Hazards of chemicals in recycled plastic. Effect-based analysis and suspect screening

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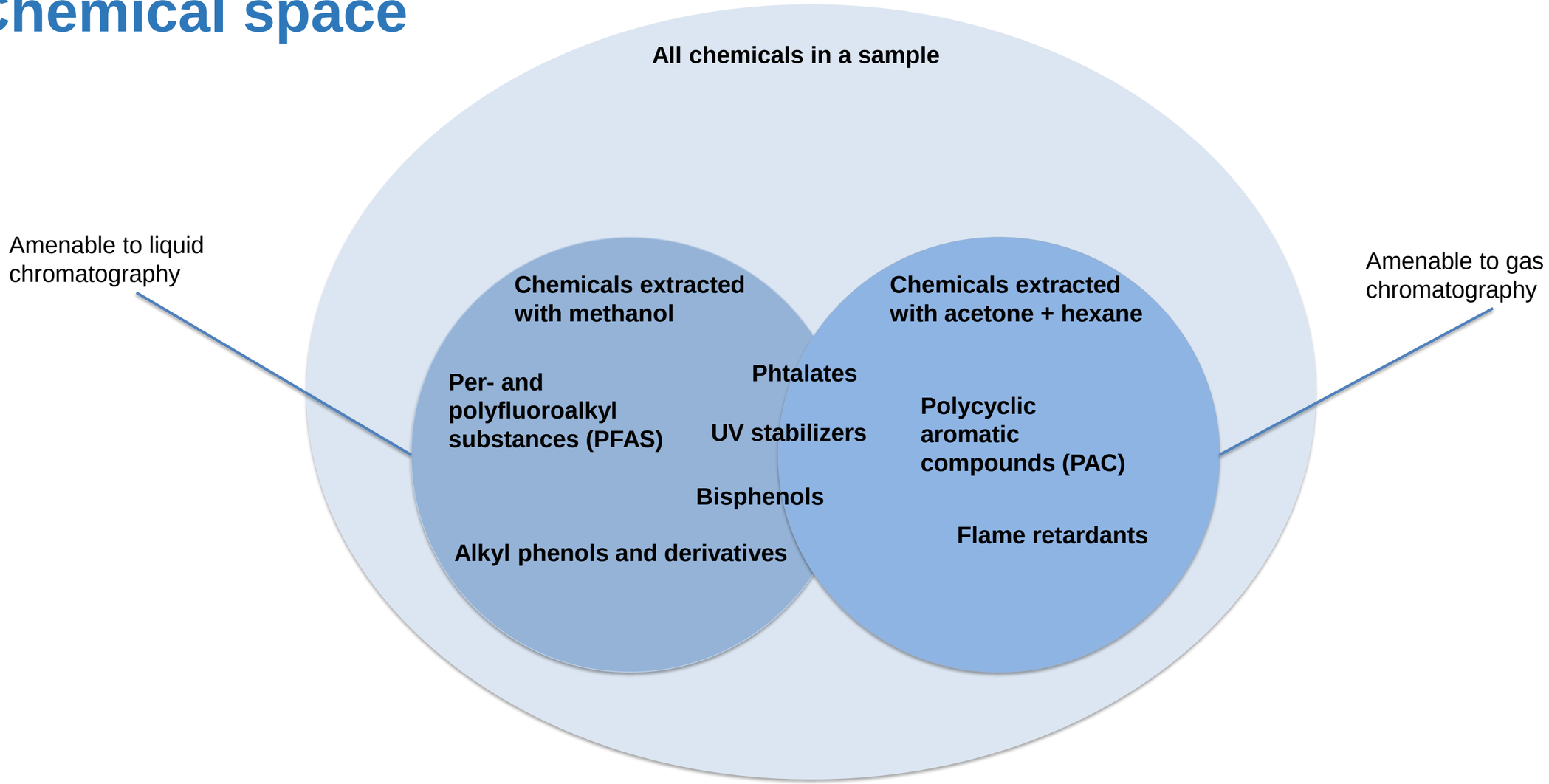
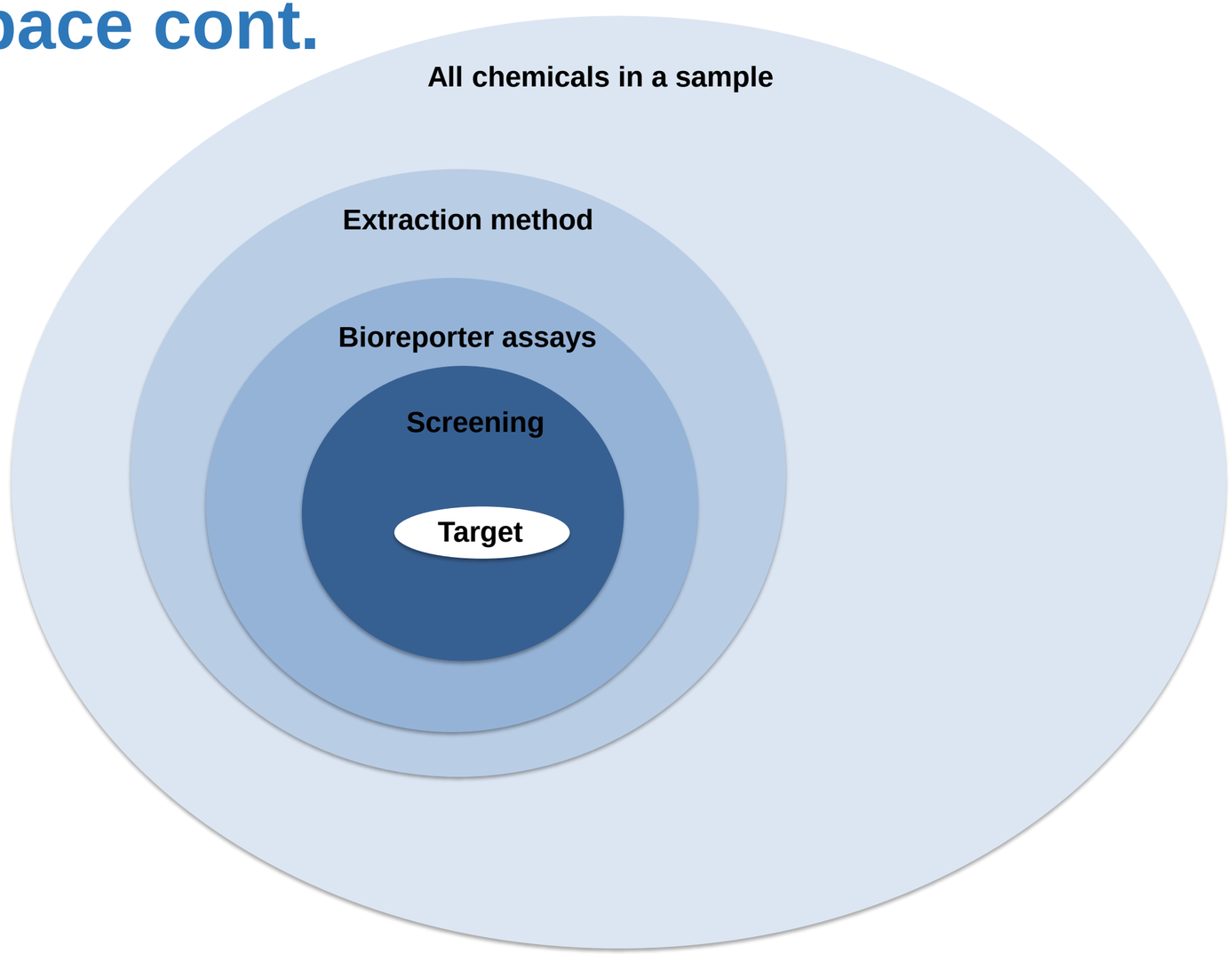
Overview chemicals in plastics

