Chemical mixture risk assessments in the past and in the future

BfR Anniversary 4th Nov 2022

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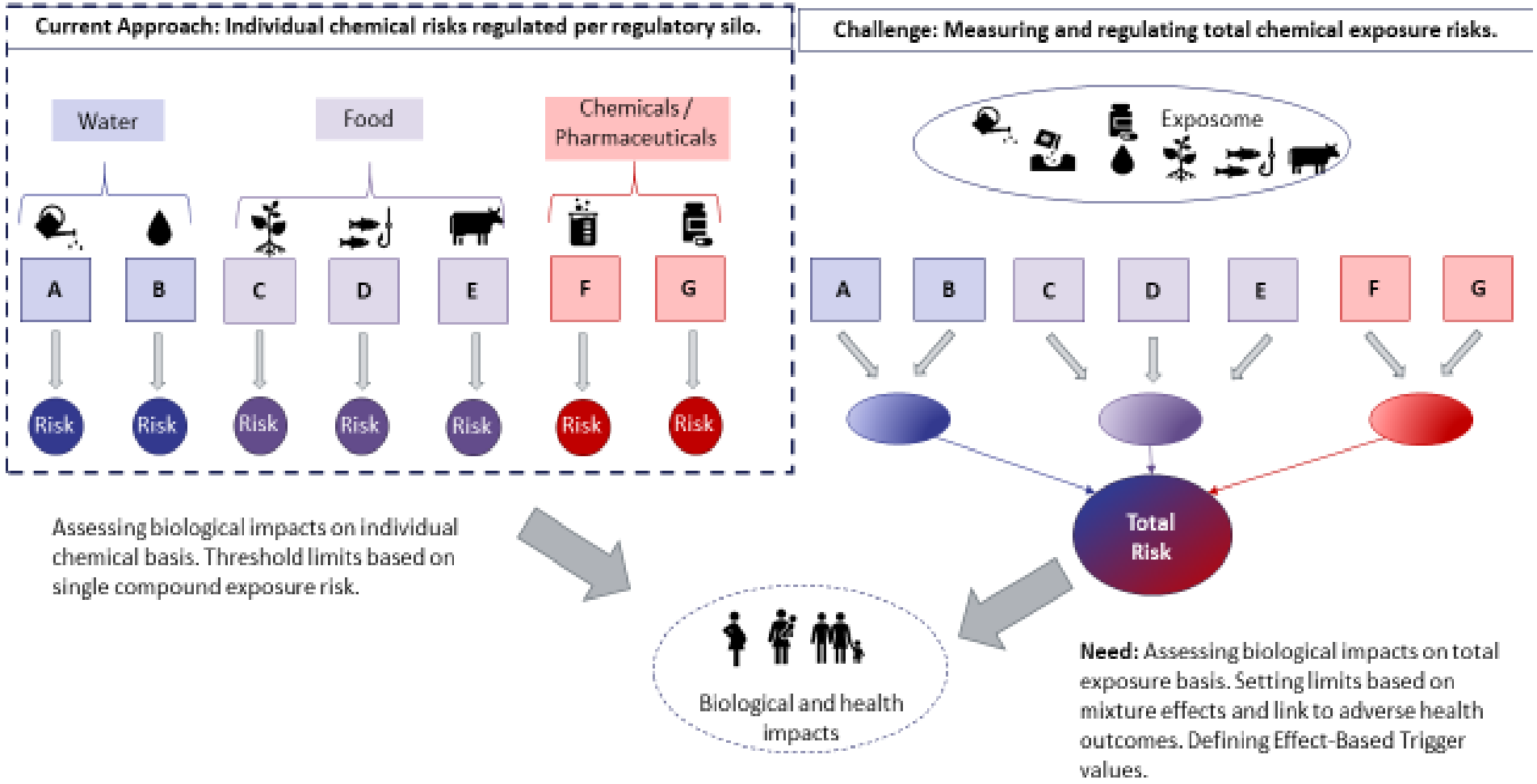
Co-exposure of chemicals in humans

Mixture risk assessment of chemicals

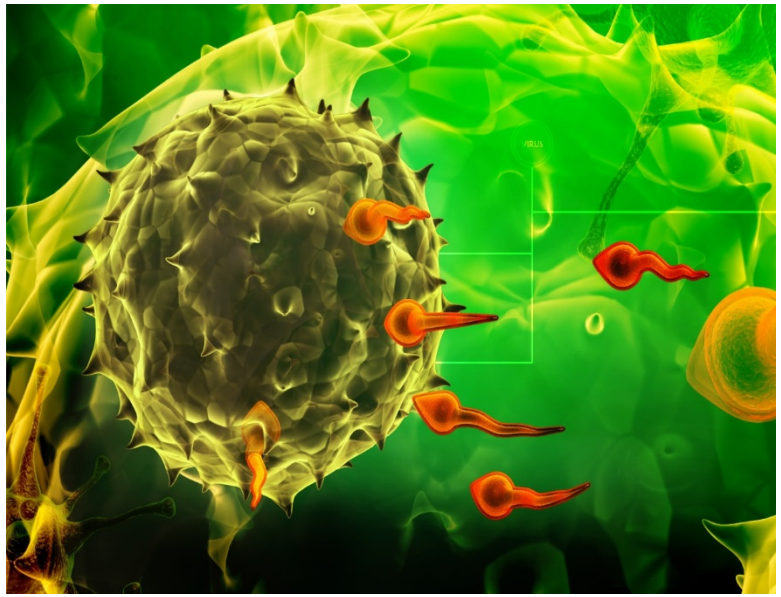
Slice-by-slice: often no risk

The whole sausage: risk?

Implementation of mixture risk assessment is a challenge



Chemical mixtures may affect human health



- Dioxins and related PCBs in food and feed (EFSA)
- Phthalates in articles (ECHA 2017)

- Pesticides in food (EFSA 2020)
- 4∑PFAS (EFSA 2020)

Is it possible to predict the effects of chemical mixtures?

