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

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1 General Introduction

1.1 Freshwater Ecosystems

Freshwater ecosystems are an essential part of the Earth's biosphere., as they serve as habitats for a diverse range of aquatic and terrestrial species, making them critical for global ecological stability (Higgins et al., 2021). Freshwater systems are highly diverse, ranging from massive rivers to small vernal pools, from wetlands to catchment areas and ground water (Albert et al., 2020; Lynch et al., 2023). A crucial part of freshwater ecosystems are smaller streams and ditches, which are often small in length and volume, but collectively make up the majority of the length of freshwater systems (Fergus et al., 2017).

They provide microhabitats, which are characterised by their high environmental heterogeneity and dynamic hydrological regimes, which support unique and specialised communities of organisms, many of which are highly adapted to local conditions (Baranov et al., 2020; Hasse et al., 2023; Sayer et al., 2025). The intertwinedness of terrestrial and aquatic environments in these systems, such as in riparian zones, creates steep ecological gradients that enhance biological diversity and productivity (Palmer et al., 2010; Moi et al., 2024; Diepens et al., 2016). These transitional areas facilitate the exchange of nutrients and energy between ecosystems, freshwater streams and wetlands serving as sinks for nutrients via surface runoff and atmospheric deposition (King et al., 2019). Moreover, freshwater systems deliver a wide range of ecosystem services that are essential to human well-being. These include water provision for irrigation, filtration of pollutants, and the mitigation of natural hazards such as floods and droughts, services that are especially critical in agricultural landscapes, as reviewed in Lynch et al. (2023). Their capacity to regulate hydrological flows also plays a

role in maintaining soil health and preventing erosion, further supporting food production and landscape stability (Dodds et al., 2013). Moreover, small streams located within heavily human-impacted landscapes serve as crucial refugia for biodiversity, supporting and sustaining ecological communities within otherwise degraded environments (Albert et al., 2020; Moi et al., 2024; Sayer et al., 2025).

Aquatic insects constitute an abundant, diverse, and functionally important component of the biota of freshwater systems (Baranov et al., 2020; Twardochleb et al., 2021). With nearly 100,000 species spending one or more of their life stages in freshwater, they are a highly ecologically important group in aquatic systems (Dijkstra et al., 2014). Aquatic insect species provide services within freshwater systems, such as nutrient cycling, secondary production, bioturbation and bioirrigation (Macadam & Stockan, 2015; Diepens et al., 2016).

1.1.1 Importance of chironomids in freshwater systems

Within the aquatic insect community, the family *Chironomidae* (Diptera) stands out as one of the most diverse and ecologically significant taxa. These non-biting midges are found globally in nearly all types of freshwater systems, ranging from pristine mountain streams to highly polluted urban ditches (Armitage et al., 1995). During their larval stages, they inhabit the benthic zone, where they contribute to nutrient cycling, organic matter decomposition, and serve as an important food source for fish and other invertebrates (Hölker & Stief, 2005; Prata et al., 2023). Additionally, as they emerge into flying adults, they serve as a link between aquatic life and terrestrial systems (Armitage et al., 1995; Karima, 2021). See Figure 1.1 for a schematic overview of chironomids life cycle.

Their life cycle begins as egg ropes laid just underneath the water surface (Beaudouin et al., 2012). After a few days, the eggs hatch and larvae are released into the water column. From there they swim to the sediment, where they embed themselves into the sediment top layer

(Osborne et al., 2000). Here, they will build and hide in tube like sediment structures using mucus and smaller grains (Stief et al., 2005). In this stage, they feed on detritus, however getting food is a trade-off between staying hidden for predators and emerging from their tubes to look for food (Osborne et al., 2000; Hölker & Stief, 2005).

The larval stage of the *C. riparius* takes around 12-17 days at 20 °C, in that time, developing through four instar phases until reaching pupation and adulthood (OECD, 2023). Importantly, the development can depend on many factors, including temperature, feeding, and gender (Klagkou et al., 2024). During pupation, they change into a smaller more compact version and their colour changes to black, while remaining mobile (Brackenbury, 2000). As adults, the males will hatch from their pupa a few days before the females (Watts & Pascoe, 1998).

