Do new generations of active pharmaceuticals for human use require an adaption of the environmental risk assessment? Part II: Case studies

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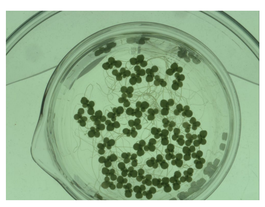
Background

In 2006, the European Medicines Agency (EMA) adopted a guideline on the environmental risk assessment (ERA) of medicinal products for human use[1]. Since then, a large number of active pharmaceutical ingredients (APIs) with specific Mode-of-Actions (MoAs) that are potentially effective in the aquatic environment in concentrations lower than 0.01 µg/L have been approved. This raised concern that the guideline does not allow a sufficiently protective environmental risk assessment for all of these new and very specific APIs and adaptations might be necessary. A test strategy proposed after a literature research conducted in a previous study (FKZ 3718 65 420 1) should enable identification of effects specifically related to the MoA of the API and/or effects which occur at concentrations lower than the endpoints derived in the current standard long-term toxicity test set on fish, daphnia and algae. During the literature research, a number of test systems with potentially high sensitivity to these new APIs were identified. These tests include the *Lemna sp.* Growth Inhibition Test (OECD 221)[2], the *Danio rerio* Fish Embryo Test (OECD 236)[3] amended with sublethal endpoints, and the comet assay[4] with environmentally relevant cell types derived from *D. magna* and *D. rerio*. With theses tests, a total of 18 substances (oncologicals, cardiologicals and statins) were investigated. The data generated according to the proposed new test strategy was compared to available data e.g. from European public assessment reports (EPARs) to evaluate the level of protectiveness.

Test systems

OECD 221 Lemna sp. Growth Inhibition Test



Test design: 7 days; static or semi-static

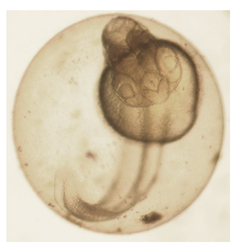
Test species: *Lemna minor*, *Lemna gibba*

Test medium: AAP medium, Steinberg medium

Measurement variables: frond number, frond area and/or dry weight

Endpoint: NOEC or EC_{10} for growth rate

OECD 236 Fish Embryo Test

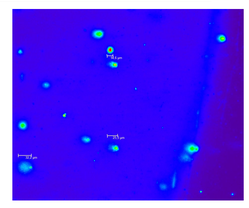


Test design: 5 days; static

Measurement variables: Coagulation, somite formation, detachment of the tail, heart beat, hatch, spontaneous movement, pigmentation of body, malformation of otoliths, oedema, deformation of spinal cord and yolk, number of somites, heart beat rate, body length.

Endpoint: NOEC or EC_{10}

Comet Assay with *D. magna* and Zebrafish liver cells (ZFL)



Test systems: Cell lysates from *D. magna*; Zebrafish liver cell line (ZFL)

Test design *D. magna*: exposure according to OECD TG 202 (48 h) with subsequent lysis

Test design ZFL: density of 100.000 cells/24-well plate, 48 h exposure

Measurement variable: Tail intensity % (TI%)

Endpoint: NOEC

Results

Substance	Mode of Action	Observed effect Lemna	Lemna vs. standard endpoints	Observed effect Comet assay	Observed effect FET
Edoxaban tosylate hydrate	Faktor Xa inhibitor; anti-coagulant	-	No effect expected	-	-
Rivaroxaban	Faktor Xa inhibitor; anti-coagulant	-	No effect expected		-
Atorvastatin calcium	HMG-CoA reductase inhibitor	+	More sensitive		
Pitavastatin calcium	HMG-CoA reductase inhibitor	+	No data on standard endpoints	+/-	
Rosuvastatin calcium	HMG-CoA reductase inhibitor	+	More sensitive	-	+
Propranolol hydrochloride	ß-blocker				+
Dabrafenib mesylate	BRAF-Serin-Threonin Kinase inhibitor	+	More sensitive	-	
Abemaciclib	CDK4/CDK6; kinase inhibitor	+	Less sensitive	-	
Palbociclib	CDK4/CDK6; kinase inhibitor	+	More sensitive	-	+
Ribociclib succinate	CDK4/CDK6; kinase inhibitor	+		-	
Methotrexat	Dihydrofolate reductase inhibitor	+	No data on standard endpoints		
Pemetrexed disodium heptahydrate	Dihydrofolate reductase inhibitor; Thymidylat synthase Inhibitor; Glycinamid ribonucleotide formyl transferase inhibitor	+	More sensitive		
Cabazitaxel	Mikrotubule inhibitor (Beta-Tubulin)	+	Less sensitive		
Paclitaxel	Mikrotubule inhibitor (Beta-Tubulin)	-	Less sensitive		
Imatinib mesylate	Thyrosin-kinase inhibitor	+	More sensitive	+/-	-
Afatinib dimaleate	Thyrosin-kinase inhibitor erbB-2	+	Less sensitive		-
Neratinib maleate	Thyrosin-kinase inhibitor	-	Less sensitive		
Cyclophosphamide monohydrate	Alkylating antineoplastic			+	+

Legend: + = effect observed; - = no effect observed; +/- effect only in one of the two test systems; no study performed

Conclusion

The test with Lemna sp. was more sensitive than the standard endpoints (available data, e.g. EPARs; [5]) for two substances with a pharmacological MoA relating to the mevalonate pathway (atorvastatin, rosuvastatin), three kinase inhibitors (dabrafenib, palbociclib, imatinib) and one dihydrofolate reductase inhibitor (pemetrexed). Thus, the Lemna growth inhibition test might be a relevant additional test for the ERA of at least some APIs, e.g. the statins or the dihydrofolate reductase inhibitors. The fish embryo toxicity test amended with sub-lethal endpoints and the comet assay were in none of the examined cases more sensitive than the currently employed apical endpoints from chronic aquatic toxicity tests (OECD 201, 211, 210) and can at best provide additional information.

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