EDC-MixRisk
safe chemicals for future generations

The Chemical Cocktail Challenge

"A workshop on results and conclusions from the two H2020-funded projects, EDC-MixRisk and EuroMix, and their implications for future needs for chemical mixture risk assessment"

26 March 2019, Brussels, Belgium

EXPOSURE ASSESSMENT OVERARCHING SECTORS AND TOOLS TO IDENTIFY MIXTURES OF CONCERN

Amélie Crépet[1], Corinne Sprong[2], Marie Vanacker[1], Angelo Moretto[3,4], Hilko van der Voet[5], Jacob van Klavaren[2]

(1) ANSES, French Agency for Food, Environmental and Occupational Health and Safety, Risk assessment department, Methodology and studies unit, 947001, Maisons-Alfort, France
(2) RIVM, National Institute for Public Health and the Environment, The Netherlands, PO Box 1, 3720 BA Bilthoven, The Netherlands
(3) ICPS, International Centre for Pesticides and Health Risk Prevention, 20157 Milano, Italy.
(4) Department of Biomedical and Clinical Sciences, Università degli Studi di Milano, Italy.
(5) Wageningen University & Research, Biometris, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands

Abstract:

The EuroMix project has developed a strategy for mixture risk assessment. In particular, it has proposed a methodology that integrates exposures and hazard information to perform a combined exposure assessment and to identify relevant mixtures of chemicals to which the European population is exposed via food.

For a combined exposure assessment, substances were classified into cumulative assessment groups (CAGs). Relative potency factors for each substance were calculated to express exposure as toxicity-equivalents of a defined reference compound. Finally, exposures expressed as toxicity-equivalents were summed for each individual in the food consumption database and margins of exposure were calculated. The method to identify relevant mixtures consists of risk-based identification of co-occurrent substances in diet for a given time frame.

The data needed and the developed methodology will be presented and illustrated with different case studies around the CAG liver steatosis performed with the EuromixToolBox.

EuroMix has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 633172

The Chemical Cocktail Challenge

"A workshop on results and conclusions from the two H2020-funded projects, EDC-MixRisk and EuroMix, and their implications for future needs for chemical mixture risk assessment"

26 March 2019, Brussels, Belgium

AGGREGATED EXPOSURE ASSESSMENT

Marc Kennedy[1], Cecile Karrer[2], Marie Vanacker[3], Natalie von Goetz[2], Amélie Crépet[3]

(1) Fera Science Ltd, Sand Hutton, York, UK
(2) Swiss Federal Institute of Technology (ETH) Zurich, Institute for Chemical and Bioengineering, Zurich, Switzerland
(3) ANSES, French Agency for Food, Environmental and Occupational Health and Safety, Risk assessment department, Methodology and studies unit, 947001, Maisons-Alfort, France

Abstract:

Populations can be exposed to chemical mixtures from one or more non-dietary sources, in addition to their dietary exposure. There is a need for general purpose tools that can quantify the combined (aggregated) mixture exposure for the affected subpopulations. The EuroMix toolbox provides a non-dietary module that can combine dietary exposures with measurements, or external model results, representing non-dietary exposures. The functionality originally developed in the EuroMix toolbox for dietary risk assessment and mixture identification can now also be applied using the aggregated exposure sources.

Results from 3 case studies developed within the EuroMix project will be presented. These illustrate the capabilities of aggregated modelling for diverse applications, compound groups and health effects. In the following examples dietary exposures and the specified non-dietary exposures were considered jointly:

- Pesticide mixtures from incidental residential exposure due to arable crop spraying. The UK adult population is considered and information on sprayed mixtures is taken from the UK Pesticide Usage Survey
- Bisphenol mixtures in personal care products, dust and thermal paper
- Pyrethroid mixtures from air, dust, veterinary usage and medicine

EuroMix has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 633172

EDC-MixRisk
safe chemicals for future generations

EuroMix

The Chemical Cocktail Challenge

"A workshop on results and conclusions from the two H2020-funded projects, EDC-MixRisk and EuroMix, and their implications for future needs for chemical mixture risk assessment"

26 March 2019, Brussels, Belgium

HUMAN STUDY AND HOW TO COMPARE REAL LIFE EXPOSURE WITH PREDICTED EXPOSURE USING THE EUROMIX CONCEPT AND TOOLS

Trine Husøy[1], Monica Andreassen[1], Friederike Sonnet[1], Hege Hjertholm, Cecile Karrer[2], Natalie von Goetz[2], Amrit K Sakhi[1], Hubert Dirven[1],