After emergence males can perform one to two fertilisations per day until three days after emergence, where the males stop their reproductive activity (Beaudouin et al., 2012). The females are assumed to live and be fertile up to three days after emergence (OECD, 2023). Among the available males the females select the male, and upon fertilisation, she hatches her eggs on the water surface the next day (Beaudouin et al., 2012).

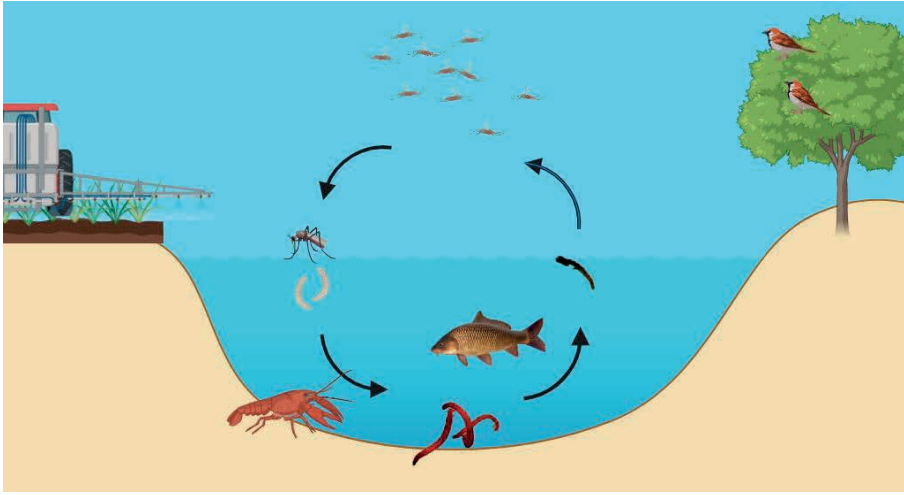


Figure 1.1 Concept drawing of chironomids life cycle including potential threads at different life-stages.

1.1.2 Exposure scenarios in freshwater environments

Many freshwater systems are in close proximity to anthropogenic activities, which can result in pollution (Geyer et al., 2017). Pollution of surface waters are a serious threat to our freshwater biodiversity, stemming from a multitude of sources. Pollutants may persist for a long time and with continuous input they can accumulate in the environment, especially sediments (Santos et al., 2010; Geyer et al., 2017; Casillas et al., 2022). Sediment-dwelling organisms, such as chironomid larvae, can be exposed to these contaminants, as well as through waterborne exposure, potentially resulting in harmful effects (Hare et al. 2001; Prata et al., 2023; Casado-Martinez et al., 2024).

Smaller streams and ditches, which are often adjacent to agricultural fields, are likely to be exposed by processes related to agriculture, including nutrients inputs and the use of pesticides (Sayer et al., 2025). Neurotoxic insecticides such as neonicotinoids and similar compounds are used in large amounts due to their high efficacy (Sánchez-Bayo et al., 2016; Costa et al., 2008). As a result, they are commonly detected in surface waters globally (Berens et al., 2021). They can enter waterways through airborne drifting, leaching, or by direct rain runoff.

They are often associated with adverse aquatic effects, as even small doses are found to cause potential impact on environmental organisms (Barmantlo et al., 2019). High water solubility, paired with prolonged degradation times, means that neonicotinoids and similar compounds may persist in freshwater systems for extended periods (Casillas et al., 2022), potentially increasing exposure for benthic organisms.

Another source of agricultural pollution are plastic particles. The use of agricultural plastics is an important source of plastic pollution, especially mulching films (Sun et al., 2020). These films degrade over time, releasing plastic particles and chemical leachate (Sun et al., 2020; Kumar et al., 2020), subsequently leaching into non-target freshwater systems (Horton et al., 2017). In aquatic environments, microplastics can cause a variety of adverse effects on non-target organisms (Tian et al., 2022; Pisani et al., 2022). These adverse effects can be caused by plastic particles itself as well as by the chemicals that are leached, depending on plastic type and environmental conditions (Luo et al., 2019; Capolupo et al., 2020), indicating highly diverse effects and mode of actions of these kinds of mixtures (Shi et al., 2024).

