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EuroMix: using (Q)SARs, TTC, molecular docking simulation and read-across as a first tier in mixture toxicity risk assessment

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Every day, we are exposed to mixtures of multiple chemicals via food intake, inhalation and dermal contact. Whether exposure to these mixtures poses a health risk depends on how the effects of different chemicals in the mixture combine, and whether there is any synergism or antagonism between them. The number of different combinations of chemicals in mixtures or co-exposures is infinite, making testing of all possible combinations practically impossible and ethically unacceptable if it involves animal testing.

The overall objective of the EU project EuroMix (https://www.euromixproject.eu/) is to establish and to disseminate new, efficient, validated test strategies for risk assessment of mixtures, and do this by using, among others, *in silico* and *in vitro* methods. The EuroMix strategy to risk assessment of mixtures follows a tiered approach, where hazard assessment in the first tier is done by applying *in silico* tools including QSARs, Read Across, and the TTC concept [1]. Exposure assessment via multiple exposure routes is performed by using the MCRA tool [2,3]. The EuroMix approach aims to extend, generalize and refine the EFSA Cumulative Assessment Group (CAG) concept [4], and make it possible to apply the CAG approach also to substances outside the EFSA determined grouping of pesticides, like environmental contaminants, food additives, industrial chemicals etc.

To achieve this goal existing (Quantitative) Structure Activity Relationships ((Q)SARs) are used in a first assessment tier to determine to which CAG(s) a substance should (potentially) belong. Existing (Q)SARs (among others DEREK, OECD QSAR Toolbox profiles, MultiCASE) are evaluated to assess whether a substance can be categorized into the CAGs *liver toxicity*, *developmental toxicity* and *endocrine disruption*. A decision strategy using multiple (Q)SARs will be presented for the example of liver toxicity. For the CAG endocrine disruption molecular docking simulations are performed in the project to predict the approximated binding free energy to the estrogen and androgen receptors.

The individual substances are subsequently prioritized on their contribution to the overall risk of the mixture or co-exposure, by assigning Relative Potency Factors (RPF) to each substance. The RPF is expressed as the simple ratio of the (effect specific) NOAEL of a substance relative to the NOAEL of a known substance in the mixture causing the same toxicological effect. Full dose additivity is thereby assumed in this first tier for substances in the same CAG. If toxicological data is available for a substance, effect specific NOAELs are used. If no data is available, Read Across is used in the first tier of the mixture assessment strategy to estimate a NOAEL. If no valid read across structures are available, the TTC concept is used to generate a plausible worst case NOAEL for the substance in the mixture. For the CAG endocrine disruption binding energy estimates from the docking models give a first estimate of the Relative Potency Factor.

These first tier estimates of both CAG membership and RPFs are ideally confirmed/refined in the following tiers where *in vitro* testing information is generated in CAG / mechanism specific assays. These further tiers in the EuroMix mixture assessment strategy will not be elaborated in this presentation.

References

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[4] EFSA Panel on Plant Protection Products and their Residues (PPR) (2014). Scientific Opinion on the identification of pesticides to be included in cumulative assessment groups on the basis of their toxicological profile (2014 update). *EFSA Journal 2013*;11, 3293, 131 pp.