Are mesocosms really not suitable for the risk assessment of plant protection products?

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Background

A recently published article on the representativity of macroinvertebrate communities in micro- or mesocosm studies used as a higher tier tool in the environmental risk assessment of plant protection products (PPPs) in the EU concluded that "*micro-/ mesocosm studies do not represent natural macroinvertebrate communities*" (Reiber *et al.* [1]). Fundamentally, the article based its conclusion on the analysis of data from 26 streams in a recent monitoring project in Germany (Liess *et al.* [3]) in comparison to taxa found in seven lentic micro- and mesocosm studies, conducted 2013 – 2018 at four test sites and submitted to the UBA, Germany. We believe that this conclusion is unfounded for multiple reasons:

- Streams are not the only type of water bodies in agricultural landscapes, there are also ditches and ponds.
- Solely based on numbers, the diversity of macroinvertebrates in 26 streams in Germany can be expected to be higher than in four test systems.
- The number of taxa, for which the Minimum Detectable Differences (MDDs) were low enough to allow a detection of direct effects in seven mesocosm studies, cannot be compared to the number of taxa just present in at least five of 26 streams.
- The SPEAR trait classification of taxa as sensitive, developed for stream macroinvertebrate monitoring with potential exposure to multiple chemicals, is not applicable to mesocosm studies on effects of one specific test item with known mode of action.

Evaluation of the mesocosm studies

Here we intend to revisit the data provided by the seven selected studies from this publication, with the objective of determining how many, and which, taxa were considered as potentially sensitive or vulnerable and allowed a meaningful statistical analysis of effects with no other constraints.

Thus far, we have re-evaluated the invertebrate data in five of the seven mesocosm studies based on the MDDs. We followed the approach by Reiber *et al.* [1] considering only the MDDs within the first 30 days after the first application to focus on direct effects.

However, the relevant period to detect direct effects depends on the number of applications, the dissipation of the test item and the type of data – emerging insects may have been exposed as larvae several weeks before.

We listed all invertebrate taxa with minimum MDD < 70 % within the 30 days instead of the mean MDD since MDDs of e.g. 99, 45, 50, 99 % during the first 4 weeks would still allow to detect a direct effect < 70% despite that the mean is > 70 %. We used the MDDs provided in the study reports, according to Brock *et al.* [2].

The four mesocosm test systems

Taxon	Family	Group	Study-20	Study 21	Study 22	Study-23	Study-43	% presence in studies	Mean MDD_{Min}%	MDD% in 5 reference streams 1)	Likely evaluable in meso	Sensitive (SPEAR) 2)	Vulnerable (SPEAR) 3)
Crustacea													
Daphnia	Daphniidae	Cladocera	66		60	50	64	80	60		X	1	
Chydorus	Chydoridae	Cladocera	69	65				40	67			1	
Simocephalus	Daphniidae	Cladocera	65				68	40	67			1	
Cyclopodiae	Cyclopodiae	Copepoda	40	37	35	61	29	100	40		X		
Diaptomidae	Diaptomidae	Copepoda		64			11	40	38				
Asellus sp.	Asellidae	Isopoda	38	46	32	51	21	100	38		X	0	0
Crangonyx	Crangoyctidae	Amphipoda				47		20	47		X 1	1	0
Gammarus	Gammaridae	Amphipoda								95		1	0
Insecta													
Chaoborus	Chaoboridae	Diptera	42	45	39	42	29	100	39		X	1	1
Chironominae	Chironimidae	Diptera	47	51		54		60	51	97	X	0	0
Tanypodinae	Chironimidae	Diptera		69		46		40	58		X	0	0
Orthocladiinae	Chironimidae	Diptera			68			20	68			0	0
Culicidae	Culicidae	Diptera				58		20	58			1	0
	Pediciidae	Diptera								83			
	Simuliidae	Diptera								95		0	0
Cloeon	Baetidae	Ephemeroptera	56	58	61	64		80	60	89	X	1	1
	Heptagenidae	Ephemeroptera								95		1	1
	Limnephilidae	Trichoptera								89		1	1
Corixidae	Corixidae	Heteroptera		62				20	62			1	0
Notonecta	Notonectidae	Heteroptera				53		20	53			0	0
Anisoptera	Anisoptera	Odonata				50		20	50			0	0
Zygoptera	Zygoptera	Odonata		49		46		40	48		X	0	0
Coenagrionidae		Odonata	57		45	66		60	56			1	1
Polycentropodidae	Polycentropodidae	Trichoptera			65			20	65			1	1
Rotifera													
Polyarthra	Synchaetidae					57		20	57				
Keratella	Brachionidae		41	54	63			60	53		X		
Trichotria	Trichotriidae			64				20	64				
Lepadella	Lepadellidae						41	20	41				
Lecane	Lecanidae						67	20	67				
Clitellata													
Tubificidae	Tubificidae	Oligochaeta	66					20	66		X 4)	0	0
Lumbriculus		Oligochaeta			68			20	68			0	0
Helobdella	Erpobdellidae	Hirudinida		61	54	67		60	61		X	0	0
Turbellaria						62		20	62		X	0	0
Dugesia	Glossiphoniidae	Turbellaria	59		40			40	50			0	0
Mollusca													
Lymnaea	Lymnaeidae	Gastropoda	47	48		39		60	45		X	0	0
Sphaeridae	Sphaeridae	Bivalvia				55		20	55			0	0
n_Invertebrates (MDD_{Min} < 70%)			**13**	**14**	**12**	**18**	**8**		**55**	**0**	**14**		
n_Arthropods (MDD_{Min} < 70%)			**9**	**10**	**8**	**13**	**6**		**54**	**0**	**9**		