Leachate from microplastic consist of a mixture of often many different compounds all existing in the environment together (Capolupo et al., 2020; Cao et al., 2024). Furthermore, entering the water ways, runoff from agriculture mixes with other sources of pollution, resulting in aquatic environments being exposed to large unknown mixtures of diverse chemicals (Oldenkamp et al., 2022).

1.1.3 Chironomids as bioindicators

Because of their wide distribution, diverse ecological tolerances and varying degrees of sensitivity to environmental stressors, members of the *Chironomidae* family are widely recognised as effective bioindicators in freshwater monitoring and water quality assessments (Crane et al., 2002; Ferrington, 2008). Especially the first instar larval phase is found to be sensitive, and due to them being free-swimming

and able to survive for days without food, this stage is ideal for acute ecotoxicity testing (OECD, 2011). Furthermore, their relatively short but complex life cycle and benthic lifestyles also make them suitable for chronic testing of persistent chemicals (OECD, 2023).

Effects of chemicals with known neurotoxic have shown high toxicities towards *C. riparius* such as lufenuron (Brock et al., 2016) and neonicotinoids (Langer-Jaesrich et al., 2010, Casillas et al., 2022). Furthermore, high sensitivity of *C. riparius* towards neurotoxins have even been potentiated by additional environmental stressors with similar modes of action, including increased temperature and predation stress (Pestana et al., 2009; Van Praet et al., 2014; Heye et al., 2019).

In addition to chemicals with known neurotoxic mode of action, *C. riparius* has been found sensitive to other stressors where the target mode of action is less obvious or where information on non-target effects remains limited. Exposure to virgin polyethylene (PE) and weathered polyamide (PA) particles caused developmental issues of the larvae (Silva et al., 2019), and emergence impairments in *C. riparius* (Khosrovyan & Kahru, 2021). The sensitivity of *C. riparius* towards multiple different stressors highlight the species as relevant in toxicity studies and prediction of actual environmental effects.

1.2 Traditional effect assessment

To effectively safeguard environmental integrity, Environmental Risk Assessment (ERA) frameworks have been developed to systematically evaluate the potential ecological consequences of anthropogenic activities (Rohr et al., 2016; Oldenkamp et al., 2022). ERA is applied to a wide range of human-induced threats to ecosystems, including (chemical) pollution, land-use change, and climate-related stressors (Suter, 2007). The foundation of an ERA consists of several key components: hazard identification, exposure assessment, and effect assessment, which together inform the risk characterisation phase (ECHA, 2023; EMA, 2024). Characterisation of risk is based on

regulatory protection targets set to protect the environmental population and not individual organisms (Pamminger et al., 2025).

ERA is often performed in a tiered approach, starting with low level acute standardised testing, adding ecological realisms and complexity in higher tiers (EFSA, 2013; EMA, 2024). Limiting testing by focusing on a few selected test species under standardised short-term exposures to begin with, can help save resources (Diepens et al., 2016; Oldenkamp et al., 2022; EMA, 2024). For these experiments, test species are selected as part of a simplified aquatic food chain to accommodate the different levels found in freshwater environments, with producers in the form of algae, primary consumers typically represented by daphnids, and secondary consumers, such as fish (Schuijt et al., 2021).

Furthermore, testing multiple species can often be very time consuming. Hence, generally, at the first tier, acute toxicity is expressed by the concentration causing lethality of 50% of the organisms during an *acute exposure*. The duration of an acute exposure depends on the biology of each organism, typically ranging from hours to days. Sublethal effects, such as adverse impacts on growth or reproduction, can also be assessed in conventional toxicity studies, often by extending the study duration. The duration of these *chronic studies* is determined in relation to the life cycle of the organisms (ECHA, 2017). Standardised procedures are in place to aid the assessments such as OECD test guidelines (OECD, *Guidelines for Testing of Chemicals*). In general, each tier adds on to the complexity, enhancing the environmental relevance of the testing, in order to best predict the actual environmental effects (Diepens et al., 2016; Gomes et al., 2021; EMA, 2024; Figure 1.2).