Design of chemical mixture studies

Chemicals & doses selected based on their effect/mode of action

Top-down approach:

Designed mixture containing model compounds

Mixture studies to test hypotheses on mathematical predictions of effects

$$X = (p_1/x_1 + p_2/x_2 \ldots\ldots)^{-1}$$

Mixture studies reflecting real-life exposure scenarios

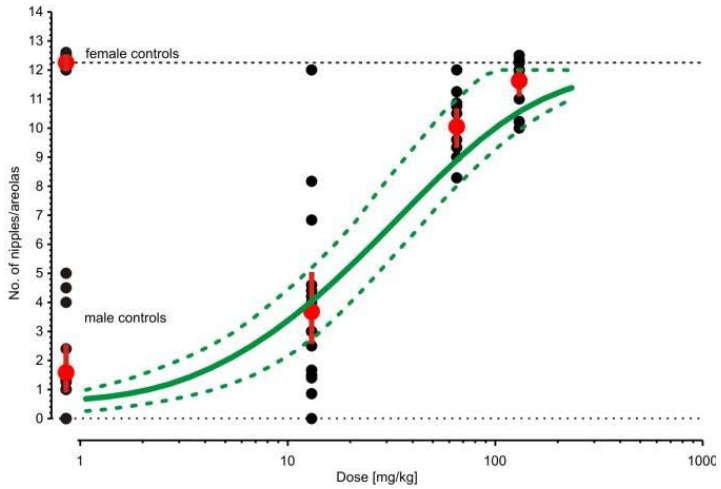
Bottom-up approach:

Composition of mixture according to real-life exposure

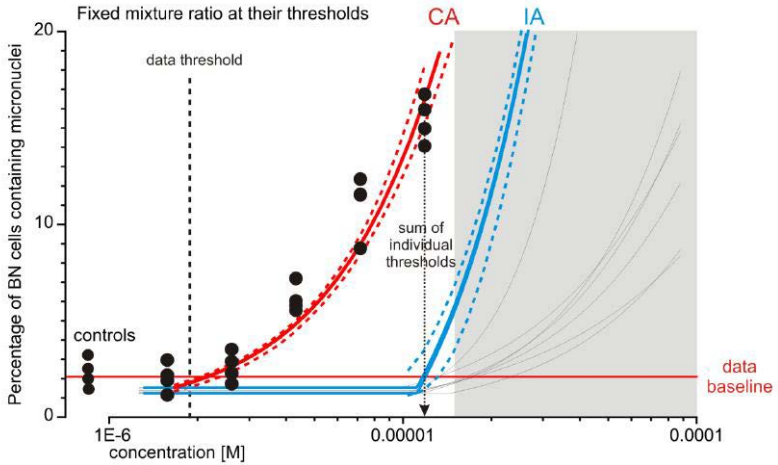
Human biomonitoring of chemical exposures

Prediction of mixture effects: Dose-Addition provides good approximations of observed effects

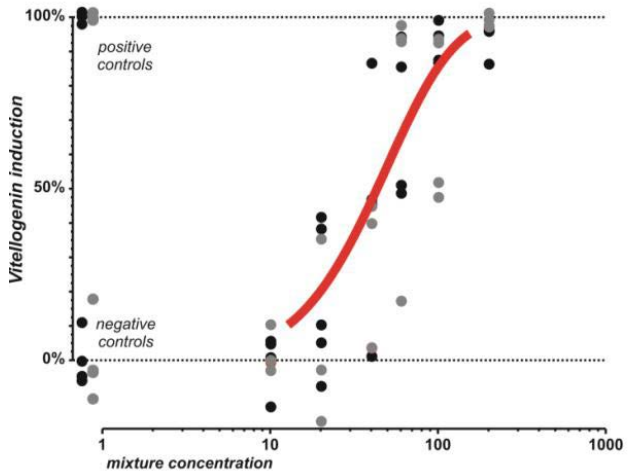
Retained nipples



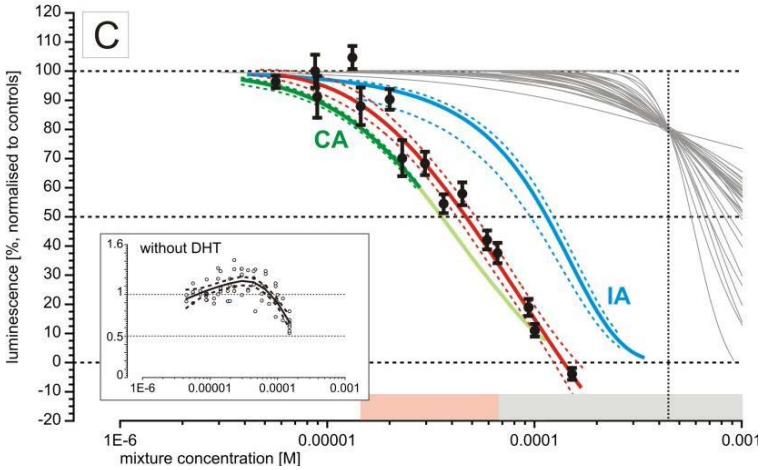
Micronuclei



Vtg induction (fish)

