high throughput RNA sequencing data with high quality, non-targeted LC-MS metabolome analyses to investigate both metabolomic effects and the subjacent regulatory pathways of exposing 5-day zebrafish embryo to bisphenol A. The results show that even a moderate coverage of the zebrafish metabolome (from 50 to 100 metabolites) may be representative for almost all affected major metabolic pathways, and that the changes on metabolite concentration were not directly predictable form transcriptomic data. This type of analyses, which involved multivariant chemometric models as well as standard tools for transcriptome analyses, can easily incorporate other non-targeted omic approaches, including lipidomics, proteomics, and/or epigenetic data at the genomic scale, among others. We conclude that only a combination of non-targeted omic approaches can correctly predict macroscopic adverse outputs from molecular data, a paradigm for predictive (eco)toxicology. *Acknowledgements* - This work was funded by the Spanish Ministry of Science and Innovation (CTM2014-51985-R) and by the Advanced Grant ERC-2012-AdG-538320737 from the European Research Foundation. LNM acknoledges a Beatriu de Pinos Postdoctoral Fellowship (AGAUR-MSCA Cofund-2013BP-B-00088).

Environmental endocrine compound concentrations and human and ecosystem health effects

370

Assessment of chronic effects of chemicals and detection of potential endocrine-disruptors for a hermaphrodite mollusk: possible endpoints in a full life-cycle bioassay.

C. Druart, University of Bourgogne Franche-Comté; F. Gimbert, University of Franche-Comte / UMR ChronoEnvironnement; R. Scheifler, University of Franche-Comte / Chrono-Environnement; A. de Vaufleury, University of Franche-Comte / Department of Chrono-Environment

For several years, the need for life cycle tests that are sensitive to chemicals and that can help detecting endocrine-disrupting chemicals (EDCs) is recognized. Partial life-cyle tests measuring apical endpoints such as fecundity that can respond to both EDCs and some non-EDCs have been recently published for freshwater molluscs. In its Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals, OECD includes these tests as a level four test i.e., a test with apical endpoints demonstrating adverse effects on whole individuals, but with little or no mechanistic value. Indeed, until now, endocrine or neuro-endocrine underlying mechanisms of reprotoxicity in molluscs are not known. For the terrestrial environment, few life cyle tests are available. The aim of the present study was to assess the chronic effects and to detect potential ED effects of chemicals on a simultaneous hermaphrodite soil organism, based on the measurement of apical endpoints (hatchability, survival, growth, fecundity, fertility, histopathology of gonads). A full lifecycle (240 days) bioassay using the terrestrial snail, *Cantareus aspersus*, allowing exposure from embryogenesis to reproduction, was used to assess the effects of Bypass®, a glyphosate-based herbicide (GlyBH). A mixture (R-A) made of diquat (Reglone®) and nonylphenols (NP, Agral®), known for its ED effects in other organisms, was also tested. At environmental concentrations, both pesticides enhanced growth but disrupted reproduction without any visible effects on gametogenesis. The R-A mixture strongly reduced the number of clutches, possibly due to a permanent eversion of the penis, suggesting a disrupting effect at the neuro-endocrine level which prevented normal mating. Bypass® had contrasted endocrine disruption effects, with a significant enhancement of growth speed followed by a marked reduction of the fertility of *C. aspersus* snails. The hormetic effect of GlyBH on growth could results of a modulation of the cell activity of the mesocerebrum involved in growth regulation whereas the decreased fertility of snails exposed from the embryogenesis could imply an alteration of the quality of eggs laid or/and of their fertilization. The presented full life-cycle bioassay provides original data on chronic effects of chemicals and offers new opportunities to progress in the challenging identification of endocrine perturbations in hermaphrodite organisms.

371

Steroid and thyroid gene transcription ontogeny during the first 32 days of zebrafish development improves our understanding of endocrine disruption

J. Periz Stanacev, E.D. Michiels, University of Antwerp / Zebrafishlab Dept Veterinary Sciences; E. Stinckens, University of Antwerp; I. Gabriëls, University of Antwerp / Zebrafishlab Dept Veterinary Sciences; 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

