

Effects of the flame retardant hexabromocyclododecane (HBCD) on the early life development and metamorphosis of echinoderms

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Introduction

Early life development and thyroid hormone-dependent metamorphosis of amphibians and fish were shown to be sensitive to short time exposure of persistent organic pollutants (POPs) [1-4]. As echinoderms have a thyroid hormone dependent metamorphosis as well, it is hypothesized that echinoderm early life stages may also be affected by exposure to POPs.

Two echinoderm species were aquacultured [5], the burrowing heart urchin *Echinocardium cordatum* and the sea urchin *Psammechinus miliaris* (Fig. 1).

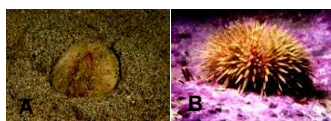


Figure 1
A) Heart urchin (*Echinocardium cordatum*);
B) Sea urchin (*Psammechinus miliaris*).

The present study describes the effects of the marine contaminants HBCD and PCB126 (dioxin-like compound) on the early development of echinoderms. Due to the extremely small size of the larvae (90-600 µm) a new test method had to be developed.

Materials & Methods

The early life stage (ELS, Fig. 2) test was performed at 19±1°C with 0,5 larvae/ml in 500 ml test beakers (duplicate) from 0-16 days post fertilization (dpf). Larvae were exposed to solvent control (SC), 9, 25, 50, 100 nM HBCD, and to SC, 0,3, 3, 30, 300 pM PCB 126. Twice a week half of the test volume was replaced. At 1, 6 and 13 dpf 20 larvae were sampled from each beaker (20x2) into a 24 wells plate (2 larvae/well) with 2 ml of test volume. Larvae condition was scored during 3 subsequent days.

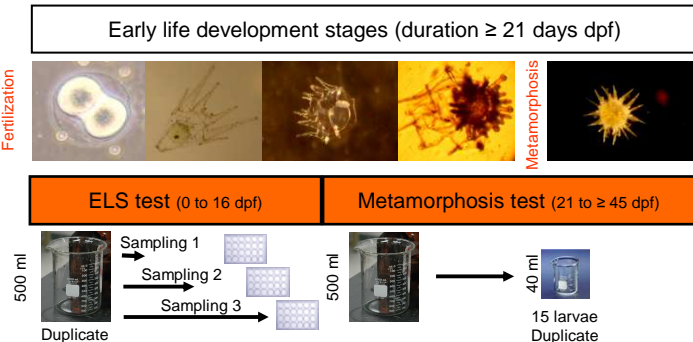


Figure 2. Experimental setup of the ELS test (left) and metamorphosis test (right) developed for echinoderm larvae (here *P. miliaris*)

For the metamorphosis test (Fig. 2), larvae were reared at 19±1°C in clean filtered sea water until 21 dpf. Larvae were exposed in beakers of 40 ml with 15 larvae each (duplicate) to SC, 9, 25, 50, 100, 200 nM HBCD, to 0, 10⁻¹², 10⁻¹¹, 10⁻¹⁰, 10⁻⁹ M thyroxin (T4), and to 0, 10⁻⁶, 10⁻⁵, 10⁻⁴, 10⁻³ M of the thyroid hormone synthesis inhibitor thiourea (TU). Twice a week 10 ml of new test solution was added. Larvae were scored 3 times per week for survival and completion of metamorphosis.

References

- [1] Foekema, E., Deerenberg, C., Murk, A. (2008). Early life stages of the marine flatfish sole (*Solea solea*) show delayed toxic effects after exposure to PCB 126. *Aq. Tox.* 90 197–203.
- [2] Gutleb, A.C. and Murk A. J. *et al.* (1999). Delayed effects of pre- and early-life time exposure to polychlorinated biphenyls_PCBs on tadpoles of two amphibian species. *ETAP* 8:1-14.
- [3] Gutleb, A.C. and Murk A. J. *et al.* (2007). Amphibian metamorphosis assay using synchronized larvae of *Xenopus laevis*. *Chemosphere* 70: 93–100.
- [4] Gutleb, A.C. and Murk A. J. *et al.* (2007). Delayed effects of environmentally relevant concentrations of 3,3',4,4'-tetrachlorobiphenyl (PCB-77) and non-polar sediment extracts detected in the prolonged-FETAX. *Science Tot. Environ.* 381, 307-315.
- [5] Schipper C.A. and Murk A.J. *et al.* (2008). Cultivation of the heart urchin *Echinocardium cordatum* and validation of its use in bioassays for salt-water environmental toxicity testing in risk assessment. *J. of Exp. Marine Biol. Ecol.* 364, 11–18.