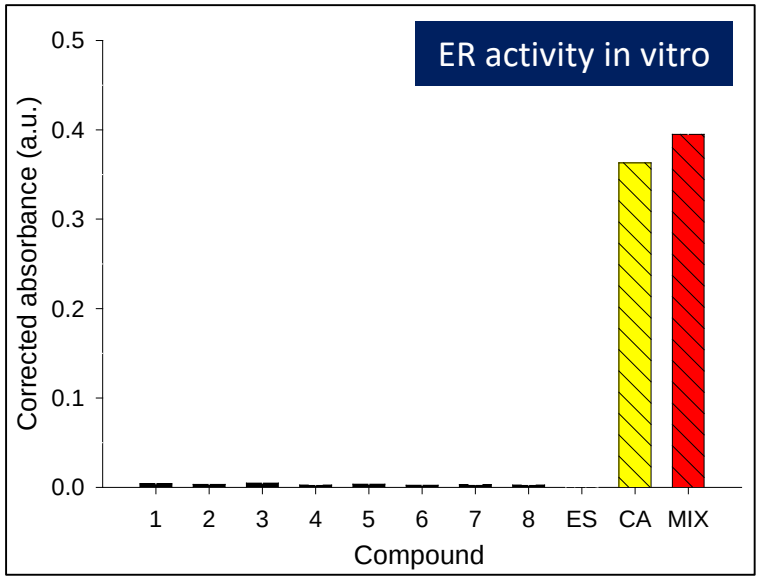


AR antagonism

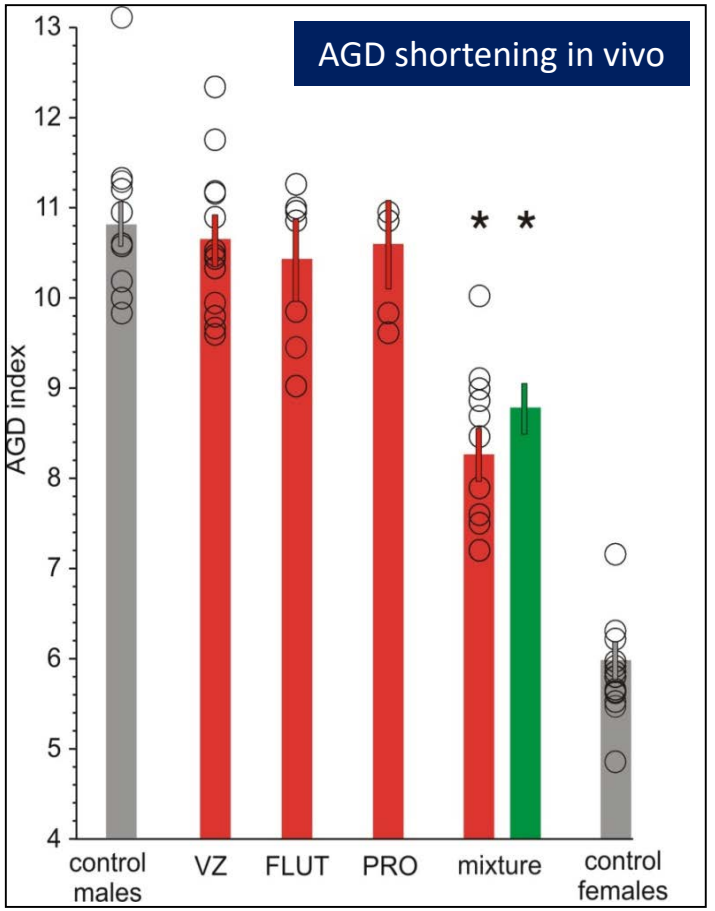


Something from ‘nothing’ observed in various test systems:

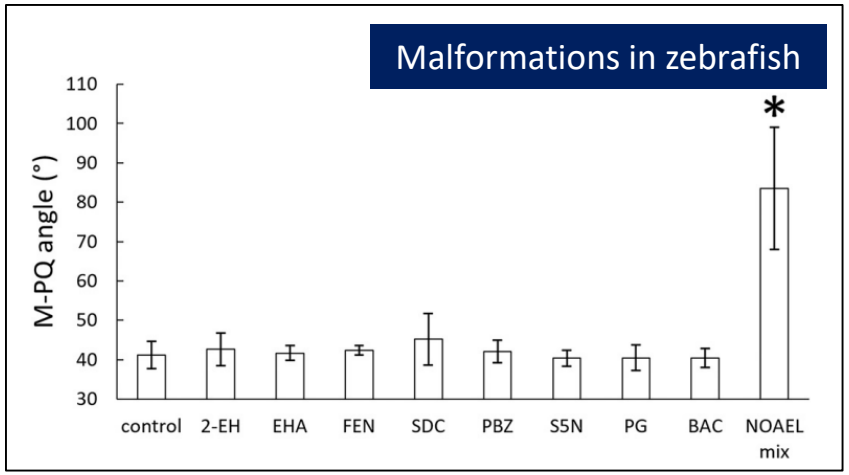
No effect of single compounds, but mixture effect at doses <NOAELs



Silva et al. (2002). Something from “Nothing” - Eight Weak Estrogenic Chemicals Combined at Concentrations below NOECs Produce Significant Mixture Effects Environ Sci Technol 36, 1751



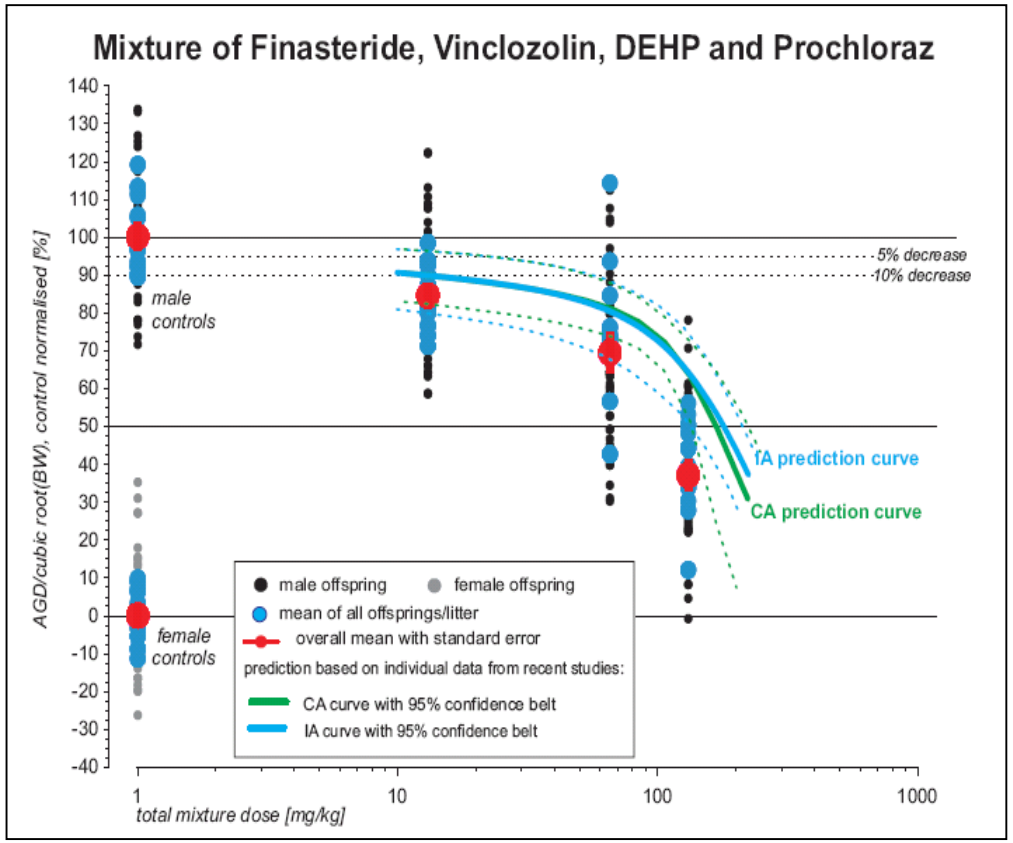
Hass et al. (2007). Combined exposure to anti-androgens exacerbates disruption of sexual differentiation in the rat. EHP 115 (1), 122–128



