EDC-MixRisk

POLICY BRIEF

The endocrine disrupting properties of chemicals, and mixtures thereof, have become a global concern. A normally functioning healthy endocrine system is essential for our ability to reproduce and develop. Endocrine disrupting chemicals (EDCs) are linked to serious health problems such as diabetes, obesity, neurodevelopmental disorders and reproductive problems. The fact that we are exposed to complex mixtures of EDCs is of particular concern.

EDC-MixRisk is an EU Horizon 2020 research project that studied the effects of prenatal exposure to mixtures of suspected EDCs on the development and health in children. Our work emphasises potential effects of EDC mixtures during foetal development and provides new tools and approaches for mixture risk assessment.

EDC-MixRisk

safe chemicals for future generations

We wish to emphasise the following Key Messages

Our **key findings** are

- Chemicals identified in pregnant women within the general population originated from different sources and application areas which are currently regulated by different pieces of European Union legislations.
- Epidemiological analysis showed that prenatal exposure to mixtures of EDCs was associated with various effects in children's health and development. Some effects were sex specific.
- The tested mixtures affected hormone-regulated and disease-relevant outcomes in a variety of experimental models at the same concentrations found in the pregnant women.
- Applying our novel whole mixture approach indicates a higher risk for children compared to risk estimated by current methods based on a single compound assessment.

Based on our findings we draw the following **overarching conclusions**

- Current regulations of man-made chemicals systematically underestimate health risks associated with combined exposures to EDCs or potential EDCs.
- Interdisciplinary collaborations and the integration of multiple methods have proven successful for developing novel approaches for testing and risk assessing EDC mixtures.
- EDCs and mixtures need to be tested in complementary models covering several molecular, cellular and organismal effects.
- The feasibility of identifying and testing a small number of chemical mixtures that are broadly relevant for the exposed population can pave a way to similar efforts at the European and global-scale.
- Our new approaches can be used to define acceptable levels of exposure based on epidemiological data or the integration of human and experimental data.
- These new approaches may also provide empirical support for estimating the size of a potential additional mixture assessment factor that could take mixtures into account in chemical risk assessment.
- Our whole mixture approaches can only be used retrospectively for chemicals for which epidemiological data are available. For "new chemicals" a prospective risk assessment is required.

We have identified the following future needs

Current, single substance risk assessment paradigm assumes that substances are released into a pristine environment. However, they enter into the environment or a human body where other chemicals are already present. Calculating risk ratios for single chemicals underestimates the risk, and hence, if we add up the risk quotients we could exceed acceptable levels of risk.

Therefore, regulatory requirements should take mixtures and combined exposures into account in all relevant EU chemicals and environment legislations.

Our research results add to the increasing scientific evidence that health risks associated with EDC mixtures are underestimated. There is an urgent need to assess the magnitude of this problem. Our novel whole mixture approach could supplement the current regulatory system to improve risk assessment of mixtures. To achieve this, we need access to appropriate data.

Therefore, we propose that future and ongoing biomonitoring efforts should include (a) analyses on complex mixtures, (b) assessment of adverse health outcomes in the same cohorts, (c) good quality toxicity data to identify hazardous chemicals, (d) long-term resources to follow time trends and evaluate risk management measures.

Interdisciplinary research initiatives are critical to bridge the current data and knowledge gaps.

We see that development and validation of sensitive and fit-for-purpose test methods with inclusion of new approach methodologies (NAM) as well as increased efforts on exposure assessment and modelling are essential.

Legislative measures should be taken to assure ***full insight to which chemicals are used in all products, materials and goods on the market.*** This would enable allocating research efforts to chemicals and mixtures of concern, increase diversity of chemicals under scrutiny and to promote consumers' informed choices of products and goods.

Given the large amounts of money invested in the implementation of chemicals legislation, it is important to ensure that regulatory processes and practices are efficient and up to date with current scientific knowledge.