Taxa with minimum MDDs < 70 % within the first 30 days after (first) application in the five evaluated mesocosm studies

1) MDDs calculated from the data of the five references sites defined in Liess *et al.* [3]; only those families with an MDD < 100 % listed
2) Taxa considered as sensitive in SPEAR, based on average acute toxicities of organic chemicals related to the EC50 of *D. magna* [4]
3) Taxa considered as vulnerable in SPEAR considering average sensitivity, generation time, exposure potential and potential of colonisation from refuges [3]
4) The amphipod *Crangonyx* can be introduced in cases where gammarids are not feasible
5) The studies did not include sediment samplings which would provide data for Tubificidae and other sediment organisms with lower MDDs

Results

- In the mesocosms, the number invertebrate families with MDD < 70% considered sensitive or vulnerable according to SPEAR is low (5 respectively 3). In the monitoring data set, MDDs < 100% can be expected for 4 sensitive and 3 vulnerable families.
- However, the mesocosms include potentially sensitive taxa which are not considered sensitive in SPEAR based on averaged sensitivity, e.g. non-arthropods which can be highly sensitive to fungicides as well as plankton species.
- MDDs were below 70 % for 12 to 18 invertebrate taxa per study and, thus, allowed to detect medium effects (as defined by [5]. For arthropods only, these were 8 to 13 taxa per study.
- Based on these example studies, 14 taxa, covering Crustacea, Insecta (4 families), Rotifera, Clitellata, Turbellaria, and Mollusca, can be expected to be evaluable for direct effects in such studies.
- Variability in the field is larger than in the mesocosms studies. Plecoptera are also hardly found in sufficient numbers in reference streams.

Conclusions

- Also several taxa not considered sensitive in SPEAR based on average sensitivity to organic chemicals can be considered relevant in mesocosm studies, depending on the specific test item. Thus, usually a sufficient number of potentially sensitive taxa can be evaluated under the Ecological Threshold Option.
- The use of isolated test systems prevents recolonization of non-flying species. Thus, such taxa are more vulnerable than in the field which could be considered conservative for the Ecological Recovery Option.
- Lentic systems represent ponds and ditches. Typical stream taxa cannot be expected, but can be tested in the laboratory or artificial streams if considered especially sensitive.
- Exposure duration in mesocosms is usually longer than expected in streams, which can be seen as conservative for stream taxa.
- Well conducted micro-/mesocosm studies provide reliable and useful higher tier data for the risk assessment of PPPs, and other chemicals, since they are the only aquatic experimental option to cover long-term as well as indirect effects under semi-natural conditions and in a community context.

References: [1] Reiber *et al.* 2022, doi.org/10.1186/s12302-022-00643-x, [2] Brock *et al.* 2015. doi.org/10.1007/s11356-014-3398-2, [3] Liess *et al.* 2021. DOI: 10.1016/j.watres.2021.117262, [4] von der Ohe & Liess 2004, ETAC 23 (1), 150–156, [5] EFSA PPR panel 2013, doi:10.2903/j.efsa.2013.3290.