Van der Ven et al. (2022). Dose Addition in the Induction of Craniofacial Malformations in Zebrafish Embryos Exposed to a Complex Mixture of Food-Relevant Chemicals with Dissimilar Modes of Action. EHP.

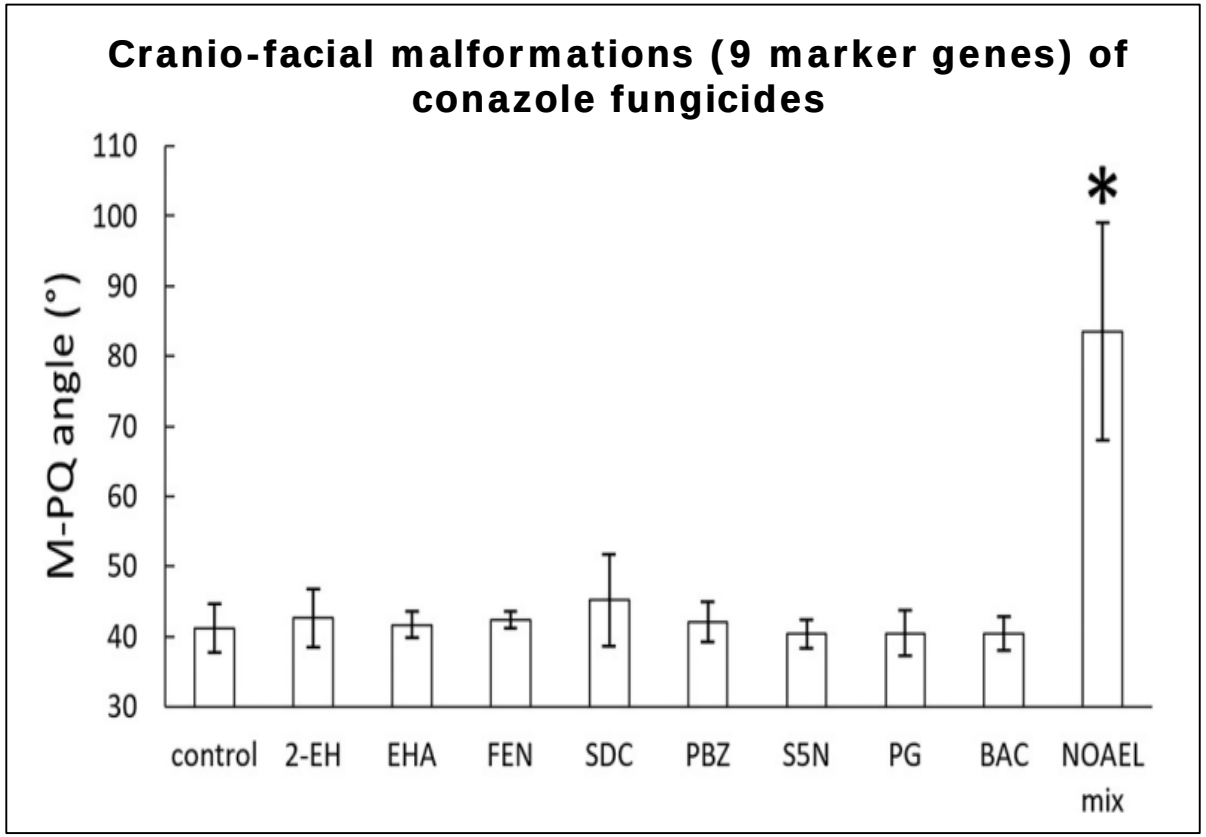
Mixture effects of dissimilarly acting chemicals can also be predicted by dose-addition