Therefore, innovations at the cross section of science and policymaking should be facilitated by creating platforms for collaborations between regulatory agencies, industry and academia.

Introduction

Humans are exposed to a large number of anthropogenic chemicals and combinations of chemicals via air, water, food, consumer products, materials and goods. In addition, pharmaceuticals, drugs, tobacco and occupational exposures add to the number and potential combinations of chemical mixtures, during the whole lifespan. Exposure to hazardous chemicals during the foetal period is of particular concern as it can lead to irreversible changes in the development of organs and tissues and increased susceptibility to diseases later in life.

Combined exposure to multiple chemicals raises significant concerns about the impacts on health and environment. Scientific evidence about mixture effects of real life exposures is accumulating, yet current risk assessment and management practices focus mainly on single substances. Neglecting combined effects can lead to underestimation of risk that is fundamentally linked to human health and wellbeing.

The EDC-MixRisk project has developed a novel approach which is based on identifying and testing real life EDC mixtures associated with adverse health outcomes in humans, in the domains of growth and metabolism, neurodevelopment and sexual development. By using epidemiology data from the Swedish pregnancy cohort SELMA, reference mixtures were created to mimic real life internal exposures. These mixtures were tested in various experimental (cell and animal) models, and the toxicological data from these tests were used to establish new methods and strategies for mixture risk assessment in order to better account for complex environmental exposures. This whole mixture approach enables more systematic integration of epidemiological and experimental evidence into mixture risk assessment strategies, and complements current risk assessment strategies based on a single compound approach or assumptions of additivity.

Highlights and key results

Epidemiology

Epidemiological data from the SELMA study were used to identify EDC mixtures associated with adverse and developmental outcomes in prenatally exposed children in the three health domains.

- Fifty-four potential endocrine disruptors were analysed in blood and urine from pregnant women in the Swedish SELMA pregnancy cohort study. Forty-one of these[1] (75%) were found above the level of detection in the majority of the analysed urine/serum samples from more than 2,300 pregnant women.
- Among the forty-one chemicals, we identified those that were associated with adverse health outcomes in three different health domains in children:
  - sexual development (measured as a shorter anogenital distance (AGD) in boys at 21 months of age)
  - neurodevelopment (measured as language delay at 30 months of age, and cognitive functions at 7 years of age), and
  - metabolism and growth (measured as birth weight and growth during the first 7 years of age).
- The identified chemicals were mixed in ratios corresponding to their geometric mean exposure concentrations in more than 2,300 pregnant women in SELMA. The resulting mixtures are our “reference mixtures” that were tested in the experimental models.

[1] The 54 chemicals/metabolites analysed included: 13 phthalate ester metabolites of seven phthalate esters plus a metabolite of DINCH, 2 metabolites of 2 polycyclic aromatic hydrocarbons (PAHs), 5 metabolites of 5 alkyl phenols (4 bisphenols and triclosan), and a metabolite of an organic phosphate esters. Further, 8 perfluoroalkyl substances, 19 polychlorinated persistent aromatics and 3 polybrominated diphenyl ethers.

Experimental in vivo and in vitro studies

Experimental investigations in animal and cell models were used to uncover molecular mechanisms underlying associations between exposure and effects seen in the epidemiological studies. The cell and animal models included human brain organoids, human cell lines, mice, tadpoles and zebrafish, which were considered relevant for the three health domains.

- Exposing the experimental models to the mixtures caused adverse effects and dysfunctions in animal and cell models.
- The experimental effects were observed at exposure levels similar to those measured in the SELMA cohort.
- The measured effects were also coherent with adverse health outcomes seen in the SELMA children, and further with endocrine disruptive modes of action. For example:
  - The mixture associated with shortened AGD interfered with production of sex hormones and led to morphological changes of the reproductive organs and to a shortened AGD in mice.
  - The mixture associated with language delay interfered with thyroid hormone signalling, altered the expression of genes involved in autism spectrum disorder and intellectual disability in human brain organoids, modified neuronal differentiation and function, and induced behavioural changes in tadpoles, zebrafish and mice.
  - The mixture associated with low birth weight interfered with thyroid hormone signalling, changed expression of genes involved in fat cell differentiation and obesity, increased differentiation of stem cells into fat cells, and lead to higher fat cell number in developing zebrafish. The mixture also associated with low birth weight in male mice.
- Our results revealed the impact of genetic background diversity on the sensitivity to chemical exposure, demonstrating the unique value of human in vitro models and underpinning their potential use for testing chemicals on genetic backgrounds that would be representative for large parts of the (European) population.

