

Predicting toxicity to fish based on *in vitro* data

Julita Stadnicka^{1,2}, Kristin Schirmer^{1,2,3} and Roman Ashauer¹

¹Eawag, Swiss Federal Institute of Aquatic Science and Technology, 8600 Dübendorf, Switzerland

²ETH Zürich, Department of Environmental Systems Science, 8092 Zürich, Switzerland

³EPF Lausanne, School of Architecture, Civil and Environmental Engineering, 1015 Lausanne, Switzerland

E-mail contact: julita.stadnicka@eawag.ch

1. Introduction

Environmental regulations require comprehensive testing and risk assessment before a chemical can be approved for use. In ecological risk assessment of chemicals in water, fish play a very important role, being the only vertebrate representative of freshwater systems [1]. Quantification of chemical toxicity is generally based on measurements of external exposure; however, in order to understand, interpret and extrapolate toxic effects, using internal concentrations of chemicals is more suitable [2]. Also the quantification of the time course of internal concentrations (i.e. toxicokinetics) in cells and whole organisms facilitates a better understanding of toxicity and may improve *in vitro* to *in vivo* toxicity extrapolation. Finally, following the tissue-residue approach, which is an idea based on toxicokinetics, one can derive the hypothesis that the concentration in a fish that causes toxicity must be similar to those concentrations in cells that cause toxicity in a fish cell line.

Therefore we aim to: (i) predict chemical concentrations in fish, (ii) measure and predict chemical concentrations in fish cells, (iii) link chemical concentrations in cells to the effect on cells, (iv) link the effect on cells to the effect on fish.

2. Materials and methods

2.1. Lethality in fish and fish cells

We measured concentrations of eleven organic chemicals in exposure medium, fish cells (RTgill-W1) and plastic of the well plate for various time points in order to study the chemicals' distribution in the *in vitro* test system. We also investigated the relation between uptake and elimination rate constants and log K_{OW} . In addition, based on previous *in vitro* toxicity studies [3], we used our empirically obtained toxicokinetic model to predict internal effect concentrations in cells which we compared with internal effect concentrations in fish gills predicted using a Physiology Based Toxicokinetic (PBTk) model [4].

2.2. Predicting sub-lethal endpoints in fish

We exemplarily studied the impact of two pesticides (Propiconazole and Cyproconazole) on survival of the rainbow trout gill cells and described it by the General Unified Threshold model of Survival (GUTS) [5]. In addition, the cell proliferation under toxicant stress was investigated and compared with sub-lethal effect endpoints measured in rainbow trout (based on unpublished data provided by Syngenta).

3. Results and discussion

3.1. Lethality in fish and fish cells

The relationships between external effect concentrations in fish (LC50) and in RT-gill cells (EC50) vs. log K_{OW} , as well as the relationship between internal effect concentrations in fish gills (ILC50) and in gill cells (IEC50) vs. log K_{OW} are presented in Figure 1. Our results show that in most cases, the difference between log LC50 and log EC50 is smaller (values closer to "0" on y-axis) than between log ILC50 and log IEC50. This could suggest that external effect concentrations might be more useful for comparison of toxicity to fish and cells than internal concentrations of chemicals; however, there was no visible relationship between the ratio of LC50 and EC50 values and log K_{OW} ($R^2 < 0.2$, $p = 0.17$), while the ratio of ILC50 and IEC50 values

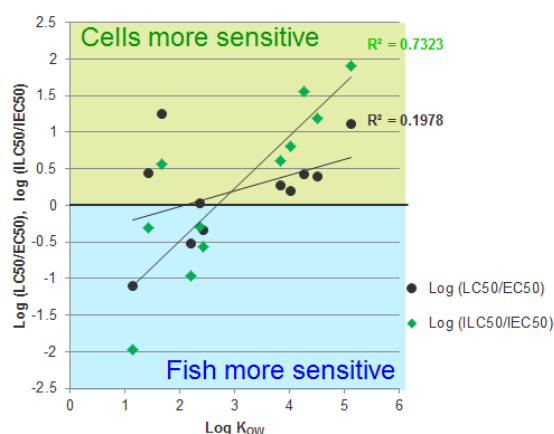


Figure 1: Relationship between external effect concentrations in fish (LC50) and cells (EC50) vs. log K_{OW} and between internal effect concentrations in fish gills (ILC50) and cells (IEC50) vs. log K_{OW} .