AGD shortening in rats



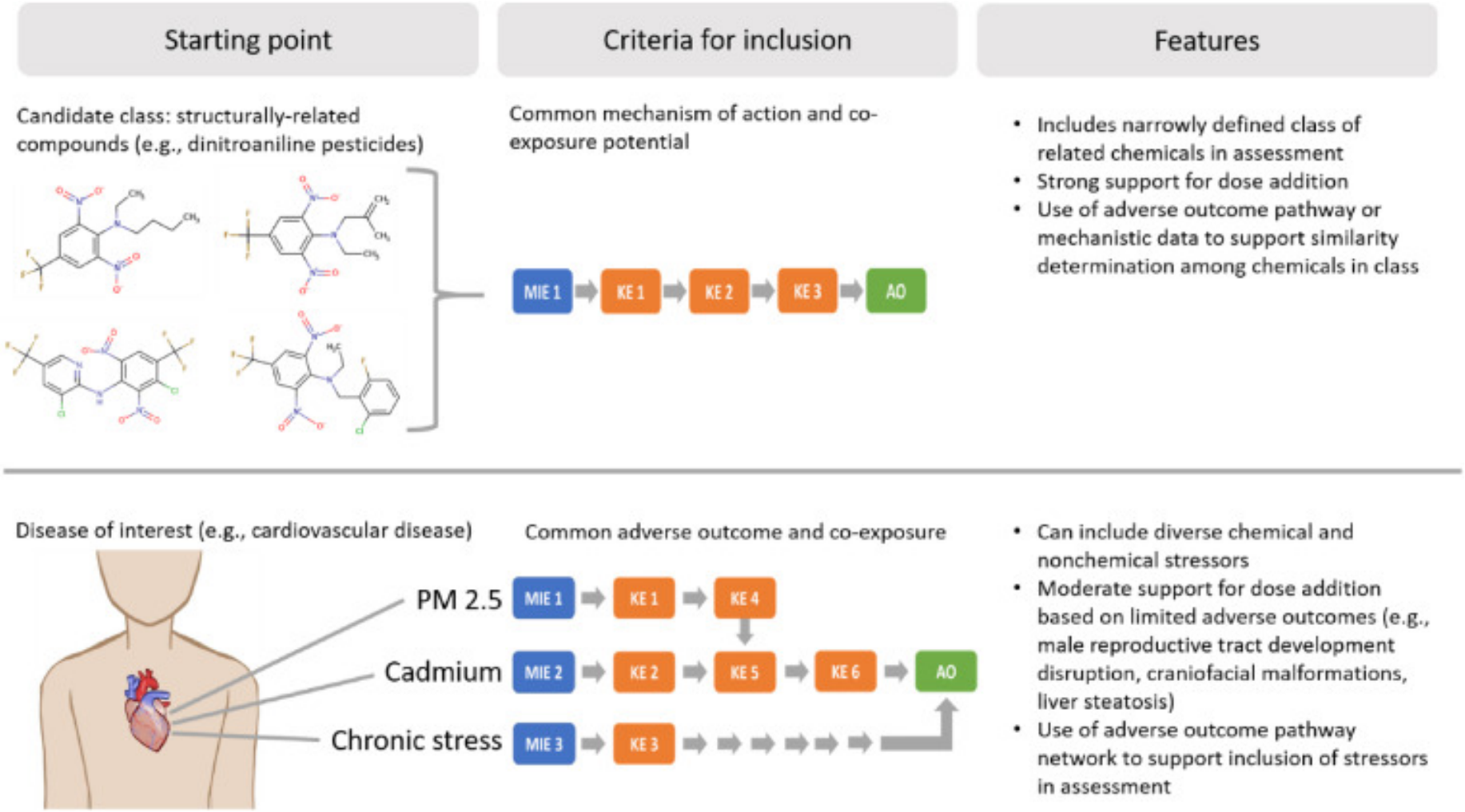
Christiansen et al. (2009). Synergistic Disruption of External Male Sex Organ Development by a Mixture of Four Antiandrogens. EHP 117, 1839