Risk assessment and societal impact

Epidemiological and experimental data from the project have been used to develop new mixture risk assessment methods and approaches that complement current strategies.

- By defining and experimentally testing a reference mixture, i.e., a whole mixture approach, we could assess how many mothers in the SELMA study are at risk for effects in their children. This was performed by applying advanced biostatistical methods[2]:
  - This novel approach indicates that a large proportion of the SELMA women had mixtures in their blood that were considered sufficiently similar to the tested reference mixtures. If generalized, this means 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 possible combinations of different mixtures.
  - When applied in the three health domains we observed:
    - When assessing sexual development, i.e., AGD, a higher rate (13%) of pregnant women were assessed as being at increased risk of giving birth to baby boys with shortened AGD, when compared with traditional risk assessment strategies based on the assumption of additivity (<5%), wor the most used single compound approach (<2%)[3] .
    - When assessing neurodevelopment, i.e., language delay at 30 months of age, 0.2% of the pregnant women were at increased risk for having a child with language delay.
    - When assessing metabolism and growth, i.e., birth weight, 23% of the pregnant women were at increased risk for having a child with lower birth weight.

- New statistical models developed in the project deliver “guideline values” for risk based on epidemiological data to inform risk assessment of mixtures. *The acceptable concentration range model*[4] provides estimates of acceptable exposures to mixtures using human biomonitoring data from cohort studies.
  - When assessing language delay and birth weight endpoints, we found that the current single compound approaches underestimated risk by a factor that ranged from 1 to 100 for different chemicals.
  - Our study shows that it is possible to derive health based guideline values from epidemiology data. Such estimates may provide empirical support for determining assessment factors for mixtures.
- The Systematic Review and Integrated Assessment (SYRINA) methodology[5] was applied with the aim to contribute to a development where the identification of endocrine disrupting chemicals and mixtures becomes systematic and transparent. We found that:
  - The SYRINA framework is suitable for assessment under the WHO definition for endocrine disrupting chemicals.
  - The assessment of mechanistic (in vitro) data is time consuming and should be modified in order to find a balance between a comprehensive and a practically feasible process.
  - The use of structured reporting of data, and data repositories designed for risk assessment purposes have the potential to make the evaluation process more efficient.

We envisage that if the new mixture risk assessment methods and approaches were incorporated in existing legislations dealing with chemical mixtures that these novel methods may significantly contribute to more relevant risk assessment and management by providing more reliable empirical information and better reflecting real life scenarios which in turn would alleviate the burden of disease and health expenditure attributed to EDC and benefit the wellbeing of future generations.