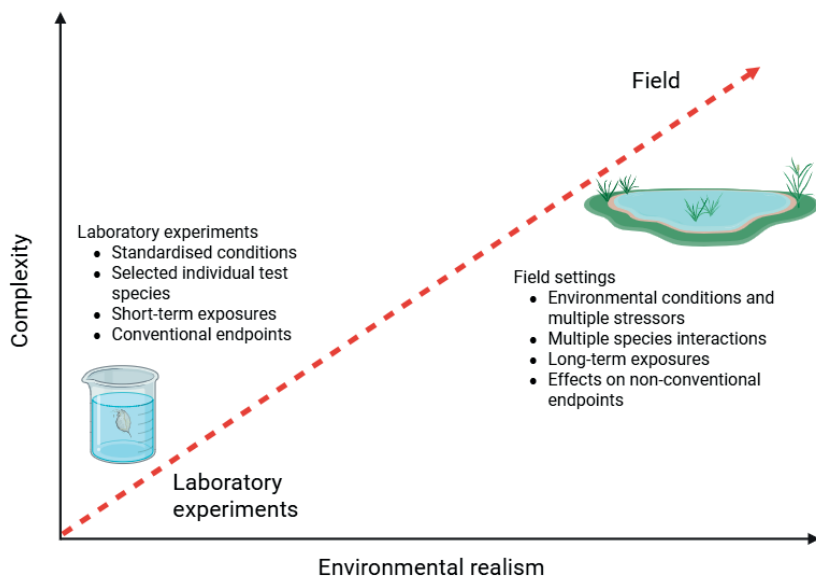


Figure 1.2 Concept drawing of the difference between standardised laboratory experiments and the actual environment measured in the field.

The lower tiers of assessment in general focusses on whole-organism responses in single-species laboratory setups using standardised conditions (Rohr et al., 2016; Schuijt et al., 2021; Harrison et al., 2025). The test methods traditionally include conventional measures on individual survival or immobilisation, growth, and/or reproduction. These endpoints are relatively straightforward to interpret, have a direct link to population fitness, which is essential for regulatory targets of invertebrate species (Moermond et al., 2016; Pamminger et al., 2025).

1.3 Novel test strategies

Standardised methods are found to increasingly fall short of predicting the environmental impacts of chemicals. Especially biologically active molecules with novel modes of actions can lead to implications for wildlife populations. These effects might be enhanced in combination with other stressors and during long-term exposures, threatening

ecosystem functioning (Rasmussen et al., 2013; Legradi et al., 2018; Oldenkamp et al., 2022).

1.3.1 Non-conventional endpoints

A growing body of scientific literature is providing stark evidence that chemicals present at low concentrations over extended timescales can contribute to wildlife population declines and community changes that threaten ecosystem function and service provision. In many cases, such effects would not have been expected for current widely used standard ERA test methods. One major contributing factor to the failure to identify potential adverse effects of these chemicals are likely the disconnect between apical conventional endpoints and the way these chemicals exert their sublethal effects, i.e. through behavioural changes that have secondary impacts on populations (CHRONIC proposal).

Non-conventional endpoints are potentially more accurate and sensitive in their ability to express environmental effects (Gomes et al., 2021; Ford et al., 2025). Here we define non-conventional endpoints *as endpoints not usually applied in regulatory frameworks for effect assessment. Furthermore, these endpoints are traditionally not standardised in commonly used guidelines such as OECD guidelines.* While abnormal behaviour for some invertebrates is included in OECD test guidelines (e.g. OECD, 2023), effects are usually only based on visual observations and only recorded qualitative. Furthermore, they are not used as primary regulatory endpoints (Ågerstrand et al., 2020). More complex or specific behavioural measures are not standardised within OECD guidelines, and according to our above definition, fall under non-conventional endpoints.

Although less standardised, these endpoints are promising especially for chemicals with novel modes of actions such as genotoxic, immune toxic, or neurotoxic, as they seem to link more directly to the actual mechanisms of these biologically active molecules (Gerhardt, 2007; Wong & Candolin, 2015; Van den Berg et al., 2019). However, their

relative importance on population impacts is less clear, challenging the consideration of many of these endpoints in quantitative risk assessments (Ågerstrand et al., 2020; Bertram et al., 2025; Pamminger et al., 2025). The table below show a simplified comparison between conventional and non-conventional endpoints (Table 1.1)

Table 1.1 Simplified list of differences between conventional endpoints and non-conventional endpoints to guide a better understanding of the distinction between conventional and non-conventional endpoints.