Malformations in zebrafish

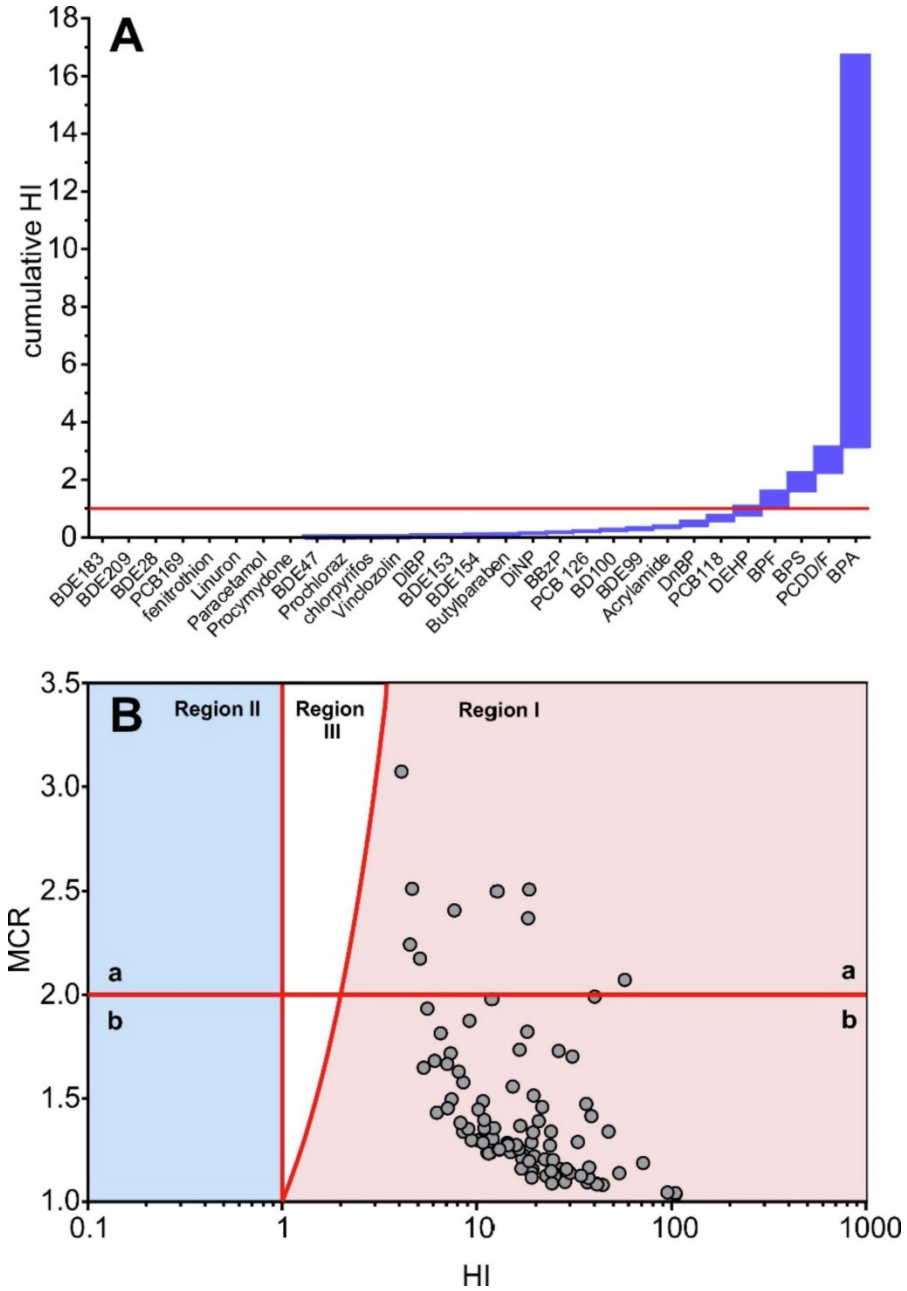


Van der Ven et al. (2022). Dose Addition in the Induction of Craniofacial Malformations in Zebrafish Embryos Exposed to a Complex Mixture of Food-Relevant Chemicals with Dissimilar Modes of Action. EHP.

Cumulative risk assessment based on chemicals **sharing mechanism of action** or **targeting a common disease**



Rider, C (2022). Mixture math: Deciding what to add in a cumulative risk assessment. Current Opinion in Toxicology 31, 100358



Mixture risk assessment for decline in sperm quality.

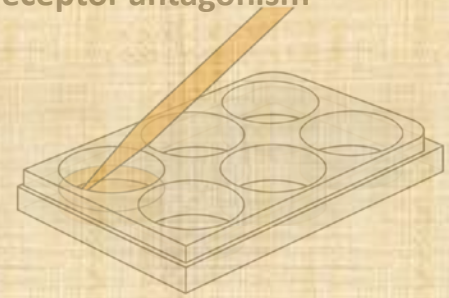
29 chemicals monitored jointly in urine from 98 Danish young men

Bisphenols (A, S, F), PCBs and phthalates (DEHP) identified as drivers of mixture risk

Kortenkamp et al. Combined exposures to bisphenols, polychlorinated dioxins, paracetamol, and phthalates as drivers of deteriorating semen quality. Env Int 165, 107322, 2022

Mixture risk assessment based on *in vitro* and human biomonitoring data: A case study on antiandrogenic chemicals

In vitro data for human androgen receptor antagonism

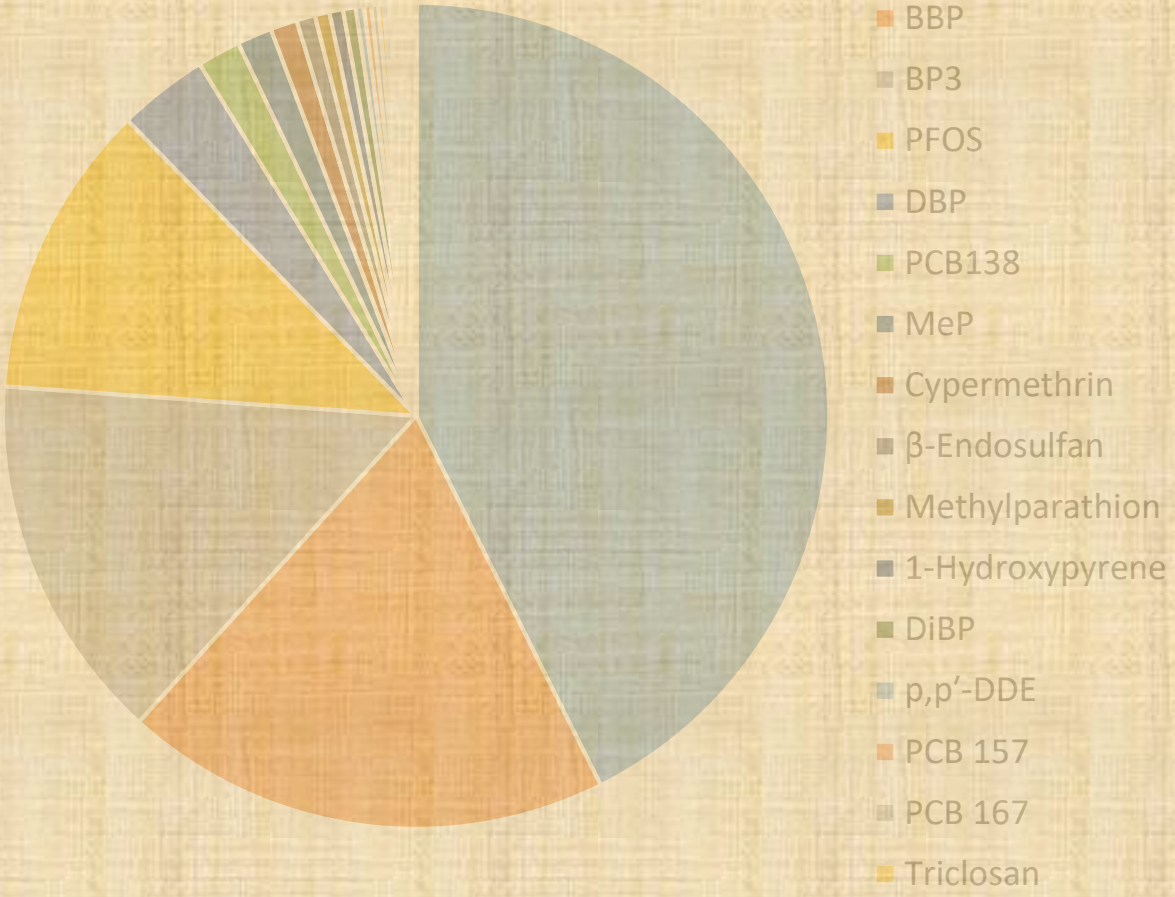


Human biomonitoring data for hazardous chemicals

HBM4EU

Calculation of Risk Quotients for 61 chemical and their combined Hazard Index

Chemical mixture drivers for adverse effects on male reproductive health



Ma et al. Human risk associated with exposure to mixtures of antiandrogenic chemicals evaluated using in vitro hazard and human biomonitoring data. Submitted.