[1]The Norwegian Institute of Public Health, Division of Infection Control and Environmental Health, 0403 Oslo, Norway

[2]Swiss Federal Institute of Technology (ETH) Zurich, Institute for Chemical and Bioengineering, 8093 Zurich, Switzerland

The Horizon 2020 EuroMix project aims to provide validated test strategies for hazard and exposure assessments of chemical mixtures. A human biomonitoring (BM) study was performed to study the exposure to chemicals present in foods and personal care products (PCPs). For two 24-hour study periods separated by 2-3 weeks, adult volunteers (44 males and 100 females) in Norway kept detailed diaries on food consumption (type/brand, weight, time and packaging material) and the usage of PCPs (type/brand of product, time and number of applications, and number of showers and hand washes). The participants also registered the number of thermal papers (TP) handled. In parallel, 24 hour urine samples were collected. Bisphenol's (BP's), parabens, triclosan (TRCS), triclocarban (TRCB) oxybenzone (OXB), imazalil and metabolites of phthalates and boscalid were measured in the urine of the first day. Concentrations (log transformed) in urine were used in a multivariate linear regression (MLR) analysis with the main food and PCP categories. Exposure to bisphenols BPA, BPS and BPF from foods, PCPs, and TP was modeled for the study population. The EuroMix toolbox was used to calculate individual external exposure from foods, aggregating dietary with non-dietary exposures from PCPs and TP, and for the comparison with BM data. The good agreement between the ranges of modeled and measured BPA amounts indicates that available concentrations, especially from the main exposure source food, mirrored the exposure situation realistically, and suggests that the model structure is applicable and considers the relevant exposure sources. For BPS and BPF, modeled amounts mostly underestimated measured amounts, which suggests that the current data situation is insufficient to represent actual exposures. Overall, the participants in the EuroMix BM study were exposed to a mixture of phenols and phthalates. A variety of food categories and PCPs were found to be possible sources of these chemicals. The results from the BM study indicate a complex pattern of exposure to numerous chemicals, originating from multiple sources depending on individual diet and PCP preferences.

EuroMix has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 633172

EDC-MixRisk
safe chemicals for future generations

EuroMix

The Chemical Cocktail Challenge

"A workshop on results and conclusions from the two H2020-funded projects, EDC-MixRisk and EuroMix, and their implications for future needs for chemical mixture risk assessment"

26 March 2019, Brussels, Belgium

LOWER TIER ASSESSMENT OF MIXTURES USING *IN SILICO* APPROACHES

A. Peijnenburg[1], J. Cotterill[3], I. Eberini[4], L. Palazzolo[4], A. Moretto[4], K. Kyriakopoulou[5], D. Nikolopoulou[5], K. Machera[5], D. Louca-Christodoulou[6], A. Beronius[7], A. Hanberg[7], C. Sprong[2], E. Rorije[2]

(1) RIKILT Wageningen University and Research, The Netherlands
(2) RIVM, National Institute for Public Health and the Environment, The Netherlands
(3) FERA Science Ltd, UK
(4) Università degli Studi di Milano, Italy
(5) Benaki Phytopathological Institute, Greece;
(6) State General Laboratory, Cyprus
(7) Karolinska Institute, Sweden

In Work Package 2 of the EuroMix project an in silico-based workflow has been set up which is proposed to be used as a first tier in the risk assessment of chemical mixtures. The workflow consists of four steps. The first step is the identification of chemicals that are relevant for food and feed and of interest for combined exposures. In the second step, the probability that a chemical belongs to a certain Cumulative Assessment Group (CAG) is determined using QSAR and/or molecular docking models. The third step concerns the assignment of a relative potency factor (RPF) to each chemical that was considered in the previous step to be part of the CAG of interest. If exposure data are available, these relative potencies can be applied to scale the exposures of all mixture components. Conservative, worst case NOAELs as the basis for RPFs can be derived using Threshold of Toxicological Concern (TTC) concepts. Possibly more realistic estimates of potency can be derived from the binding energies calculated by molecular docking, and ultimately NOAELs from read-across based on chemical similarity to a data-rich compound should give the most realistic estimate. Finally, in the fourth step, the mixture risk is determined by adding up all the scaled exposures of the mixture components that are predicted to be part of a CAG. The in-silico workflow and all the data obtained with the models for three CAG endpoints - hepatotoxicity, developmental toxicity and endocrine disruption - are implemented in the EuroMix MCRA software tool. The models in the in silico mixture risk assessment workflow can also be used for prioritization and ranking for further testing, i.e. to identify the substances that contribute most to the calculated risk for a specific CAG.

