Introduction of a New Dosing System for Chronic Fish Tests Conducted with Difficult Substances

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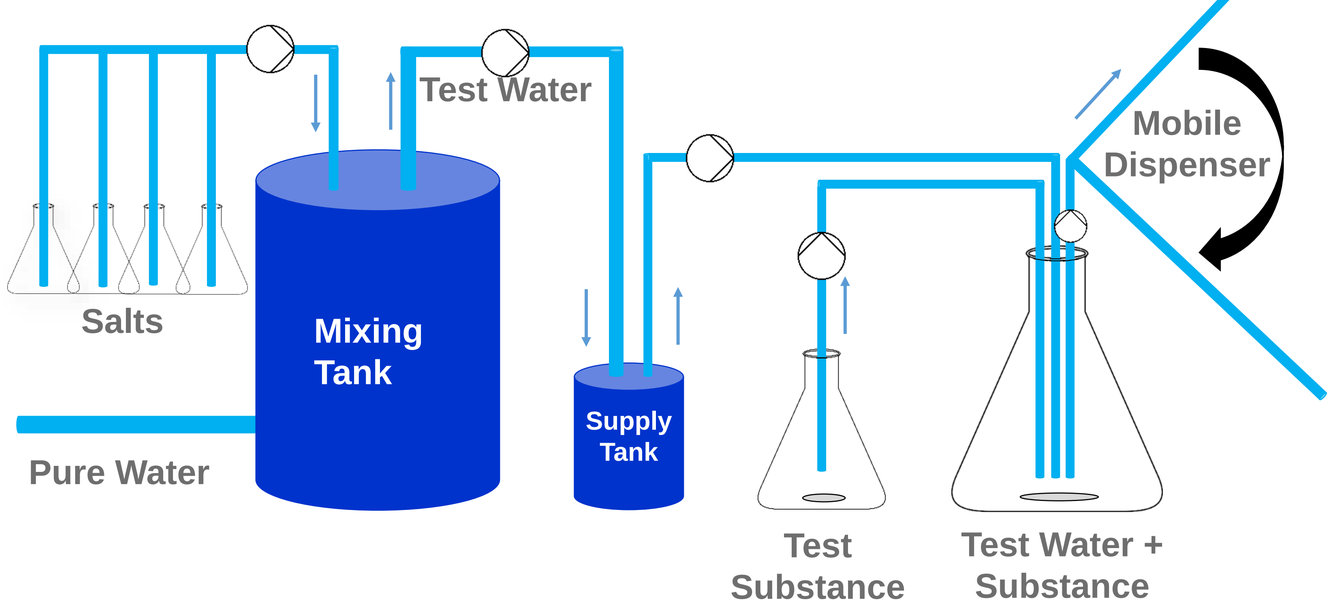
Background

Chronic toxicity tests with fish are required for the risk assessment of plant protection products, pharmaceuticals and chemicals (depending on the tonnage and characteristics of the chemical). As chronic standard toxicity test the fish Early Life Stage (ELS) test following the OECD Guideline (GL) 210 has to be conducted, in certain cases this test system can be extended to an OECD GL 229, 230, 234 or to a full life cycle test. All these tests include the evaluation of sublethal effects on the test fish. This extension is recommended in case an influence of the substance towards the endocrine system (and finally the reproduction) cannot be excluded. The addition of some "endocrine endpoints" avoids additional tests, which may be requested at a later stage from the competent authorities. Typically, for these chronic fish tests very low concentrations have to be tested and in many cases the substance to be tested can be classified as difficult according to the OECD criteria, for instance low water solubility, high toxicity to fish, volatility or degradation during the test. For chronic fish tests preferable a flow through test design is used and difficult test items with specific properties as described above request a highly sophisticated flow through test device to guarantee the success of the test. In cooperation with an external company specialized on providing flow through technique to science and industry, IES developed a new, highly flexible dosing system. This computer controlled dosing device is a modular system which provides several new technical features for important steps during the test e.g. dosing of the test substance, preparation of the test media and distribution of the test medium to replicates. In this presentation several examples for the testing of difficult substances are shown and the advantages of this dosing system are explained. The biological and analytical results demonstrate that difficult test items can be successfully handled using all available technical options, which are provided by the presented flexible flow through dosing system. Considering the increasing complexity of ecotoxicological tests and the methodical challenges during the testing of difficult substances, this presentation also intends to underline the importance of a continuous improvement of the technical setup for a successful performance of ecotoxicological test.