Effects of endocrine disruptors (EDCs) are usually well described at the morphological level, but underlying mechanisms are still poorly understood. Transcription patterns of steroid and thyroid genes during normal development can be used as a reference dataset. By combining this information with knowledge on morphological effects of EDCs, we can hypothesize and validate molecular mechanisms of endocrine disruption. Studies using zebrafish embryos reported a wide range of effects of 17α-ethinylestradiol (EE2), including skeletal malformations, pericardial and yolk-sac oedema and an uninflated swim bladder. First, we described the timing of the normal embryonic transcriptional activation of the thyroid and steroid hormone synthesis machinery and associated receptors. We isolated RNA at 25 time points between 0 and 32 days post fertilization of zebrafish development. mRNA levels of 20 genes involved in the steroidogenic pathway and 9 genes related to the thyroid metabolism, were measured by QPCR. Second, we created an overlay of the ontogeny data of the estrogen receptors and vitellogenin 1 with morphological effects after EE2 exposure in order to identify specific transcriptional events that are associated with adverse developmental effects. These associations will be formulated as working hypotheses, which will be validated in a next set of experiments. We observed different transcriptional patterns of estrogen receptors during development. E*sr2a* is maternally transferred. While *esr1* is abundantly transcribed around the time of embryonic genome activation (6 hpf), transcription of *esr2a and esr2b* increases later in development. Transcription of *vtg1* shows peaks which correspond to elevated transcription of *esr1*. In exposures to EE2, we observed dose-dependent skeletal malformations as early as 54 hpf, compared to the controls. Our results indicate an increase in transcriptionof *esr1* and *vtg1* in the period of embryonic genome activation (~3 hpf). These data highlight the possible importance of *esr1* in the estrogen signalling transduction in the early developmental period. These ontogeny results will improve our fundamental understanding of the role of steroid and thyroid hormones during early life stages of the zebrafish and will be applicable to the zebrafish research community. Furthermore, the approach of combining ontogeny data with exposure data will facilitate a more thorough understanding of the molecular mechanisms of endocrine disruption.

372

Combined exposure to EDCs resulting in neurodevelopmental disorders

D.A. Sarigiannis, Aristotle University of Thessaloniki / Chemical Engineering; K. Polanska, W. Hanke, Nofer Institute of Occupational Medicine; A. Salifoglou, A. Gabriel, Aristotle University of Thessaloniki; N. Papaioannou, Aristotle University of Thessaloniki / Chemical Engineering; E. Handakas, Aristotle University of Thessaloniki / Chemical Engineering / Environmental Engineering Laboratory; S. Karakitsios, Aristotle University of Thessaloniki / Chemical Engineering

It has been well established that organic compounds such as phenols, PCBs phthalates exert endocrine disrupting activity, however, the contribution of heavy metals is more questionable. In the current study, phthalate and heavy metals (Pb and Hg) prenatal exposure was determined measuring 11 phthalate metabolites in urine, Pb in blood and Hg in hair of (n=149) mothers during the third trimester of pregnancy (prenatal exposure) and from their children at the 24th month of age (postnatal exposure). Urine untargeted metabolomics analysis was also carried out in a Thermo Orbitrap LC/MS-MS. Psychomotor development was assessed in children at the age of 2 years by the Bayley Scales of Infant and Toddler Development. Associations were investigated using the linkage disequilibrium method of EWAS, while pathway analysis was mapped with the Mass Profiler Pro (Agilent Technologies). Exposure levels to both phthalate metabolites and metals were far below the respective biomonitoring equivalent values, while from the EWAS analysis, it was found that child cognitive development was inversely associated with natural log concentrations of metabolites of DEHP, BBzP and DiNP in the urine, as well as the Pb in blood and the Hg in hair collected from mothers. With regard to post-natal exposure statistically significant association was the inverse correlation of Hg in hair and cognitive functions for females. Metabolic pathway analysis revealed that alterations in urine metabolites are related to the TCA cycle, suggesting impaired mitochondrial respiration; the latter is central to energy metabolism and cellular signaling and plays fundamental roles several cellular processes. Inhibition of mitochondrial oxidative phosphorylation could also cause a defective mitochondrial energy production during the process of fetus formation and development that are reflected in early life motor development. The latter is enhanced by the oxidative stress induced by heavy metals. This defective mitochondrial energy production during the process of fetus formation and development is reflected in early life motor development. The key finding of the study is that although phthalates and metals affect mitochondrial respiration through different mechanisms (endocrine disruption and oxidative stress respectively), this synergistic effect is essential for the deployment of neurodevelopmental defects.

373

Using cats as model for indoor exposure of thyroidogenic compounds

J. Weiss, Stockholm University, ACES / Department of Aquatic Sciences and Assessment; A. Bergman, Stockholm University / Department of Environmental Science and Analytical Chemistry ACES

Household pets such as cats spend a significant time indoors. This and in combination with their intense cleaning of their fur by licking, makes them particularly exposed to house dust and chemicals associated to the dust. Cats are exposed to dust through inhalation but more importantly through ingestion. Recently it has become evident that dust is a significant source of exposure to POPs such as PBDEs, and an association has been demonstrated between domestic cat's thyroid health status and levels of brominated flame retardants in their blood. Thyroidogenic disruption is of growing concern for human health and the