were correlated with $\log K_{OW}$ ($R^2 > 0.7$, $p = 0.0008$). Thus, it could be possible to predict effects on fish based on internal effect concentrations in cells. In addition, the difference between LC50/EC50 and ILC50/IEC50 was mainly caused by different toxicokinetics in fish and in fish cells. Our results showed that chemical bioconcentration in fish is more sensitive to changes in $\log K_{OW}$ than bioconcentration in cells. For chemicals characterized by higher values of $\log K_{OW}$ (above 3), $\log BCF$ in cells was lower than in fish. This could be explained by chemical circulation to fatty tissues for these compounds which can take place only in fish (cells used in our study were not lipid storage cells). On the other hand, for chemicals with lower values of $\log K_{OW}$ (below 3), $\log BCF$ in cells was higher than in fish. This finding might be explained by the fact that for these chemicals binding to proteins plays a more important role and that this process may be more important in cells than in fish.

3.2. Sub-lethal endpoints

The scheme of our work is presented in Figure 2. Based on unpublished data provided by Syngenta (i.e. concentrations in water and the effects on fish), we predicted the chemical concentration in fish gills by using the PBTK model. Assuming that this concentration is the same as in gill cells, we predicted the chemical concentration in exposure medium which we could dose to carry out *in vitro* experiments (e.g. testing cell proliferation). Figure 2 shows the impact of a low concentration (LC50 for rainbow trout is 20 mg/L) of Cyproconazole on fish and the RTgill-W1 cell line. The rainbow trout gill cells were proliferating significantly slower ($p < 0.0001$) under exposure to 1.5 mg/L Cyproconazole.

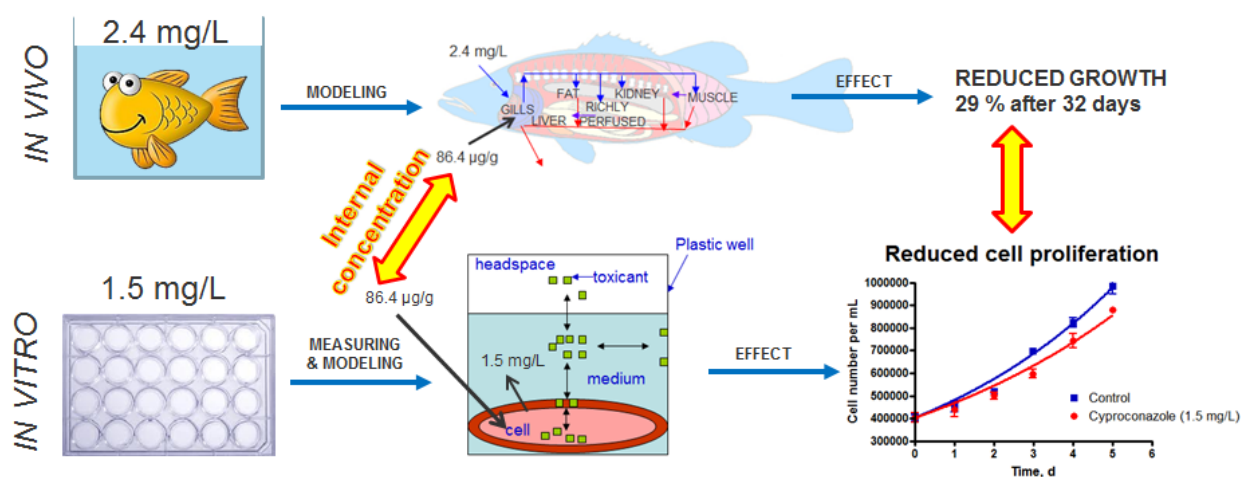


Figure 2: Toxicity of Cyproconazole to fish and fish cells.

4. Conclusions

Our study shows that modeling and experiments on fish cell lines can be used to obtain internal concentrations of various chemicals. These concentrations can be linked to concentrations in fish and to effects on fish and on cells. This suggests possible applications in ecotoxicology and ecological risk assessment.

5. References

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Acknowledgement - This research is financially supported by the European Union under the 7th Framework Programme (project acronym CREAM, contract number PITN-GA-2009-238148).