International impact

Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Toxicology

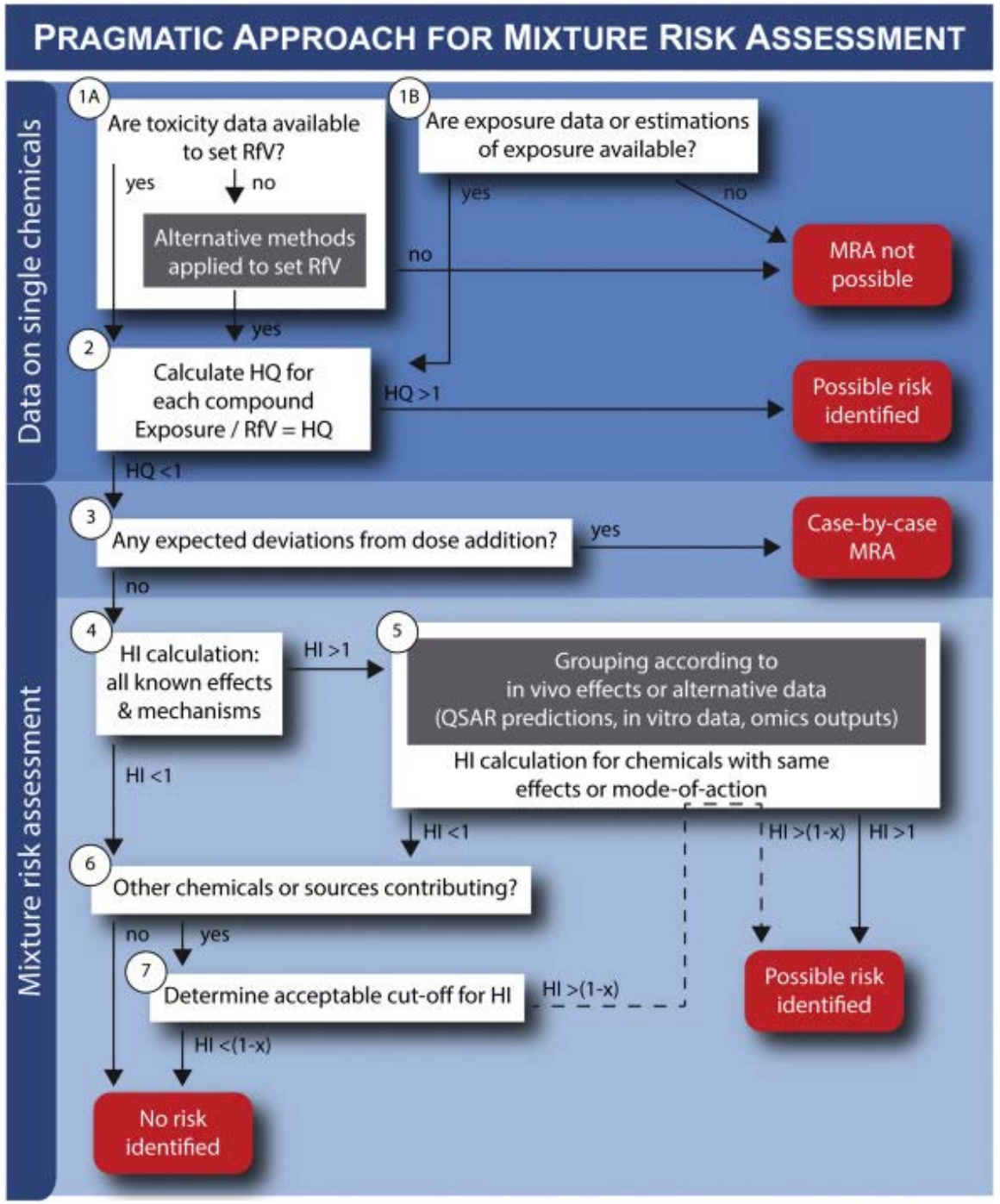
A pragmatic approach for human risk assessment of chemical mixtures

Julie Boberg, Marianne Dybdahl, Annette Petersen, Ulla Hass, Terje Svingen and Anne Marie Vinggaard

- Input to EFSA's mixture work
- EU projects (EDEN, Contamed, HBM4EU, PANORAMIX, PARC)

'Chemical Mixture Calculator'

BOBERG ET AL. Chemical Mixture Calculator - A novel tool for mixture risk assessment. Food Chem Toxicol 152, 112167, 2021



Rie Vinggaard, DTU

https://panoramix-h2020.eu/

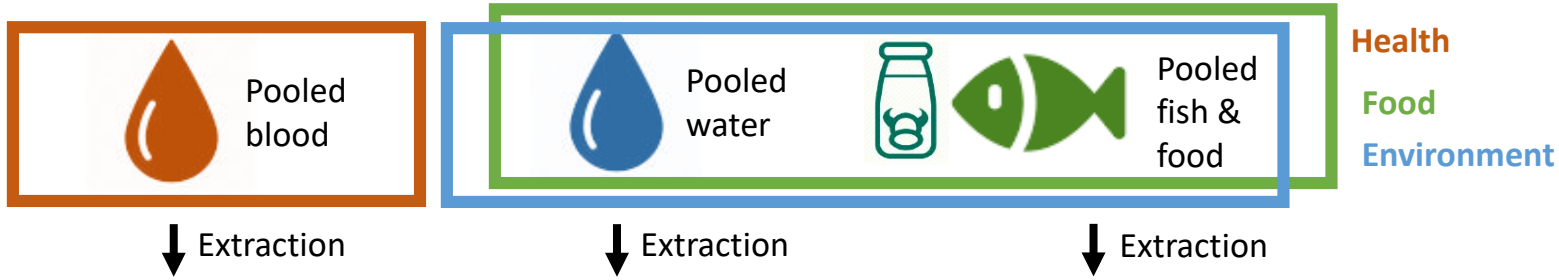
Providing risk assessments of complex real-life mixtures for the protection of Europe's citizens and the environment

This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement 101036631

- 11 partners from 6 European countries, coordinated by DTU
- 4 yrs, Nov 2021-2025
- Budget: ~ 4.5 million €

