Advancing environmental risk assessment: investigating the relevance of non-conventional endpoints for effect prediction

Rasmussen, A.S.B.

Summary

Our modern society thrives on chemicals, from agrichemicals to pharmaceuticals. From the sales of these chemicals, we can see that the demand is growing with the years. However, with increasing demand, so come potentially increasing ecological consequences of these man-made chemicals. Chemical pollution is among the most important human-induced threats we are facing currently. Stark evidence is staggering, showing that chemicals present in our environments at low concentrations over extended timescales can contribute to wildlife population declines. In many cases, these effects are not predicted by our current widely used standardised methods to assess environmental risk.

Standardised testing often relies on conventional apical endpoints such as growth, reproduction, and mortality. However, for many chemicals, a disconnect between these measured endpoints and the way chemicals exert their sublethal effects likely contributing to a failure in identified adverse effects. The difficulty lies not only in establishing clear connections between apical and sub-lethal mechanistic endpoints, but also in the limited consideration of non-conventional endpoints, such as altered biomechanics (explicitly behavioral changes), which can significantly impact population survival.

To improve chemical effect assessment and limit further potentially adverse effects of novel chemicals, we urgently need to rethink current standardised methods. Therefore, the combined results of my thesis will aim at *investigating the relevance of non-conventional endpoints in predicting potential effects of chemical-induced stress.*

In **Chapter 2** we executed a Hackathon with students following the master course 'Ecotoxicology' at Leiden University. We had them work on identifying sensitive organisms and endpoints outside the standardised toxicity tests, that can quickly and efficiently detect the effects of a neurotoxic substance. This approach provided creative and realistic insights into how to measure non-conventional endpoints. We

demonstrated that a scatter-gun approach correctly identified sensitive and relevant endpoints such as changes in locomotion of *Chironomus riparius* larvae. After exposure to the neurotoxicant Sulfoxaflor, the average speed of the larvae in the water declined; and this endpoint was detected at doses 5 to 10 times lower than those causing mortality.

In **Chapter 3**, we wanted to investigate whether the behaviour of *C. riparius* could also detect sensitivity to the neurotoxic substance in combination with environmental factors. We found that conventional endpoints do not pick up on possible stronger effects of Sulfoxaflor in combination with environmental stressors: Increased temperature and predation stress. Instead, Sulfoxaflor induced increased effects in combination with the environmental stressors on the size of emerged adults, exploration and swimming behaviour of the larvae.

In **Chapter 4** we further wanted to assess if similar non-conventional endpoints would still show enhanced sensitivity compared to conventional endpoints, when *C. riparius* are exposed to mixtures of chemicals of unknown mode of action. To this end, we conducted a life-cycle experiment, exposing *C. riparius* larvae to particles from both conventional plastic mulches and a biodegradable alternative in leached and unleached form, as well as their respective leachate. Both conventional and non-conventional endpoints showed none to negligible responses of the *C. riparius* larvae exposed to the different types of plastics. Utilising similar setup between chapter 3 and 4, we saw that the differences in chemicals' mode of action impacts the sensitivity of selected endpoints. Therefore, known or unknown mode of actions of chemicals should be considered for endpoint selection in risk assessment, ideally on a case-by-case basis.

In **Chapter 5** we conducted a meta-study to investigate whether organisms exhibit altered responses after multigenerational exposure to chemicals with different mode of action compared to a single generation. Furthermore, we addressed whether endpoint selection including conventional and non-conventional endpoints might

influence the prediction of effects over time. Analysing, a total of 176 individual multigenerational exposures, only 37% of all observed effects were found to be consistent over time. Chemical toxicity increased in 39% of all instances and 23% of all observations showed signs of acclimation over time. Overall, this review emphasized the variability in multigenerational ecotoxicity testing and advocates for continued research to enable broader, more predictive assessments beyond case-by-case evaluations.

Lastly, in **Chapter 6** we synthesize the findings in this thesis and discuss potential future prospectives. As current risk assessment schemes fail to predict adverse environmental effects of all novel chemicals, we urgent the need to rethink how we assess environmental risk. While standardisation ensures streamlined regulatory processes, it comes with the loss of complexity necessary for specific and novel chemicals. A tailored approach based on environmental relevance, allowing the incorporation of non-conventional endpoints and flexible testing requirements can enhance environmental protection urgently needed for effective risk assessment. This chapter further explores the tension between involved stakeholders. Rethinking environmental risk assessment as this thesis highlights is not an easy task; however, it is crucial for how we effectively assess the risk of novel chemicals in the future.