

***In silico* prioritization for endocrine active substances (EAS) and their *in vitro* validation**

Ivano Eberini¹, Luca Palazzolo^{1,2}, Ad A.C.M. Peijnenburg³, Uliano Guerrini¹, Chiara Parravicini¹, Angelo Moretto², and Toine F.H. Bovee³

¹Dipartimento di Scienze Farmacologiche e Biomolecolari and ²Dipartimento di Scienze Biomediche e Cliniche "L. Sacco", Università degli Studi di Milano, Milano, Italy; ³RIKILT-Institute of Food Safety, Wageningen University, Wageningen, the Netherlands

In silico molecular docking can be a cheap and fast strategy to estimate the binding free energies, and consequently the dissociation constants, for a set of compounds with respect to their putative targets. Interesting targets for EAS are the ligand binding domains (LBD) of the human nuclear receptors for the sex hormones, i.e. the estrogen, androgen, progesterone, and (gluco)corticoid receptor.

The Horizon 2020 project EuroMix (<http://euromixproject.eu>) aims to establish and disseminate new, efficient and validated strategies for the risk assessment of mixtures, while limiting the use animal tests. This presentation deals with a part of EuroMix that is intended to set up a testing approach for mixtures of endocrine disrupting chemicals, focusing on estrogenic and anti-androgenic effects. For that purpose, a combined adverse outcome pathway (AOP) was constructed, including molecular initiating events, key events, and adverse outcome (reproductive dysfunction). Using this combined AOP as framework, cognate *in silico* and *in vitro* tools as well as the *in vivo* confirmation studies were selected, i.e. *in silico*: h-ER and h-AR docking; *in vitro*: cell-based ER and AR transcriptional activation bioassays and the H295R steroidogenesis assay; and *in vivo*: the fish sexual development test (FSDT, OECD Test No. 234) and a rat study, examining in (male) offspring a number of parameters, such as anogenital distance, cryptorchidism, and nipple retention.

Preliminary *in silico* and *in vitro* tests of selected reference chemicals show for individually tested compounds a great correlation between the *in silico* determined binding energies and the *in vitro* measured hormonal activities.

1 Toine F.H. Bovee, Jos P.M. Lommerse, Ad A.C.M. Peijnenburg, Elsa Antunes Fernandes, Michel W.F. Nielen. A new highly androgen specific yeast biosensor, enabling optimisation of (Q)SAR model approaches. *Journal of Steroid Biochemistry and Molecular Biology* 108 (2008) 121–131.