- ~13,000 chemicals identified as associated with plastics and plastics production (UNEP, 2023)
- ~3,200 of which have one or more properties of concern
- Several thousands are uncharacterized toxicologically
- Groups of chemicals identified as being of major concern
    - Flame retardants
    - UV stabilizers
    - Per- and polyfluoroalkyl substances (PFAS)
    - Phtalates
    - Bisphenols
    - Alkyl phenols and derivatives
    - Polycyclic aromatic compounds (PAC)
    - Biocides
    - Certain metals

Chemical space



Chemical space cont.



Recycling of plastics

- Chemical complexity with thousands of additives and non-intentionally added substances (NIAS)
- General lack of traceability of the chemicals
- Absorption of chemicals during use
- Contamination possible and not controlled for in recycled plastics
- Conflict between the goal of circularity and chemical safety

Method

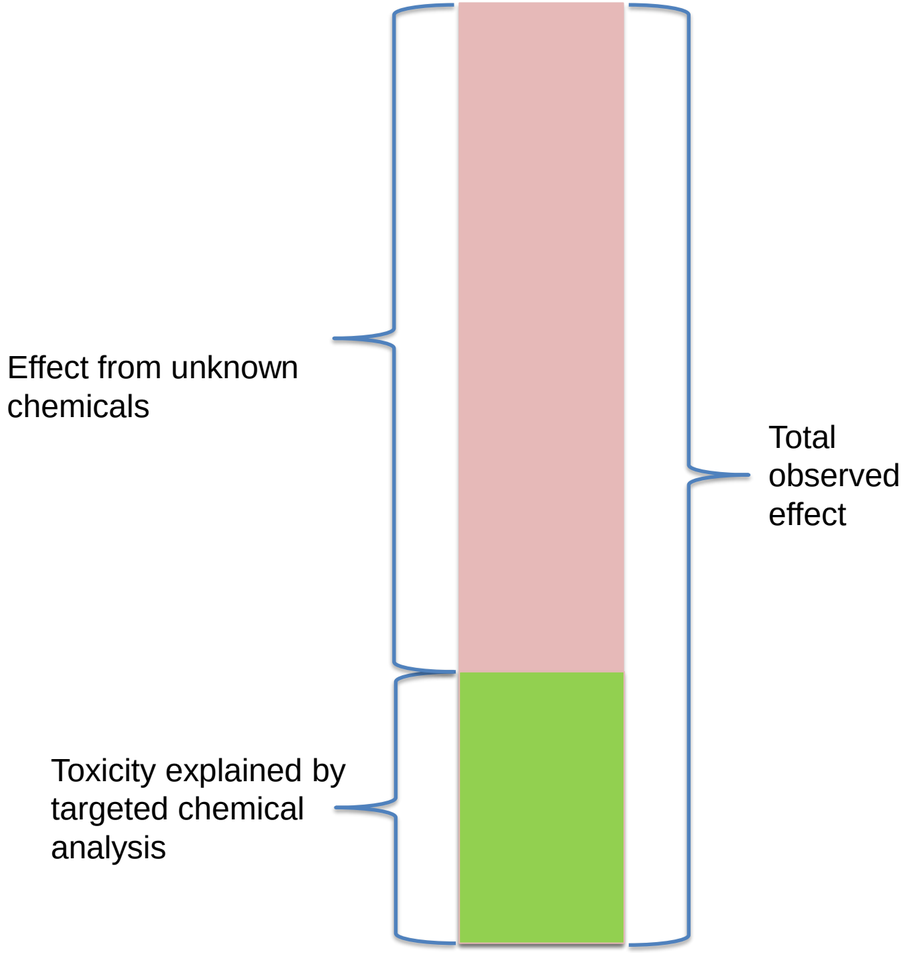
- Ultrasound assisted extraction
- Hexane and acetone (3:1) → non-polar, volatile compounds
- Methanol → (semi)-polar compounds
- Recycled and virgin PP, PET, PETG, and PP-C pellets (n=13)
- Plastic products of TPR, TPU, and PMMA (n=6)
- Plastic pellets of TPR, TPU, and PMMA (n=6)
- More samples to be added

Approach – bioreporter assay in conjunction with suspect screening

- Bioreporter assays measure effects of chemical mixtures toward specific end-points
- High-resolution mass spectrometry based suspect screening – identification of chemicals with less a priori information
- Effect-directed analysis – fractionate samples, measure effect and screen for chemicals in fractions with high effect
- Goal: find the chemical drivers for the observed toxicity in plastics

Approach – bioreporter assay in conjunction with suspect screening cont.

