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POLICY BRIEF

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

Linking Environmental Contamination to Human Exposure: *In Vitro* Evidence for Chemical Mixture Transfer

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Humans and ecosystems are exposed to complex mixtures of thousands of synthetic chemicals across the environment, food, and biological systems. Even when individual chemicals are deemed safe, their combined effects can contribute to adverse health outcomes. Current regulatory frameworks face significant challenges in assessing these mixtures due to high chemical diversity and co-exposure patterns, and because chemical analysis alone cannot capture the full spectrum of chemicals.

New approach methodologies (NAMs), especially high-throughput animal-free *in vitro* bioassays, provide a complementary solution by measuring the total biological activity of all extracted chemicals in a sample, including unknown substances. When combined with chemical analysis and mixture modelling, these tools enable a more comprehensive and effect-relevant assessment of risk, provided that the NAMs used have a clear link to adverse health outcomes.

In the PANORAMIX Project (Figure 1), we collected samples in Europe across the environment–food–human continuum, including:

- Water (wastewater, surface, drinking, bottled)
- Food (milk, fish)
- Human matrices (serum, breast milk)

Extraction protocols for water and serum were harmonized with a focus on water-soluble chemicals, while milk and fish were delipidated after extraction. Suspect screening identified 547 chemical features across all extracts, 45 of which were also semi-quantified. We tested the extracts in a battery of 22 *in vitro* bioassays (cell-based, cell-free, and organismal) that target key environmentally and health-relevant endpoints including neurodevelopment, thyroid hormone system disorders as part of endocrine disruption, developmental neurotoxicity, reproductive disorders and genotoxicity. A reconstituted mixture of 24 chemicals quantified with sufficient certainty, representing a curated subset of the much larger pool of detected and partially identified chemicals, was prepared at the concentration ratios measured in the samples and tested in six selected *in vitro* bioassays.

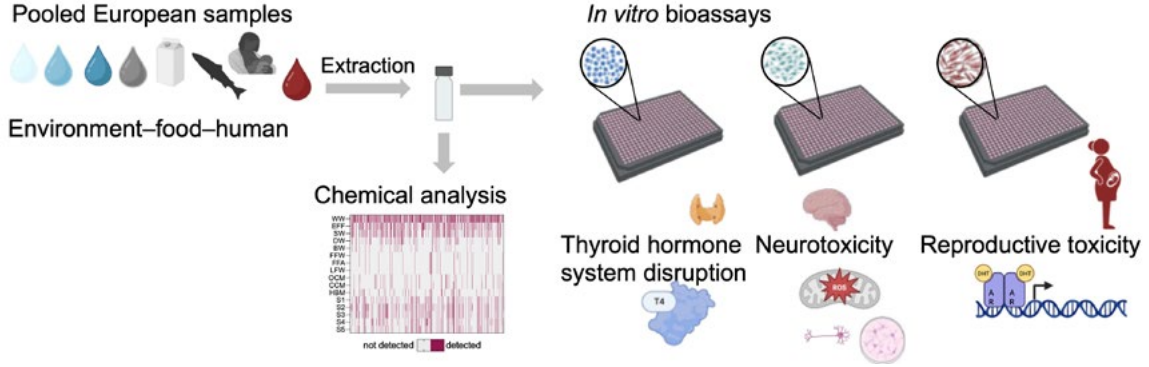

Figure 1. Approach to follow chemical mixtures from environment over food to humans: Extraction of pooled samples followed by non-target and target analysis and testing with a battery of in vitro bioassays. Adapted from Escher et al. 2026

Key findings:

Widespread bioactivity was detected across all sample types, strongest in wastewater, fish, and human samples. Effects were most pronounced for:

- Thyroid hormone system disruption (e.g., interference with thyroid hormone distribution)
- Neurotoxicity (e.g., impaired neurite growth, mitochondrial dysfunction)
- Reproductive toxicity (e.g., anti-androgenic activity)

Low genotoxicity (via structural DNA breaks) was detected in wastewater and one fish extract.

Mixtures containing up to 24 measured chemicals, combined at the same ratios found in the samples, were well predicted by concentration addition models. This suggests that the chemicals mostly acted additively.

However, even mixtures designed from up to 17 identified chemicals explained only a small part of the total bioactivity observed. This indicates that much of the effect came from unknown or insufficiently characterized toxicants including bioactive endogenous chemicals, consistent with the 547 detected but unaccounted-for chemicals and the many additional unresolved chromatographic peaks. Bioassay-specific and matrix specific effect-based trigger values were proposed to differentiate acceptable from concerning mixture effects.

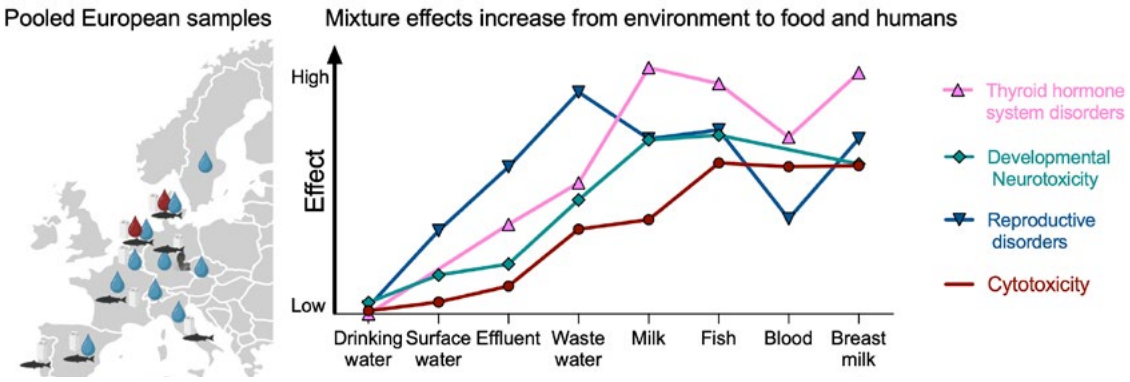

Figure 2. Levels of adverse effects observed in 22 bioassays and clustered into developmental neurotoxicity, including thyroid hormone system disorders, and reproductive disorders in various water, food and human samples. Adapted from Escher et al. 2026

Implications:

- Synthetic organic chemicals, such as industrial chemicals, consumer product chemicals, biocides and pesticides, spread through water systems and the environment, accumulate in living organisms, and ultimately reach humans
- The majority of mixture effects is driven by unknown or unmonitored chemicals, representing a critical blind spot in current risk assessment
- Sole reliance on chemical monitoring leads to systematic underestimation of real-world risks
- Integrating bioassays with chemical analysis enables:
  - o Detection of cumulative mixture effects
  - o Identification of key toxicological drivers
  - o Translation of complex exposures into actionable metrics (e.g., effect-based trigger values)

Conclusion

This works has policy considerations for the following EU legislation such as chemicals (REACH), food (plant protection products), endocrine disrupters (CLP) and the wastewater Directive. Protecting human health requires a shift toward mixture-aware monitoring frameworks. Combining chemical and bioanalytical approaches is recommended to capture the true scale and impact of chemical exposure across the environment–food–human system.

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