	Conventional endpoints	Non-conventional endpoints
+	Standardised in protocols to ensure high reproducibility	Can often relate directly to specific modes of actions
	Relates directly to population measures	Show high sensitivity towards specific stressors
		Add realism in higher tiers
-	Fail to predict non-apical effects	Not typically standardised
		Complicated to link to population responses
	Examples: mortality, growth, and reproduction of a few standard species	Examples: behavioural and physiological sublethal endpoints, immune responses, and molecular biomarkers.

There is a clear consensus that currently the use of non-conventional endpoints in risk assessment can be improved, enhancing our mechanistic understanding of chemicals impact on the environment (Forbes et al., 2006; Bownik and Wlodkowic, 2021; Ford et al., 2025). Endpoints selected for effect assessment should ideally be relevant to the test system, including the mechanism of action of the chemical and biology of the test species (Moermond et al., 2016; van den Berg et al., 2019). This ensures more targeted ERAs, increasing the detection limit

and, in the end, enhancing environmental protection (Rivetti et al., 2025).

1.3.2 Relevance of behavioural endpoints for neurotoxins

Despite the increasing number of environmental contaminants with potential neurotoxic action, such as neurotoxic insecticides, direct neurotoxicity assessment of environmental species still lacks (Legradi et al., 2018; Bownik and Wlodkowic, 2021; Casillas et al., 2022). Behavioural endpoints such as locomotion and mobility are expected to show higher sensitivity compared to conventional endpoints for these kinds of compounds (Augusiak and Van den Brink, 2016; Raby et al., 2018). By incorporating the mode of action, novel methods on neuro-behavioural phenotyping directly focusing on the expected target of the compounds, can greatly add to our understanding of impacts (Bownik and Wlodkowic, 2021).

Neonicotinoids and associated compounds are produced to modulate nicotinic acetylcholine receptors (nAChRs) in target insect species with the purpose of pest control (Cutler et al., 2012). The nAChR competitive modulators work by continuously stimulating the postsynaptic cell, leading to sustained muscle activity. At high enough concentrations, this can lead to neurological impairments, including hyperactivity or paralysis (Wei et al., 2020). Although the specific binding sites are still debated, nAChRs are commonly found across an array of species, including non-target insects (Watson et al., 2017). It hence should not come as a surprise that these specific compounds can likewise impact the mobility of non-target insect species (Azevedo-Pereira et al., 2011; Barmantlo et al., 2019).

Textbox 1: Tracking of animal movement

Testing the mobility of non-target species can be done in a multitude of different ways (Bownik and Wlodkovic, 2021). For aquatic species, using cameras to video-track swimming activity has shown promising results (Augusiak and Van den Brink, 2016). Tracking software working by monitoring an organism's location over time, is developed to efficiently and precisely estimate individual behaviour (Noldus, 2001; Chiara & Kim, 2023). Specific biological mechanisms in each species behaviour are important to consider for these methods to be optimal (Bownik and Wlodkovic, 2021). For the standardised test organisms within the *Chironomidae* family, the chironomids larvae uses a 'figure-of-eight' style of swimming, produced by side-to-side flexion of the body, in order to move around in open water. In addition, they perform a distinct slower form of moving, when 'crawling' at sediment surface (Brackenbury, 2000). By recording the animals and tracking them, locomotive endpoints such as mean velocity, swimming capabilities, exploration areas or angle of movement can be investigated depending of the research question (Chiara & Kim, 2023).

As environmental risk assessment revolves around the protection of natural populations, linking laboratory testing outcomes to population dynamics is key for selecting protection limits (Rohr et al., 2016; Schuijt et al., 2021; Brooks et al., 2024). As mortality and reproduction are easily linked to population level effects, their use remains popular in traditional ecotoxicity testing. To increase the use of highly sensitive non-conventional endpoints, such as behavioural endpoints, their linkage to population level responses is crucial (Ågerstrand et al., 2020). There is in general a broad consensus of the relevance of behavioural endpoints on population dynamics (Ford et al., 2025). Clear evidence has demonstrated how certain behaviours of organisms are fundamental for several important processes related to an organism's neurological state (Bownik and Wladkovic, 2021). Limited mobility is associated with severed feeding abilities, directly impacting the individual growth (de Alkimin et al., 2020; Prata et al., 2023).

