EDC-MixRisk - Summary of Progress

Context and overall objectives of the project

Today we know that the endocrine system is of greatest importance for a healthy development and life - from the time of conception until death - for both animals and humans. It is of global concern that the entire human population, fetuses, infants, children and adults, are constantly exposed to low levels of anthropogenic chemicals, some of which are endocrine disrupting chemicals (EDCs), others are potential EDCs that may interact with our natural endocrine functions. This will potentially lead to adverse health effects in humans unless these chemicals are eliminated from human exposure, likewise the EDCs will potentially cause adverse effects in wildlife.

There are an uncountable number of common consumer products, articles and materials that contain actual or potential EDCs among which many migrate out of the materials, over their lifespan. In fact, everyone is exposed. Global biomonitoring data has shown that EDCs and/or their metabolites are routinely detected in e.g. urine, blood, breast milk, and amniotic fluid. Exposure to EDCs during windows of susceptibility, even at low doses, is of particular concern for developmental programming and trans-generational effects on the proteome, transcriptome and epigenome. These changes underlie disorders that may manifest later in adult life and contribute to "diseased ageing" with a multitude of chronic diseases.

The long reaching goal of EDC-MixRisk is to move forward and meet the societal need of improved decision making regarding human exposure risks to mixtures of anthropogenic chemicals over the whole life span. Hence the project will determine and assess the risk for multiple adverse health outcomes based on molecular mechanisms involved, after early life exposure to complex mixtures of endocrine disrupting chemicals (EDCs). The project relies on interaction between advanced expertise in exposure assessment, epidemiology, toxicology and risk assessment, i.e. toxicological sciences. The overall objectives of the project are:

- Identification of mixtures of EDCs that are associated with multiple adverse health outcomes in three health domains (growth and metabolism, neurodevelopment and sexual development) in two epidemiological pregnancy cohort studies by use of novel and advanced bio-statistical methods.

- Identification of molecular mechanisms and pathways underlying the associations between exposure to EDC mixtures and adverse health outcomes by the use of experimental animal and cell models.

- Development of a transparent, consistent and systematic framework in risk assessment – sometimes referred to as a weight of evidence approach - for integrating epidemiological and experimental research to facilitate risk assessment for EDCs and mixtures.

Main results achieved so far

The overall concept underpinning EDC-MixRisk is that early life exposure to EDC mixtures induces changes in the organism that underlie increased susceptibility to diseases during the entire life span where three health domains will be covered (growth and metabolism, neurodevelopment, and sexual development). The project integrates research from three relevant scientific modules: 1) epidemiology, 2) experimental systems and 3) risk assessment and societal impact. These modules interact as follows: In the epidemiological module, mixtures of EDCs are identified, exposure to which is associated to adverse health outcomes in the three domains. These mixtures are subsequently composed and tested in different experimental systems relevant for the respective health outcomes. To test mixtures that are composed based on epidemiological data is a novel strategy to tackle the mixture issue. The experimental data are then on one hand, integrated into the risk assessment methods developed in module 3, and on the other hand, fed back into module 1 where they are used to refine the biostatistical analyses.

The Swedish mother-child pregnancy cohort SELMA was used to identify the first relevant EDC mixtures, one for each health domain. The mixtures are based on analyses of 20 EDCs from mother's urine and serum at pregnancy week 10. As health outcomes, birth weight (growth and metabolism), language delay at age 2.5 (neurodevelopment), and anogenital distance (AGD) in boys (sexual development) were chosen. All of these outcomes are early signs for adversity in the respective domains. Using these data and a novel biostatistical method, we identified so-called bad actors, chemicals that contribute to the association between exposure and adverse health outcome. These bad actors were mixed in ratios corresponding to their (geometric) mean exposure concentrations to compose a stock solution to be used in the experimental systems. We call these mixtures G0 (growth and metabolism), N0 (neurodevelopment), and S0 (sexual development) and, in the respective dilution, they reflect a "typically-measured" mixture of EDCs in the SELMA mothers. Additionally, three more complex mixtures (mixtures I) have been established based on analyses of 54 chemicals in the mothers. Interestingly, most of the bad actors identified for mixtures 0 are found in the mixtures I (one) as well. Mixtures I will soon be prepared and distributed to be tested in the experimental systems. The German cohort LIFE Child will be used to validate the main findings in the SELMA study. In LIFE Child, sampling as well as chemical analyses are ongoing.