Test System: Fish Flow-Through

Schema 1: Fish Flow-through Systems. Test water produced by dosing highly concentrated salt solutions in pure water. Temperature adapted in supply tank before the substance is mixed into the tempered test water. Finally the substance is dosed into the four replicates à 20 individuals.



Picture 1: One of the flow-through systems. Control and five treatments

Case Studies

Test Organism	Zebrafish (ZF)	Fathead minnow (FM)	Zebrafish	*ZF, FM, Rainbow trout, Bluegill sunfish, Medaka*
Dosage via..	DMF	**Water**	Ethanol	*Water and organic solvents*
Dosage Volume	**30 µL/L**	**10 mL/L**	60 µL/L	*30 µL/L – several mL/L*
Recoveries	75 -112%	85 -105%	**81 - 115%**	*80 -120%*
Tested Range	10 - 1000 µg/L	70 - 700 µg/L	**0.10 - 10 µg/L**	*0.01-10'000 µg/L*
Comment	**Very low amount of solvent (world record?)**	Special study design based on OECD 210	**Under fume hood**, highly toxic	*Anything is possible*

Table 2: Flow-through Fish Early Life Stage Tests. Shown are three case studies, the challenges are marked in **bold** and red encircled**.** In *italic* you find a summary of the possibilities of the new system.

	Zebrafish	Fathead minnow
Hatching Rate	95 +/- 4	97 +/- 2
Survival after 30 Days	97 +/- 3	96 +/- 3
Wet Weight [mg]	41 +/- 8	51 +/- 12
Dry Weight [mg]	9 +/- 1	10 +/- 1
Length [mm]	17 +/- 1	18 +/- 1

Table 3: Biological endpoints of the new Fish early life stage flow-through tests with Zebrafish and Fathead minnow. Shown are representative examples.

Results, Discussion & Summary

Flow-through systems for fish toxicity or endocrine disrupter tests are tricky for several reasons **(Picture 1,2)**. The test water has to fulfill special requirements. It has to be excluded that toxic trace substances or substances with endocrine disruptive potential pollute in the test water. Thus for the production of the testing water ultrapure water is used and the salts are added as shown in **Schema 1.** All materials have to be build up of inert material such as glass, stainless steel or PTEE. This is very important as small parts containing toxic substances for fish (e.g. copper) can result in adverse effects to fish and thus to an invalid test. The system introduced in this presentation is completely free of dangerous substances for fish. This led to a very high hatching rate, a very high survival rate of the fish after 30 days (control) and a consistent growth of the individuals over the testing period (**Table 3**).

From a technical point of view, a redundant water supply and reliable, flexible and highly accurate pumps are required to have the chance to adapt dosing volumes to the different test designs and to find the best test design to answer the sometimes complicated questions, which are coming from the risk assessors at the competent authorities **(Table 2)**. In addition the dosage, the renewal rate and the feeding scheme must be adaptable to the specific characteristics of the test substance, to the tested fish species and to the age of the fish.

In one case the combination of highly toxic to fish (and humans), volatile and low water solubility resulted in the development of a fish flow-through system under a large fume hood **(Table 2)**.

The use of a sophisticated flow-through system for fish tests as presented here avoids the repetition of tests (reduce), enables new test designs (replace, refine) and thus follows exactly the principles for the 3R (**reduce**, **refine**, and **replace** the use of animals in experiments).

Picture 2: Fish flow - through systems: Mobile dispenser.

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