Moreover, impacts on mobility in aquatic prey species significantly lower their fleeing responses resulting in reduced survival chance in the presence of a predator (Langer-Jaesrich et al., 2010). Additionally, altered mating behaviour as a result of impacts on locomotion can significantly decrease reproductive rates, essential for maintaining an environmental population. These examples highlight the relevance of mobility, when extrapolating from laboratory setups to potential impacts on population dynamics (Langer-Jaesrich et al., 2010; Legradi et al., 2018; Bertram et al., 2025).

1.3.3 Adding environmental complexity: multi-stress and mixtures

Lower-tier risk assessments often lack environmental realism, as laboratory studies often focus on single-stress exposures and optimal test conditions (Backhaus, & Faust, 2012; Schuijt et al., 2021). However, in the environment species are seldom exposed to one stressor at a time, rather a multitude of stressors in combination impact the overall welfare of the organisms (Jackson et al., 2016). The addition of multiple stressors might alter the sensitivity of test species, which is

Textbox 2: Interactions between multiple stressors

In general, the effects of exposure to multiple chemicals and stressors are predicted based on single-stress effects (Backhaus & Faust, 2012). This can be achieved by concentration addition, if the chemicals have a similar mode of action (Bliss, 1939), or by the independent action model, for stressors acting through separate mechanisms (Loewe 1926). Both models assume no interactions between the individual stressors. However, exposure to several compounds simultaneously could end in a ‘cocktail’ effect where the effects of each compound are enhanced by the presence of the others. This is known as synergistic effects, where the combined effects are greater than the sum of their individual effects. On the other hand, multi-stress might also result in combined effects lower than additive, denoted as antagonistic effects.

not accounted for under the current scheme (see review by Holmstrup et al., 2010).

Temperature is an important environmental stressor, where depending on optimal temperature both lowering or increasing the temperature outside of this range can change body functions of organisms (Bonacina et al., 2023). With expected increase in water temperatures due to climate change, this stressor is only set to become even more relevant in the future (Hardenbicker et al., 2017). Increased water temperatures can influence vital organismal responses, such as higher metabolism (Shah et al., 2020) and faster emergence of aquatic insect species (Heye et al., 2019). Furthermore, impacts on the non-conventional endpoint; locomotion, are found for Chironomidae larvae, where traveled distances and swimming speed increased after just 24 hours of exposure (Lencioni et al., 2021). More importantly, research shows that environmental stressors such as increased water temperature can alter chemical sensitivity of aquatic species (Scherer et al., 2013; Macaulay et al., 2020). Not incorporating relevant environmental conditions could result in actual environmental effects of chemicals being underestimated, and potential adverse outcomes going unnoticed (Holmstrup et al., 2010; Jackson et al., 2016).

Similarly, environmental population are often exposed to a mixture of chemicals at a time, where the combined toxicity of the complex mixture might not be known (Backhaus, & Faust, 2012; Harrison et al., 2025). While mixtures with few components often follows the predictions of additive modelling (See textbox 2), more complex mixtures or environmentally relevant settings are not accounted for in traditional testing (Kidd et al., 2024). As an example, commonly used plastics usually are added a plethora of chemical additives, which might leak into the environment (Wiesinger et al., 2021). The exact composition of this mixture of leached chemicals can depend on the plastic type or environmental conditions (Luo et al., 2019; Capolupo et al., 2020). Whereas chemical risk assessment relies on individual testing of each component, testing on the whole mixture is a relevant

option, since a complete characterisation of all co-occurring toxicants is still troublesome (Luo et al., 2022; Oldenkamp et al., 2022).

1.3.4 Multigenerational testing in chronic effects

For many compounds, slow degradation or continuous release can contribute to the exposure of natural aquatic ecosystems over prolonged periods. Tests conducted over longer periods of exposure may hence be more representative of conditions that organisms are likely to encounter in natural environments.