Bioassay testing of complex real-life samples

Phase 1: Screening environment-food-human continuum



Panel of *in vitro* bioassays addressing the MIEs underlying

Neurodevelopment

Neurotoxicity
Neuronal cells SH-SY5Y
- ACHE inhibition
- Neurite outgrowth inhibition
- Mitochondria toxicity
- Zebrafish neurotoxicity

Thyroid hormone system disruption
- TPO, DEHAL
- TTR/TBG binding
- GH3-TRE-luc
- Iodine transport

Reproductive function

Reproductive toxicity
- ER agonism,
- AR antagonism
- hIPSC-based developmental toxicity

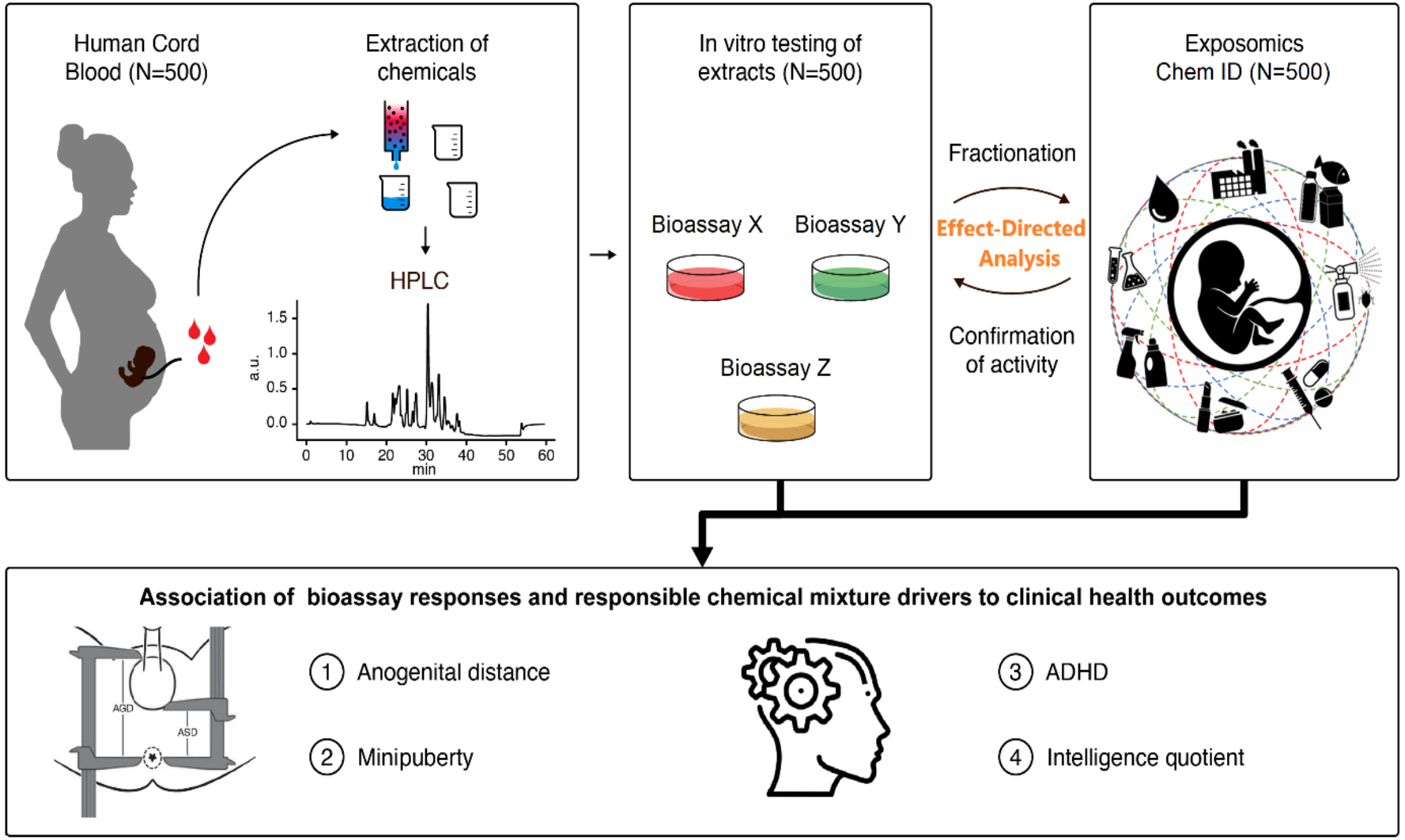
Genotoxicity and stress responses
- γH2AX/pH3 assay
- oxidative stress response (Nrf2-ARE)
- Metabolism (AhR, PPAR)

Bioassay Selection Criteria

Relevance for developmental disorders (AOP)
- **S**pecificity, **S**electivity, **S**ensitivity

Escher et al. Mixture risk assessment of complex real-life mixtures – the PANORAMIX project. Int J Exp Res Publ Health, 2022

Phase 2:
Screening 500 cord blood samples with a 3-6 HTS *in vitro assays*


Human Cord Blood (N=500)
Extraction of chemicals
HPLC
a.u.
1.5
1.0
0.5
0.0
0
10
20
30
40
50
60
min
In vitro testing of extracts (N=500)
Bioassay X
Bioassay Y
Bioassay Z
Fractionation
Effect-Directed Analysis
Confirmation of activity
Exposomics Chem ID (N=500)
Association of bioassay responses and responsible chemical mixture drivers to clinical health outcomes
AGD
ASD
1 Anogenital distance
2 Minipuberty
3 ADHD
4 Intelligence quotient

Take home messages

- One chemical at a time underestimates the risk
- The dose-addition principle can be applied for both similarly and dissimilarly acting compounds
- **YES WE CAN** predict mixture effects in most cases, if we have adequate hazard and exposure data for single compounds
- Usually additivity, synergism/antagonism in rare cases
- Risk at high-end human exposures to certain chemical mixtures
- Top-down designs from the past, bottom-up designs for the future
- *In vitro* methods for whole-mixture assessments