Review: Fraunhofer IME **Opportunities and Limitations of Aquatic Invertebrate Behavior as Relevant Endpoint** in Ecotoxicological Testing

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Background

Behavioral responses of aquatic invertebrates have recently gained attention by ecotoxicological scientists. Behavioral endpoints are a promising tool in assessing ecologically relevant effects of contaminants1. Most regulatory guidance documents focus on mortality, immobilization, growth or reproduction, however behavioral endpoints are a promising approach for more sensitive endpoints. Recently, automated tracking systems have become available and simplify data analysis. Opportunities collection and and limitations of behavioral endpoints of aquatic invertebrate model organisms and their use in ecotoxicology are to be discussed in this minireview.

Opportunities

- Higher sensitivity compared to classical endpoints in guidance documents⁸
- Short-term responses, noninvasiveness⁸
 - Ecological relevance: endpoints are related to e.g. feeding, reproduction or overall fitness⁸
- Allowing for repeated measures and time-dependent data analysis⁸
- Shorter duration than classical endpoints⁸
- Linkage between behavioral endpoints and biochemical biomarkers¹

Limitations & Need of Further Research

- Lacking standardization in setting parameters (e.g. recording time, light/dark conditions etc.)^{8,22} & inconsistency in terminology⁴
- Poor simulation of natural conditions⁴, testing of, e. g., sediment dwelling organisms holds difficulties
- Methodological gap in ecotoxicology: assessment of 3D parameters⁴
- Biological variability results in high behavioral variability⁴ and requires large sample sizes
- Insufficient understanding of baseline unconditioned behavior^{14,22} and sex-specific behavioral responses²²
- Inter-species differences in behavioral responses¹⁴
- Most sensitive endpoint seems to be contaminant-dependent^{1,22}
- Suitable holding for behavioral measurements might cause stress in organisms leading to affected behavior

Possible Endpoints in Behavioral Ecotoxicology

Table 1: Overview over possible behavioral endpoints in model organisms tracked by using automated tracking systems in literature.

tracking systems in literature.									(Locomotion	
Inver	tebrate Model		Possible Behavioral Endpoints						Swimming Path Distance		
Organism		Guideline Test	Locomotion	Taxis	Avoidance	Respiration	Startle Response	References		Activity Exploration Speed Turning Angles	
	Daphnia magna	OECD 202 & 211				*		4, 5, 7, 13, 19, 20, 27	-	TaxisPhototaxis RheotaxisThermotaxis ThigmotaxisSpatial OrientationAvoidancePredatorHeat	
	Gammarus pulex	OCSPP 850.1020				*		9, 14, 22, 23, 26			
	Asellus aquaticus	_				*		3, 6, 10, 12; picture: 16; Preliminary tests at Fraunhofer IME (unpublished)		Startle Response Contaminant	
	larvae of Chironomus riparius	OECD 218, 219, 233 & 235						2, 11, 15, 18		Respiration Frequency Surface Reaching [Consumption Rate]	
	Lymnaea stagnalis	243						17, 21, 24, 25		Startle Response Vibration Touch Noise	
* only possible by O ₂ measurements, not by automated tracking devices										Light	

*: only possible by O₂ measurements, not by automated tracking devices **Outlook & Research Aims**

- Standardization of testing conditions, endpoints (validation criteria) and terminology
- **Development of 3D tracking technologies**
- Further investigations regarding background unconditioned behavior, sex-specific behavioral responses to contaminants
- Further studies comparing contaminant effects on behavior of different species
- Establishment of behavioral responses as future additional endpoint in regulatory guidance documents or as toxicity screening tool
- Behavioral responses provide the opportunity to develop more sensitive endpoints