But, due to practical reasons, chronic testing is usually confined to a single life cycle. However, over multiple generations, long-term effects may manifest differently, with delayed consequences. Maternal transfer resulting in bioaccumulation or transgenerational changes in organisms' responses is a potential influence that multigenerational exposure can have on the toxicological outcome (Zhao et al., 2022). As a result, carry-over effects or subtle impacts altering populations after prolonged exposure might be missed, whereas chronic testing of multi-generational exposures may constitute a more sensitive means of assessment (Heugens et al., 2003; Sánchez-Bayo, & Tennekes, 2020).

1.4 The CHRONIC network

This PhD project is part of the ITN Marie-Curie network CHRONIC, developed to address some of the aspects mentioned in this introduction. The project consists of 13 early-stage researchers looking into the challenge of “*need to better understand how chemicals affect species biology under chronic exposure to support chemical stewardship, environmental protection, protect corporate reputation and inform the public*”. This is done through a multidisciplinary and intersectional approach with an intensive training and research network.

As different toxicity mechanisms such as (epi)genetics, immune response or neurotoxicity, these will be the main focus points under low

chronic exposures, multigenerational and multiple stressors scenarios (CHRONIC proposal).

1.5 Objectives and outline of this thesis

Traditional ecotoxicity testing might fall short in predicting all possible adverse effects of (novel) anthropogenic pollutants. For better environmental protection, we need to rethink current standardised methods. Therefore, the overarching aim of this thesis is to *investigate the relevance of non-conventional endpoints in predicting potential effects of chemical-induced stress*.

Specifically, we set four research objectives to evaluate:

1. Screen for potential sensitive non-conventional endpoints using a range of organisms exposed to a neurotoxin.
2. Assess the sensitivity of *Chironomus riparius* in a multifactoral chronic test focusing on conventional vs non-conventional endpoints for a neurotoxin under increased ecological realism.
3. Assess the sensitivity of non-conventional behavioural endpoints of *C. riparius* compared to conventional endpoints in a chronic test for mixtures of chemicals with unknown mode of action, leached from plastics and their biodegradable counterpart.
4. Understand how individual effects translate into multigenerational testing schemes and whether non-conventional endpoints might contribute to enhanced predictions of actual environmental effects.

We aim to obtain this through the next chapters.

Chapter 2 revolves around whether a hackathon event with MSc students from Leiden University could be used to detect potentially sensitive aquatic species and endpoints when exposed to sulfoxaflor and to develop best practices and methods to get large and fast amounts of reliable data on potential sublethal effects induced by neurotoxic chemicals. Secondly, based on the data collected in the Hackathon, a subset of endpoints was tested by researchers to validate the results and to determine more accurate dose-response relationships.

Chapter 3 further explores to what extent concurrent exposure of the non-target benthic organism *C. riparius* larvae to environmental stressors and a nicotinic acetylcholine receptor (nAChR)-modulating insecticide, sulfoxaflor. We investigate the impact on both lethal and sublethal endpoints and compare the sensitivity of conventional endpoints to behavioural responses. To this end, we used a multifactorial environmental stressor approach and hypothesised that the sensitivity of the organisms increased under stress-on-stress conditions.

Chapter 4 compares the effects of artificially weathered conventional linear low-density polyethylene (PE) and starch-based biodegradable polybutylene adipate terephthalate (PBAT) as well as their leachate on the non-target *C. riparius* larvae in order to assess toxicity of a whole-mixture with unknown mode of actions. Potential adverse effects are examined via traditional life cycle endpoints such as mortality, growth, and emergence, alongside behavioural endpoints including larval locomotion and burrowing abilities.

Chapter 5 takes the prolonged effects of multigenerational testing into account when addressing how well long-term environmental effects are predicted. The study aims to examine long-term effect patterns in order to aid in the prediction of potential environmental effects that manifest over multiple generations. We have compiled secondary literature of the impacts of chemical multigeneration exposure, with a particular

focus on sensitivity patterns between types of chemicals, organisms and tested endpoints, including the predictability of conventional endpoints compared to novel non-conventional endpoints.

Chapter 6 brings together the findings of this thesis and discusses it.