EuroMix has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 633172

EDC-MixRisk
safe chemicals for future generations

EuroMix

The Chemical Cocktail Challenge

"A workshop on results and conclusions from the two H2020-funded projects, EDC-MixRisk and EuroMix, and their implications for future needs for chemical mixture risk assessment"

26 March 2019, Brussels, Belgium

EUROMIX TEST STRATEGY: USE OF *IN VITRO* DATA TO IMPROVE AND/OR COMPLETE HAZARD DATA COLLECTION FOR CHEMICALS RELEVANT FOR CUMULATIVE EFFECT ON AOP LIVER STEATOSIS

Alfonso Lampen[1], Dajana Lichtenstein[1], Claudia Luckert[1], Albert Braeuning[1], Georges de Sousa[3], Sigrid Durinck[4], Efrosini S. Katsanou[5], Parthena Konstantinidou[5], Kyriaki Machera[5], Ad Peijnenburg[2], Roger Rahmani[3], Andreja Rajkovic[4], Deborah Rijkers[2], Geert Stoopen[2]

(1) German Federal Institute for Risk Assessment, Dept. Food Safety, Berlin, Germany
(2) RIKILT Wageningen University & Research, Wageningen, The Netherlands
(3) National Institute for Agricultural Research, INRA Unit 1331, TOXALIM, France
(4) Department of Food Technology, Food Safety and Health, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium
(5) Benaki Phytopathological Institute, Athens, Greece

Abstract:

The EuroMix project test strategy for steatosis is a mechanism-based strategy, using the concept of adverse outcome pathways (AOPs). An AOP describes, in a linear fashion, linkages (key events relationships) between chemically-induced adverse effects (key events, KEs) at various levels of biological organization, progressing from a molecular initiating event (MIE) to an adverse outcome (AO) that is relevant to risk assessment and regulatory decision-making. Therefore, an *in vitro* assay toolbox was developed to evaluate the role of mode of action (MoA) and key events of chemicals. The bioassay toolbox contains optimal *in vitro* assays to detect MoA for liver toxicity and maps to molecular initiating events (MIE) and key events (KE) of the AOP for steatosis. Exposure-based as well as *in silico* (QSAR) proposed substances were assessed according to this AOP-wise testing strategy.
When assessing combined exposure, it is essential to define the potency of each component (relative potency factor) which allows the appropriate testing of each compound being assessed. Therefore, relative potencies were computed by using benchmark dose modelling via PROAST software. Equipotent mixtures were designed and tested in all assays of the developed EuroMix *in vitro* toolbox. In the EuroMix approach, the default assumption is that the model of dose addition applies to all substances that cause the same adverse outcome, i.e. steatosis. This assumption could be confirmed for all substance tested so far in the AOP-wise *in vitro* assay toolbox.

EuroMix has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 633172

EDC-MixRisk
safe chemicals for future generations

EuroMix

The Chemical Cocktail Challenge

"A workshop on results and conclusions from the two H2020-funded projects, EDC-MixRisk and EuroMix, and their implications for future needs for chemical mixture risk assessment"

26 March 2019, Brussels, Belgium

EUROMIX TEST STRATEGY: USE OF *IN VITRO* DATA TO DEFINE THE CUMULATIVE ASSESSMENT GROUP FOR CRANIOFACIAL MALFORMATIONS

Moretto A[1], Bois F[2], Crepet A[3], Di Renzo F[1], Eberini I[1], Kumar V[4] Kyriakopoulou A[5], Machera K[5], Menegola E[1], Metruccio F[6], Palazzolo L[1], Rorije E[7], van der Ven LTM[7]

(1) UMIL, Università degli Studi di Milano, Italy
(2) INRA, France
(3) ANSES, French Agency for Food, Environmental and Occupational Health and Safety, Risk assessment department, Methodology and studies unit, 947001, Maisons-Alfort, France
(4) URV, Universitat Rovira i Virgili, Spain
(5) BPI, Benaki Phytopathological Institute, Greece
(6) ICPS, ASST Fatebenefratelli Sacco, Italy
(7) RIVM, National Institute for Public Health and the Environment, The Netherlands