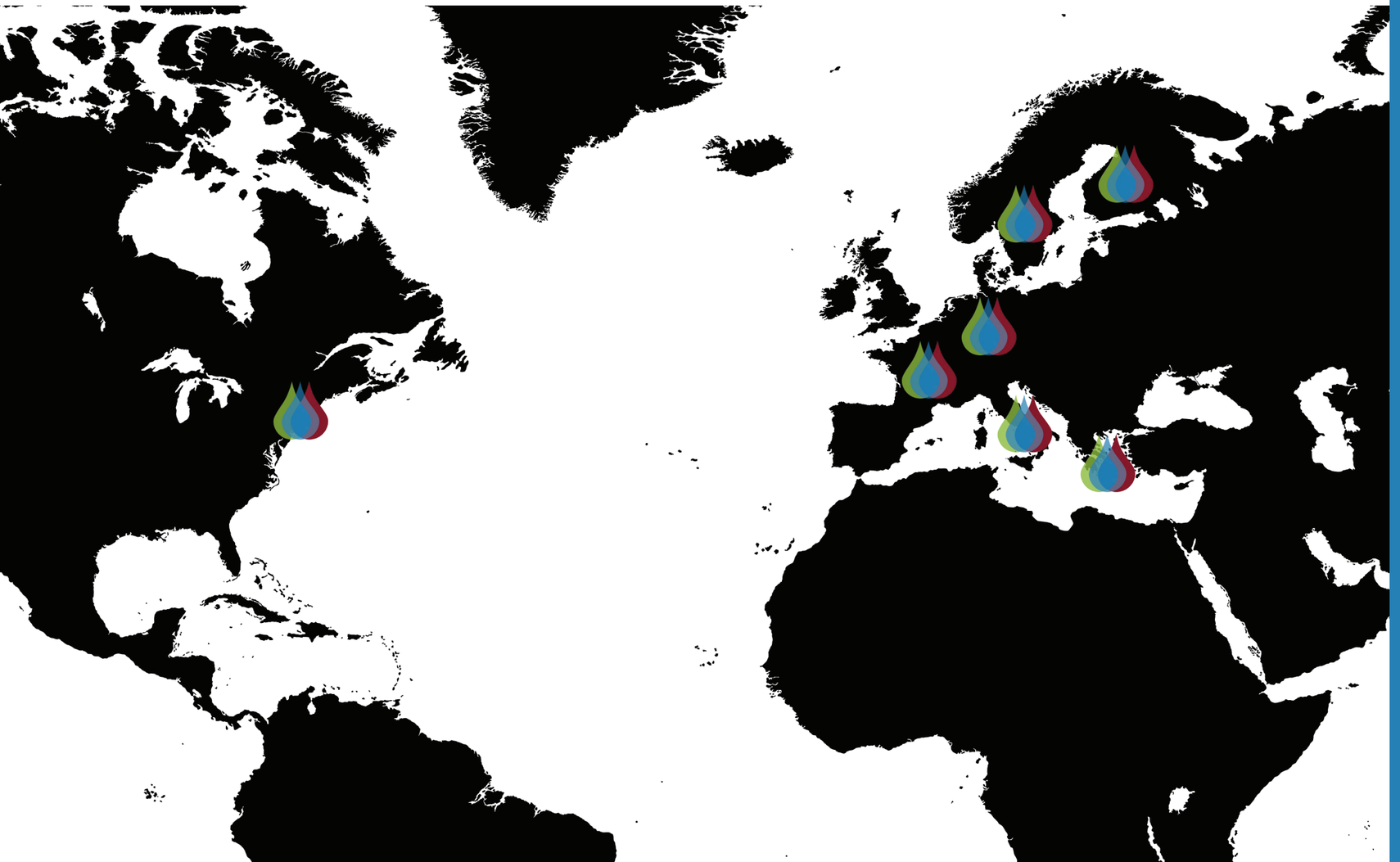
[2] Marshall et al., 2013. An empirical approach to sufficient similarity: Combining exposure data and mixtures toxicology data. Risk Analysis. Volume 33(9), pages 1582-1595.

[3] Bornehag et al., 2019. A novel approach to chemical mixture risk assessment - Linking data from population based epidemiology and experimental animal tests. Risk Analysis, in press.

[4] Gennings et al., 2018. Incorporating regulatory guideline values in analysis of epidemiology data. Environment International. Volume 120, Pages 535-543. doi: 10.1016/j.envint.2018.08.039

[5] Vandenberg et al. 2016. A proposed framework for the systematic review and integrated assessment (SYRINA) of endocrine disrupting chemicals. Environmental Health 15:74. https://doi.org/10.1186/s12940-016-0156-6

EDC-MixRisk participant locations



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