

**STSM Scientific Report**

**COST STSM Reference Number:** COST-STSM-FA1205-20685

**COST Action:** FA1205

**STSM Applicant:** Ms Ievgeniia Gazo, Faculty of Fisheries and Protection of Water, University of South Bohemia, Vodnany (CZ) , gazo@frov.jcu.cz

**STSM Topic:** The carcinogenic and reprotoxic effects of vinclozolin and bisphenol A on eggs and embryo development of tunicate (*Phallusia mammillata*)

**Purpose of the STSM:**

- To assess the potential effect of water pollutants on reproduction of aquatic animals in collaboration with colleagues from the Marine Station in Villefranche-sur-Mer, Université Pierre et Marie Curie, France.

**Description of the work done and the main results:**

During my stay in the Marine Station in Villefranche-sur-Mer I had a unique opportunity to work with the best specialists in the field of biology of marine animals. Due to their help and collaboration I was able to study the effect of endocrine disrupting chemicals (EDCs) on gamete fusion and embryo development of ascidian (*Phallusia mammillata*). During our study vinclozolin was replaced by another antiandrogen, Diuron. Moreover, the effects of other xenobiotics, such as clorothalonil, bisphenol A (BPA), diethylstilbestrol (DES) and 4-hydroxytamoxifen (4HT) were studied as well. Results of this study are summarized in the Table 1. A specific effect of BPA on sensory vesicle development was observed with BPA affecting otolith and ocellus formation. A literature search suggests that BPA effects are occurring through interaction with estrogen related receptor (ERR). To confirm this we assessed the effect of known ERR antagonists (DES and 4HT) on embryo development. No similar phenotype was observed. In order to study an interaction of xenobiotic with pregnane X receptor (PXR), Venus-tagged PXR1b probes were injected into eggs of *Phallusia* and PXR1b nuclear translocation was recorded for 10 h. However, no effect of BPA on PXR nuclear translocation was observed. Results of in situ hybridization (ISH) with probes toward PXR1a indicate that this gene is expressed in tailbuds in the region close to sensory vesicles (Picture 1).

Table 1. The dose-dependency of xenobiotics effects. No toxicity – no obvious effect on embryo development; teratogenic effect – malformations occurred at certain doses of xenobiotics; cytotoxic effect – cell division was blocked rapidly.

	No toxicity	Teratogenic effect	Cytotoxic effect
BPA	<1 $\mu\text{M}$	1-20 $\mu\text{M}$	>20 $\mu\text{M}$
Diuron	<50 $\mu\text{M}$	50-100 $\mu\text{M}$	$\geq$ 200 $\mu\text{M}$
Clorothalonil	$\leq$ 10 $\mu\text{g/L}$	50-60 $\mu\text{g/L}$	>60 $\mu\text{g/L}$
DES	unknown	0.1-2 $\mu\text{M}$	$\geq$ 5 $\mu\text{M}$
4HT	<1 $\mu\text{M}$	2-50 $\mu\text{M}$	>50 $\mu\text{M}$

**Future collaboration with host institution:**

Due to the present COST action we continued and developed our cooperative work with the Laboratoire de Biologie de Developpement in the Villefranche Marine Station. Preliminary results obtained during 6 weeks of my stay in host institute will help a lot in writing a project proposals for a post-doctoral position in the Laboratoire de Biologie de Developpement in the Villefranche Marine Station.

**Confirmation by the host institution:**

Dr. Remi Dumollard hereby confirms that Ms. Ievgeniia Gazo visited the Laboratoire de Biologie de Developpement, University of Pierre and Marie Curie, Villefranche-sur-mer, France from 22<sup>nd</sup> September to 31<sup>st</sup> October 2014.



Signed: Dr. Remi Dumollard



Signed: Ievgeniia Gazo, M.Sc.