Euromix explored the possibilities and options that are available to carry out the risk assessment of combined exposures to substances that cause craniofacial malformations. While exposure considerations should take prominence in the identification of the compounds for which the combined assessment should be prioritized, for the purpose of Euromix this part of the activity focused on the adverse outcome. Hazard identification and characterisation need to be put into an AOP to devise a strategy for both grouping compounds and testing mixtures. Also, it is essential to define the relative potency factor (RPF) and the capability of quantitative extrapolation of in vitro data (in vitro in vivo extrapolation, IVIVE) and the extrapolation from experimental animals to humans by means of Physiologically Based ToxicoKinetics (PBPK). A tentative AOP has been developed based of imbalance of the retinoic acid pathway supported by *in silico* and *in vitro* tests, that included QSAR considerations and molecular docking, morphological observations and gene expression assays in the embryonic stem cell test, the rat whole embryo culture, and the zebrafish embryo (ZFE) toxicity assay. On the basis of the *in silico* and *in vitro* data the following compounds were chosen for the *in vivo* study: cyproconazole, triadimefon (same molecular initiating event, MIE) and valproic acid (different MIE). The question that has been asked relates to the level of conservatism that is introduced by the inclusion in the cumulative assessment group of compounds with dissimilar MIE but same phenotypic adverse outcome and the application of the dose-additivity model.
.

EuroMix has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 633172

EDC-MixRisk
safe chemicals for future generations

EuroMix

The Chemical Cocktail Challenge

"A workshop on results and conclusions from the two H2020-funded projects, EDC-MixRisk and EuroMix, and their implications for future needs for chemical mixture risk assessment"

26 March 2019, Brussels, Belgium

EUROMIX TEST STRATEGY: USE OF *IN VITRO* DATA TO IMPROVE AND/OR COMPLETE HAZARD DATA COLLECTION FOR ENDOCRINE DISRUPTION

T.F.H. Bovee[1], L.T.M. van der Ven[2], I. Eberini[3], E. Katsanou[4], V. Kumar[5], M. Torrente Torné[5], A. Peijnenburg[1], J. van Benthem[2], M. Luijten[2], Corinne Sprong[2], A. Moretto[3], L. Palazzolo[3], K. Machera[4], J. Zilliacus[6]
(1) RIKILT, Institute of Food Safety, Wageningen University and Research, The Netherlands
(2) RIVM, National Institute for Public Health and the Environment, The Netherlands
(3) UMIL, Università degli Studi di Milano, Italy
(4) BPI, Benaki Phytopathological Institute, Greece
(5) URV, Universitat Rovira I Virgili, Spain
(6) KI, Karolinska Institute, Sweden

An adverse outcome pathway (AOP) is needed for both grouping and testing of compounds and mixtures thereof, as understanding the molecular initiating events (MIE), the key events (KE) and the KE relationships are especially fundamental when assessing combined exposure. In addition, it is essential to define the potency of each component (relative potency factor, RPF) which allows the appropriate weighing of each compound being assessed. This part of the EuroMix approach focusses on testing compounds and mixtures that cause endocrine effects, i.e. feminisation, by either targeting the estrogen receptor (ER) or androgen receptor (AR). It was shown that *in-silico* molecular docking can be applied qualitatively in first instance to predict the affinity of compounds to a specific receptor. Subsequently, *in-vitro* transcriptional activation (TA) assays were used to establish the ER and AR agonistic and antagonistic properties and the cognate RPFs. However, receptor docking, ER and AR TA assays do not allow determination of RPFs for compounds with a dissimilar mode of action (MoA). Therefore, the fish sexual development test (FSDT) was used to establish RPFs of compounds with a dissimilar MoA by examining the gonadal phenotype as an endpoint of ER and AR MIEs, and expression of vitellogenin in zebrafish liver as a marker of the MIE "ERalpha activation". Testing of selected compounds and mixtures thereof in the rat development test is ongoing. Preliminary results are in line with the expected effects of flutamide regarding anogenital distance, nipple retention and cryptorchidism. In addition, marker genes for KEs in the ERalpha and/or AR part of the AOP network, were selected from literature. Testis tissue was collected for gene expression analysis (testing and analysis are ongoing).

EuroMix has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 633172