[P29] Structure, function and ligand interactions of the ecdysone receptor from *Daphnia magna*

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Background: Endocrine disrupting chemicals (EDC) is a group of naturally or synthetic compounds that have the ability to mimic hormones and occupy binding site of hormone receptors. These chemicals can modulate endocrine processes and cause adverse effects in exposed individuals. Effects of EDC are well established in mammals and aquatic vertebrates, but knowledge concerning effects on invertebrates is limited. The majority of animal species are invertebrates, and information regarding effects of EDC is necessary to develop a proper risk assessment.

Aim: The goal of the study was to predict the 3D structure of the ecdysone receptor (EcR) from the invertebrate arthropod *Daphnia magna.* The models were used to predict interactions between the receptor and potential EDC. An experimental method was established to validate the prediction. The knowledge gained in this study, can contribute to linking the mode of action (MOA) to adverse outcome pathways (AOP) as an approach for risk assessment of single- and complex mixtures of chemicals.

Methods: Two homology models of the EcR in the sea flea *D. magna* were constructed from the resolved x-ray structure of the homologue EcR ligand-binding domain of the fly *Bemisia tabacil* (sequence identity 71%) and the moth *Heliothis virescens* (sequence identity 58%). The models were evaluated by docking studies using 19 active compounds and 155 decoys and ROC-curves were made. VS of a compound library containing 655 potential EDC (obtained in house) were used to select 4 chemicals for experimental testing (in addition to positive/negative controls). An *in vitro* two-hybrid reporter assay was transferred from NIBB, Japan, and established at NIVA, Oslo, as an attempt to support predicted interactions. Binding and activation of the assay requires the chemicals to be agonists.

Results: Docking and scoring of active and decoys gave acceptable results in both models. *In vitro* results of two of the active compounds, Ponasterone A and 20-hydroxyecdysone, confirmed that the compounds bind to the EcR. The assay only responds to agonists that bind and activate the receptor. The 4 chemicals selected from the VS did not induce any response, indicating that they do not function as agonist. However, it cannot be ruled out that these chemicals are antagonists.