Results – ELS Test

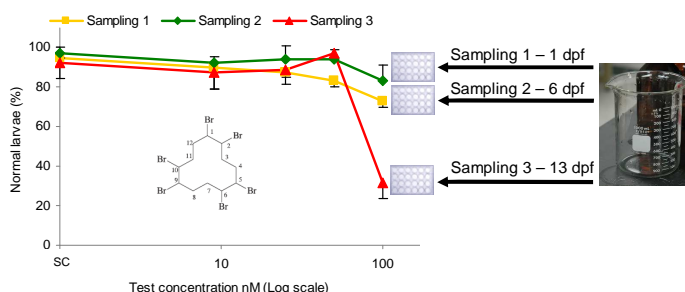


Figure 3. Percentage of normal *P. miliaris* larvae exposed to HBCD (SC, 9, 25, 50, 100 nM) from dpf 0 -16. Each data point represents the means of 3 scorings for each sampling (duplicate) plus the standard deviation.

Conclusions

- With the newly developed test systems for early development and metamorphosis, it was possible to quantify the lethal and sub-lethal effects of toxic compounds on the development echinoderm larvae.

- HBCD induced mortality with a very steep dose response curve (NOEC 50 nM), both in the ELS test (after 13 days) as well as in the metamorphosis test (already after 4 days of exposure) (Figs. 3 and 4).

- T4 enhanced metamorphosis, while TU delayed it (Fig. 5) supporting evidence that *P. miliaris* and *E. cordatum* have a thyroid hormone (T4) dependent metamorphosis just like frogs (T3) [3].

- In echinoderm larvae PCB126 did not induce any developmental effects (data not shown, tests repeated 2x), in contrast to reports for fish and frog larvae [1,2].

Results – Metamorphosis Test

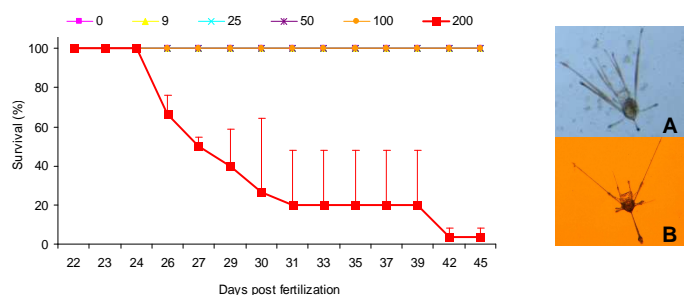


Figure 4. Survival of *E. cordatum* larvae exposed to HBCD [nM] from dpf 20-45 (mean+ stdev, duplicates, 15 animals per beaker). Pictures: A – healthy larvae; B – damaged larvae (after 1 day exposure to 200 nM HBCD)

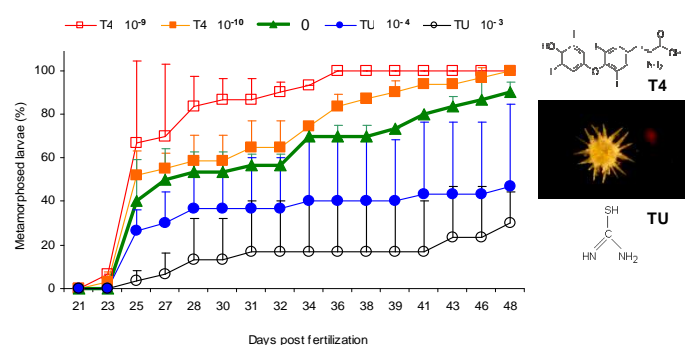


Figure 5. Percentage of metamorphosed *P. miliaris* (picture) larvae exposed to T4 [M] and TU [M] from dpf 20-48 (mean+ stdev, duplicates, 15 animals per beaker). (Results from 10⁻¹², 10⁻¹¹ M T4 and 10⁻⁶, 10⁻⁵ M TU not shown for sake of clarity).