more severe pathological conditions. Particularly when handling with novel chemicals, assessment of cytotoxic parameters can be use as start to understand mechanisms of cellular responses. Thus, further studies on $MgPhen(Hesp)_2$ effects on the cells are extremely important to point out integration between *in vitro* and *in vivo* responses.

TH045

A Temporal High-Resolution Investigation of the Ah-Receptor Cascade during Early Development of Zebrafish (Danio rerio) after Chemical Exposure

H. Meyer-Alert, RWTH Aachen University / Institute for Environmental Research BioV; K.J. Ladermann, RWTH Aachen University; H. Hollert, RWTH Aachen University / Department of Ecosystem Analysis; S. Keiter, Orebro University / MTM Research centre

The fish embryo acute toxicity test (FET) has become a well established method for the assessment and evaluation of chemicals for regulatory purposes. It is not only implemented in an OECD-guideline (no. 236) but can also be used to investigate specific modes-of-action of chemicals during the early development of zebrafish (Danio rerio). In the present study zebrafish embryos were used to investigate the arylhydrocarbon receptor (AhR) signaling pathway in very short time intervals of 4 h from 2 hours after spawning until 118 hpf. The embryos were continuously exposed to different substances inhering various properties (*beta*-naphthoflavone (BNF), polychlorinated biphenyl 126 (PCB126), benzo[a]pyren (BaP)). \nBy using qPCR we measured the expression of all genes involved in the signaling pathway of the AhR. In addition, we quantified the activity of induced biotransformation enzymes (cyp) after exposure using a kinetic EROD-assay. In qPCR, we saw that the cyp-genes were regulated after exposure to the tested chemicals. For PCB126 the gene expression pattern is alternating with several maxima, maybe due to the persistency of the compound. All the co-factors of the pathway (HSP90ß, ARNT1b, ARNT1c, AIP, AhR-Rb and AHR2 itself) remained unaffected. By contrast, after BNF exposure gene expression was significantly raised after 12 h of exposure, climaxed around 28 h and stayed on a lower level until the end of exposure. This observation is most likely due to the fact that BNF was biotransformed and thus induced transcription only in the beginning of exposure. As for PCB126, the other co-factors did not display alternated expression after BNF exposure. qPCR data of BaP will be complete soon. Regarding activity of the cyp proteins, PCB126 caused an elevation beginning at 60 h, whereas BNF did not alter enzymatic turnover. This supports the assumption that cyp1a also possesses a physiological role during the early development of the zebrafish. Its basal activity might be sufficient to metabolize BNF whereas PCB126 is at 60 h as potent as before and therefore causes increased activity. Experiments with BaP are in progress. \nOur results provide a new insight into the regulation of the AhR-pathway and show that it is very important to consider the time slot of exposure when performing biotests like the FET to interpret results properly. The authors thank the German Environmental Foundation (DBU).

TH046

Ontogeny of steroid and thyroid hormone metabolism gene transcription during zebrafish embryo-larval development

J. Periz Stanacev, I. Gabriëls, E.D. Michiels, University of Antwerp / Zebrafishlab Dept Veterinary Sciences; E. Stinckens, University of Antwerp; C. Pype, University of Antwerp / Applied Veterinary Morphology, Dept of Veterinary Sciences; E. Verbueken, University of Antwerp / Applied Veterinary Morphology, Dep of Veterinary Sciences; S.J. Van Cruchten, University of Antwerp / Applied Veterinary Morphology, Dept Veterinary Sciences; A.L. Van Nuijs, University of Antwerp / Toxicological Centre, Dep of Pharmaceutical Sciences; L. Vergauwen, D. Knapen, University of Antwerp / Zebrafishlab Dept Veterinary Sciences

The zebrafish has an impressive range of possible applications as a vertebrate model in both fundamental and applied research. It is increasingly used for incorporating different technologies in understanding the toxicity pathways of various chemicals. The Fish Early Life-Stage (FELS) Toxicity Test (OECD TG 210) is one of the primary ecotoxicological testing guidelines. Gene transcription analyses during the first 32 days of normal development offer us a dynamic picture and may provide us additional information for ecotoxicological tests. Here we describe the gene transcription profiles of the thyroid and steroid hormone synthesis machinery and associated receptors, during the first 32 days of zebrafish development, which has never been done so far. We isolated RNA at 25 time points between 0 and 32 days post fertilisation of zebrafish development covering the most important events during the embryonic and larval stages.We quantified mRNA levels of 20 genes involved in the steroidogenic pathway and 9 genes important for the thyroid systemusing QPCR. Our results show that mainly the enzymes at the beginning of the steroidogenesis pathway are maternally transferred. This suggests an important role of steroid hormones in programming the earliest stages of zebrafish development before the embryo's genome is activated around 3hpf (hours post fertilisation). Further, distinct transcriptional patterns of estrogen receptors are noticed during development. *Esr1* is abundantly transcribed from the time of embryonic genome activation and transcription of *esr2b* increases during the formation of the immature gonad. High transcript levels of *esr2a* due to maternal transfer soon drop and increase later during gonad differentiation. Interestingly, transcription *of isozymes involved in steroidogenesis differs.*Possibly, one is required for early development, whereas the other is important later during development. Transcription of *dio1, dio3a and dio3b* increases at 120hpf. This may be related to inflation of the swim bladder around this time point. *Dio2* start to be abundantly transcribed only after 12 days. These results will improve our fundamental understanding of the role of steroid and thyroid hormones during early life stages of the zebrafish. Information we provided here will be used as a reference dataset for the development of new testing methods targeted at identifying various endocrine disrupting compounds which may act by altering these transcriptional patterns.