Mixtures 0 are now being tested in various animal and cell models that have been carefully established to give relevant read-outs for the respective health domains. E.g. for mixture G0, differentiation of stem cells into fat and bone cells is measured, and weight, growth and fat accumulation in animal models is analyzed. These readouts are chosen as low birth weight is associated with increased risk for obesity and metabolic disturbances. Further, the cell and animal models are used to identify molecular actions of the mixtures that could underlie their adversity. First results have been obtained in mice, tadpoles, zebrafish, and cell models. Most of them indicate effects of the mixtures on the experimental models on the level of both molecular events and adverse outcomes. This is e.g. observed in tadpoles, in a short term assay (3d) significant disrupting effects of mixture G0 and N0 on thyroid hormone signaling at actual concentrations (1X) measured in the SELMA cohort. In the presence of exogenous thyroid hormone, anti-thyroid effects were seen at 100x and 1000x concentrations measured in mothers (still below the recommended daily intake for the single chemicals) suggesting that disrupting effects could occur over a large range of mixture concentrations. Thyroid hormone signaling during embryonic development is crucial for both growth and brain development. Indeed, the tadpoles show also changed behavior upon exposure, which is also seen in the

zebrafish model. Additionally, mixture G0 induced differentiation of stem cells to fat cell at concentrations that reflect the levels measured in the SELMA mothers. In the mice, we found clear effects on sexual organ development with mixture S0, also here in concentrations that correspond levels measured in the SELMA mothers. These findings are being confirmed and extended to include other experimental systems and readouts. Further, chemical analyses on the exposed models will be performed to assess actual exposure levels and chemical ratios.

The risk assessment and societal impact module focuses on improving the regulatory risk assessment of mixtures and policy impact. A systematic process has taken place for selecting case study chemicals that included setting up criteria for selection as well as consultation of all partners of EDC-MixRisk and other stakeholders including relevant Governmental Agencies. Additionally, a database for chemical-related toxicity data has been constructed and is now ready to be made available to all partners for their contributions into the database. Further, a number of dissemination tools have been set (Web page, Information leaflets in each language of the partners' of EDC-MixRisk and Dissemination guideline document). The activities have included contacts and meetings at several levels within the EU including physical meetings with EU officials, participation in preparation of a consensus document regarding EDCs, other written articles and interviews. To increase the impact on policy-relevant stakeholders, the output of EDC-MixRisk will be synthesized into a statement document in form of a white paper. A preliminary outline has been set for the white paper and for the working process.

Progress beyond the state of the art and expected potential impact

By integrating epidemiological data into experimental research, EDC-MixRisk will progress beyond the state of the art. In contrast to the vast majority of studies that focus on one chemical and one physiological outcome at the time, EDC-MixRisk has developed a multiple-exposure-to-multiple-outcome approach, which mimics the real life situation much better. Our first results demonstrate that EDC mixtures associated with adverse health outcomes in population based epidemiology evoke relevant molecular and physiological effects in experimental systems in cells and animals, even at low concentrations. This demonstrates the validity of our approach in interacting between epidemiology and experimental toxicology and the need to take mixture effects into account for risk assessment. The major innovative potential of EDC-MixRisk lies within the improved risk assessment methodologies directly linked to the data obtained in the project, and strategies to systematically engage policy-relevant stakeholders. Improved regulatory processes will be important for the general populations globally, for national regulatory agencies and organizations; for chemicals manufacturing industry and down-stream users of these chemicals. Concretely, EDC-MixRisk will generate:

- Improvement of testing strategies and risk assessment of EDC mixtures which can serve as basis for more accurate risk management, i.e. decisions in order to reduce health relevant EDC exposures.

- More efficient strategies for risk assessment that will simplify the work for industry and agencies in their respective roles in regulatory risk assessment.

- More efficient approaches for risk assessment that will reduce the need and costs for testing of chemicals which is important when considering the large resources

invested in regulatory chemical control.

- Identification of relevant model systems by using proposed OECD model system as well as others evaluating their relevance for analyzed health outcomes. This can serve as basis for developing novel tools to screen chemicals for their endocrine disruptive potential.

In summary, the project refines and strengthens the existing knowledge by exploiting the interaction of epidemiologists and experimentalists and by identifying novel modes of actions and relevant test model systems to test EDCs. This will result in novel strategies for risk assessment and ultimately impact on policy-making.