- Project utilizes an effect based screening approach
- Bioreporter assays cover a wide portion of chemical space
- Targeted chemical analyses usually fall short in explaining the observed toxicity → need for broader chemical characterization

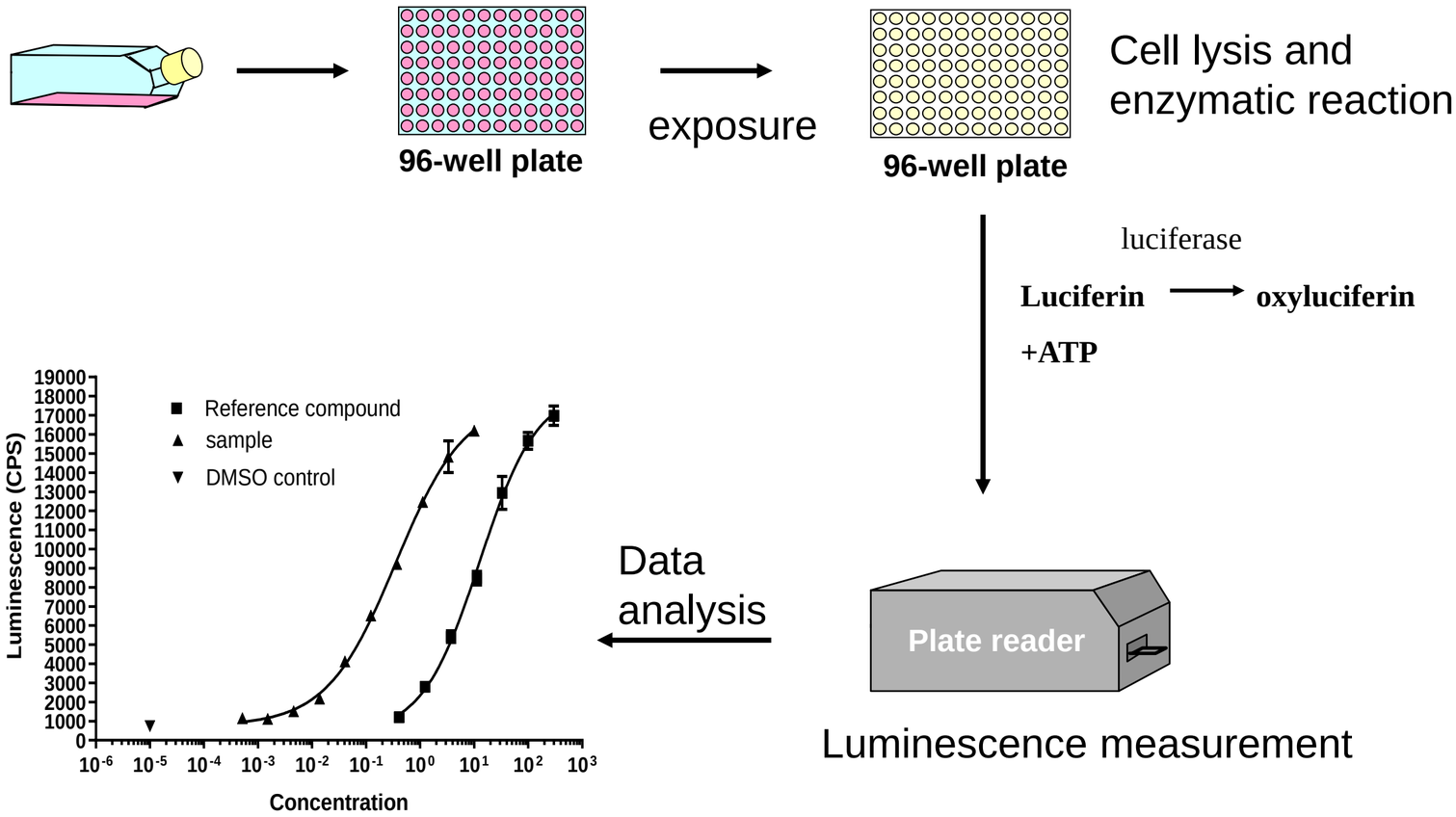


Bioreporter assay

Measure specific mechanisms behind toxicity

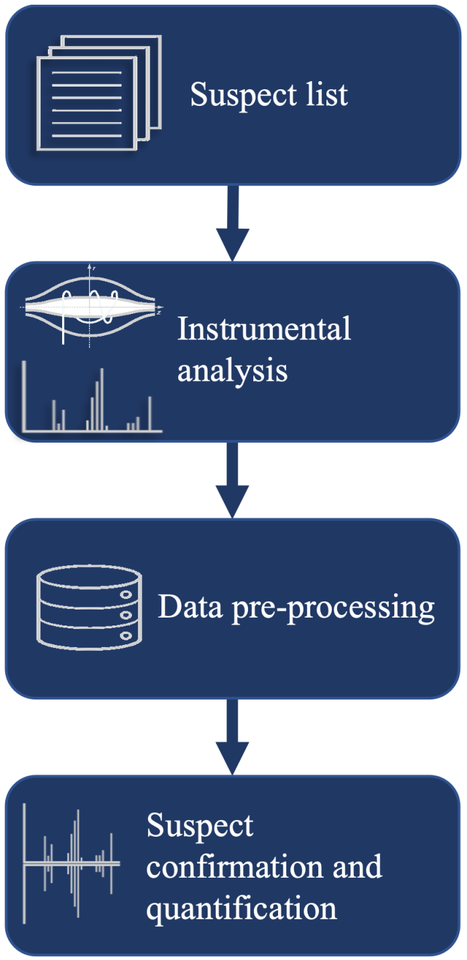
Three endpoints (receptors)

- Dioxin or aryl hydrocarbon receptor (DR/AhR)
- Estrogen α receptor (ERα)
- Androgen receptor (anti-AR)



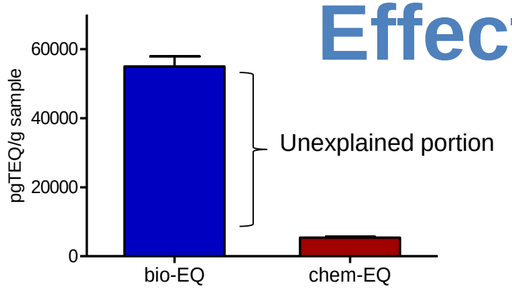
Bioassay derived equivalents (bioEQs) = $\frac{\text{EC25 Reference compound}}{\text{EC25 sample extract}}$ (pg/g)

Chemical screening - workflow



- Suspect list compiled from literature – chemicals in plastics and general chemicals of concern
- Analysis on both gas- and liquid chromatography coupled to high-resolution mass spectrometers.
