EDC-MixRisk
safe chemicals for future generations

Summary of Project Progress
(01 May 2015 - 30 April 2018)

Objectives of EDC-MixRisk

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 interfere with our natural endocrine functions. This will potentially lead to adverse health effects in humans and in wildlife.

There are an uncountable number of consumer products and materials that contain actual or potential EDCs. Many of them migrate out of the materials, and 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 this multitude of EDCs during windows of susceptibility, even at low doses, is of particular concern for developmental programming and long lasting 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 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 for integrating epidemiological and experimental research to facilitate risk assessment for EDCs and mixtures.

Overview of progress and 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.

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 the project, and, on the other hand, used to refine the biostatistical analyses.

Two sets of mixtures have been established for metabolism and growth (G), neurodevelopment (N) and sexual development (S). The first set of mixtures (mixtures 0) is based on exposure data for 20 chemicals, the second set (mixtures 1) on data for 45 chemicals, including phthalate- and PAH metabolites, bisphenols, chlorinated and non-chlorinated pesticides, PCBs, brominated flame retardants and diphenyl phosphate. The mixtures are based on data from the Swedish mother-child pregnancy cohort SELMA including chemical analyses from mother's urine and serum at pregnancy week 10 and the following health outcomes of their children: birth weight (growth and metabolism), language delay at age 2.5 (neurodevelopment), and anogenital distance (AGD) in boys (sexual development). 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 for the experimental systems. Interestingly, most of the bad actors identified for mixtures 0 were also found in the mixtures 1.

Additionally, extensive health examinations on the SELMA children have been conducted at the age of 7 years for each of the three health domains. These data are now being used for refining the associations between mixture exposure and health outcomes. Furthermore, serum and urine samples from the German LIFE Child cohort have been analysed for 330 mothers for the same 45 chemicals. These results combined with the health data available in this cohort will enable us to assess how generalizable our findings from the SELMA study are.

In the experimental module, mixtures 0 and 1 are tested in various animal and cell models that have been carefully established to give relevant read-outs for the respective health domains. The cell and animal models are used to identify molecular actions of the mixtures that could underlie their adversity. Results obtained in mice, tadpoles, zebrafish, and cell models show that mixtures 0 for all the health domains induce negative effects on the molecular, cellular, and organismal level. In some of the assays, effects were observed even at the lowest concentrations tested, which correspond to the actual levels of the SELMA mothers. Interestingly, the mixtures disrupted common signalling pathways in cell and in animal models, which enabled us to link the molecular effects to adverse outcomes such as increased

adipose tissue, behavioural changes, and disruption of sexual organ development. Some of the molecular signatures identified in the experimental models will now be analysed in the SELMA samples and associations with exposure and health outcomes in the children investigated.

Selected single chemicals were also tested and their effects compared to the mixtures. In most cases, the single compounds did not have an effect at concentrations comparable to the mixtures. Moreover, testing of mixtures 1 has started.

An important part of the project is the improvement of the regulatory risk assessment of mixtures as well as science-to-policy interaction. Three different novel mixture risk assessment methods have been established and are now being elaborated on by conducting case studies using EDC-MixRisk and published data. Additionally, a report on inclusive strategies for the regulatory implementation of risk assessment tools for EDC mixtures has been generated.

In order to improve the impact of EU-level mixture efforts and science-to-policy interface, EDC-MixRisk has initiated knowledge exchange and interaction between various EU funded research projects.(EuroMix, EU-ToxRisk, HBM4EU, and SOLUTIONS) as well as with Commission services and relevant EU agencies.

Progress beyond the state of the art and expected potential impact

The EDC-MixRisk approach of identifying EDC mixtures associated with adverse health outcomes in a pregnancy cohort, preparing artificial mixtures of the bad actors for toxicological testing and using the experimental data for risk assessment is a novel approach and one of the major outcomes of the project. More specifically, this proof-of-concept, will enable more systematic integration of epidemiological and experimental evidence into mixture risk assessment strategies. By applying the novel approach, which is based on real-life exposure data, we could find a higher rate of pregnant women at risk when compared with more traditional models of additivity. This adds to the evidence that cocktail effects of man-made chemicals are not properly taken into account in risk assessment and management of chemicals. More systematic approaches are needed, both in terms of science and regulations.

Furthermore, we have a positive indication that it is possible to identify and test a rather small number of chemical mixtures that are relevant for a large proportion of the population. This is a significant step forward as it is not feasible to test all the possible combinations of potential mixtures. The improved testing strategies and risk assessment methodologies developed in the project are important for the regulatory processes to protect public health and to avoid hazardous chemicals, whether they come in mixtures or as single substances.

EDC-MixRisk is generating:

- 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 effective strategies for risk assessment that can guide 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.

Thus, the project 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. The project has developed new knowledge and strengthened 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 policy-making.

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