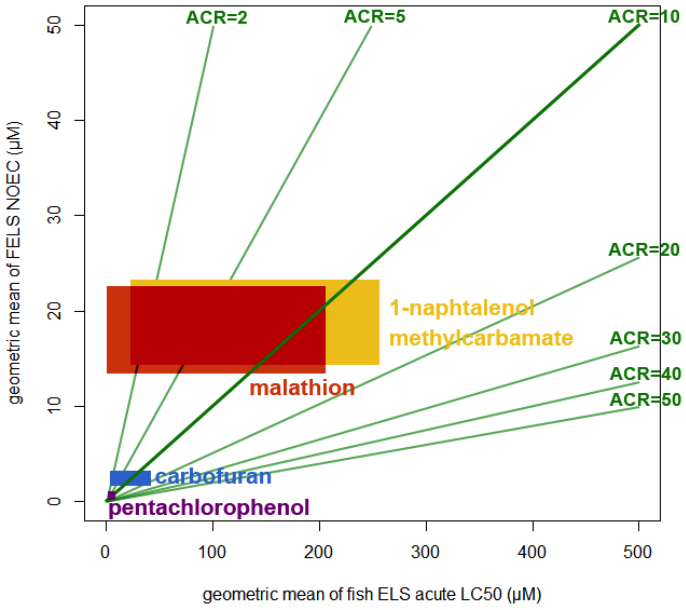
Depending on the AOP, QSARs may offer a first source of information. For example, it has been shown that acute toxicity of non-polar narcotics can be fairly accurately predicted. However, studies investigating the accuracy of more recently developed chronic QSARs are few. We performed a meta-analysis correlating acute and chronic fish toxicity data available through the US EPA ECOTOX database and OECD QSAR Toolbox, to acute and chronic ECOSAR toxicity predictions for a set of non-polar narcotics. We observed stronger correlations between experimental and predicted toxicity data for early life-stages compared to other life-stages.

In any case, before applying these chronic QSARs a critical step remains to classify a chemical as non-polar narcotic. We propose to use the 120 hpf ZFET test to confirm agreement between acute experimental toxicity and QSAR predictions for specific chemicals in order to decide whether the chronic QSARs may be applicable.

3. Acute-to-chronic relations

Secondly, if there is a consistent relationship between acute and chronic early life stage toxicity for a specific AOP, acute toxicity data gathered using the 120 hpf zebrafish embryo test may be used as a starting point for the prediction of FELS toxicity. In a first step, we constructed correlations between acute (3 to 5 days exposure) and chronic toxicity data (25 to 35 days of exposure) of non-polar narcotics including different fish species. Again, the correlations were stronger when the data was limited to early life-stages.

Next to the already existing concept of acute-to-chronic ratios (ACR), we propose the acute-to-chronic surface (ACS, four example chemicals in Figure 1). The width of the ACS informs on the spread of the available acute toxicity data, while the height represents the spread of the available chronic toxicity data. The total size of the ACS is a measure of the uncertainty of the ACR (ACR of malathion and 1-naphtalenol methylcarbamate is less certain). The position of the ACS informs on the toxicity of the chemical (carbofuran and pentachlorophenol most toxic) and the value of the ACR relative to the ACR of



10 currently used in hazard classification. For all example chemicals in figure 1 the ACR is lower than 10. Together with our correlation analyses of non-polar narcotics this suggests that the use of an ACR of 10 is a conservative approach.

4. Refined zebrafish embryo test

The previous two techniques are based on acute data limited to mortality observations (LC50, from acute ELS tests) and chronic data mostly limited to mortality and growth observations (NOEC, from FELS tests). To move to a more mechanistically-based risk assessment we propose to add a refined 120 hpf ZFET test including AOP-specific endpoints. For example, we propose a putative AOP for non-polar narcosis leading to respiratory failure. We will investigate the predictive potential of endpoints in the 120 hpf ZFET test representing key events along this AOP.

5. Conclusions

Industry, academia and regulatory decisionmakers generally agree that the mechanistic basis of fish toxicity testing should be improved. The current FELS and FET test are both mostly limited to lethal endpoints and therefore don't offer the mechanistic information necessary for sound risk assessment.

Figure 1: The acute-to-chronic surface (ACS) concept for visualizing ACRs. For four chemicals the relationship between available acute and chronic data is shown. The acute-to-chronic surface forms a rectangle ranging between the minimal and maximal values of acute and chronic toxicity and therefore informs on the uncertainty of the ACR. The green lines indicate different ACRs for comparison.

We realize that due to the complexity of chemical working mechanisms, one general method for prediction of chronic toxicity is at this point not achievable. We present a combination of several 3R compliant testing methods such as *in vivo* alternative methods, *in vitro* and in silico methods to develop a mechanistically based alternative testing strategy for prediction of fish chronic toxicity.

6. References

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