TH047

Behavioral analysis and toxicoproteomics profiling of mianserin exposure of zebrafish

K. Van Camp, Systemic Physiological and Ecotoxicological Research (SPHERE), University of Antwerp / Biology; G. Baggerman, Flemish Institute for Technological Research VITO / Department of Biology SPHERE; D. Valkenborg, R. Blust, University of Antwerp; S. Husson, University of Antwerp / Biology

Pharmaceuticals are widely used by humans, for food production or for veterinary purposes, but they may also enter the environment. Unfortunately, many pharmaceuticals have unknown mode of actions in the different environmental niches. Especially neuro-active drugs are of particular concern when acting on non-target species as the neural system is essential for the regulation of various physiological processes and behaviors. The occurrence of antidepressants in surface waters may lead to reduced anxiety of fish, which affects their abilities to deal with predators. In this research we will study effects of the pharmaceutical pollutant mianserin in aquatic environments, on different levels of complexity. In this respect, we will use the zebrafish (*Danio rerio*) which is known as a valid ecotoxicological model. We aim to study altered zebrafish behavior using 3D video tracking as a consequence of exposure to the psychoactive drug mianserin. Doing so, we found a significant effect of swimming angle, as well as the average swimming speed and the time that the fish spend in upper, middle and lower zone in the aquarium. Next, by adopting a differential proteomics approach, we aim to reveal mechanistic information of toxicity at the molecular level, or at least aim to provide a picture of (biochemical) pathways that are affected. While previous proteomics studies in ecotoxicology mainly employed labor-intensive gel-based methods, we explored the use of gel-free strategies. Neuroproteomes of individual brains of exposed zebrafish were compared with their non-exposed controls. Proteins from the entire brain region of the zebrafish were extracted using standard protocols and the resulting proteomes were treated with trypsin to yield thousands of small peptides. A tandem mass tag (TMT) labeling method was used to analyze and quantify six different samples (e.g. three control samples and three samples from exposed fishes) in one single nanoLC-MS experiment to obtain differential proteome maps. Finally, we aim to correlate obtained behavioral parameters with molecular fingerprints from differential proteomics datasets to obtain mechanistic and functional insights underlying aversive effects of exposures to mianserin.

TH048

Neurodevelopment related transcriptome alterations in zebrafish embryo after exposure to valproic acid

S. Lee, Seoul National University / System Toxicology Center; H. Chun, S. Yoon, W. Kim, Korea Institute of Toxicology, KIT / System Toxicology Center

Autism is a complex neurodevelopmental disorder characterized by deficits in social interaction, impaired communication, and repetitive behavior. The prevalence of autism spectrum disorder has recently been increased in Asia, Europe, and United States. Valproic acid (VPA) is used as an anti-epileptic drug and mood stabilizer. However, prenatal exposure to VPA has been linked to the incidence of the autism spectrum disorder. Though VPA has been used as a chemical to induce autism in many experimental studies, the mechanism of VPA is still unclear. Transcriptome analysis would be helpful to understand the underlying mechanism of VPA for autism spectrum disorder. In this study, therefore, we conducted transcriptome analysis using a Next Generation Sequencing approach and observed behavioral (total travelled distance and actively moved duration) and developmental (hatching rate, time to hatch, and malformation) changes in early developmental stage zebrafish after VPA exposure (0, 12.5, 25, 50 and 100 μM). Hatching rate was significantly reduced at 100 μM and time to hatch was significantly increased at 50 μM of VPA exposure. In addition, total travelled distance and actively moved duration of zebrafish was decreased at 50 μM of VPA exposure. In transcriptomic analysis, differentially expressed genes were associated with neurogenesis, nervous system development and sensory perception etc. Specifically, transcripts in GABA receptor modulator, dopamine receptor, parvalbumin were significantly reduced in zebrafish exposed to all the concentration of VPA. Overall, our transcriptome data imply that VPA exposure caused transcriptome changes related to various neurodevelopment in early development stage of zebrafish. The transcriptome profiling of zebrafish embryo after exposure to VPA are also expected to improve our current understanding of the molecular mechanism of VPA. This study could also contribute to the zebrafish model development for autism research.

TH049

Analysis of the relationship between effects in the locomotor activity and