- Library and in-silico fragmentation aided annotation
- Purchase of interesting standards for confirmation and quantification

Effect-directed analysis (EDA)



Bioreporter (H4IIE-luc) and chemical derived TEQs of a soil extract

Bio-EQ >> chem-EQ → fractionation of extract

Fractionation

Separation of chemicals in sample extracts with high bioassay activity

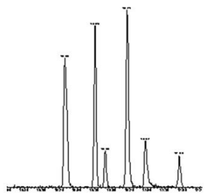
LC—fractionation system

GC-fractionation system

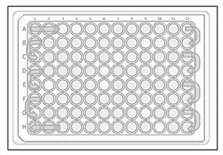
Analysis

MS detector (MSD)

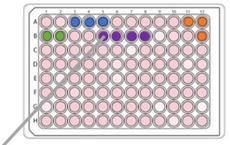
MSD data

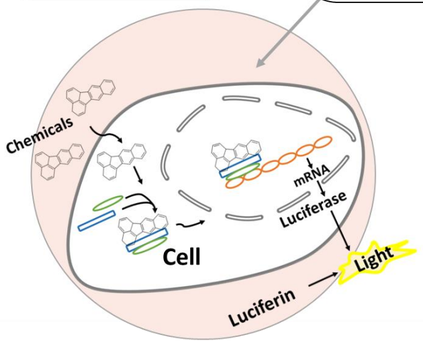


Collection of fractions



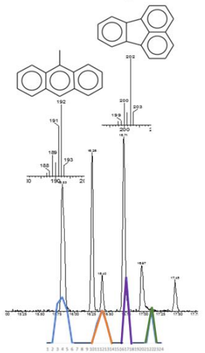
Seeding of cells in fractionated wells (bioassay analysis)



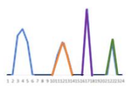


Identification

Overlapping MSD and bioassay chromatograms

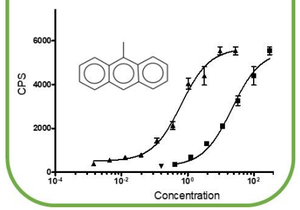


Bioactive fractions are transformed to bioassay chromatogram



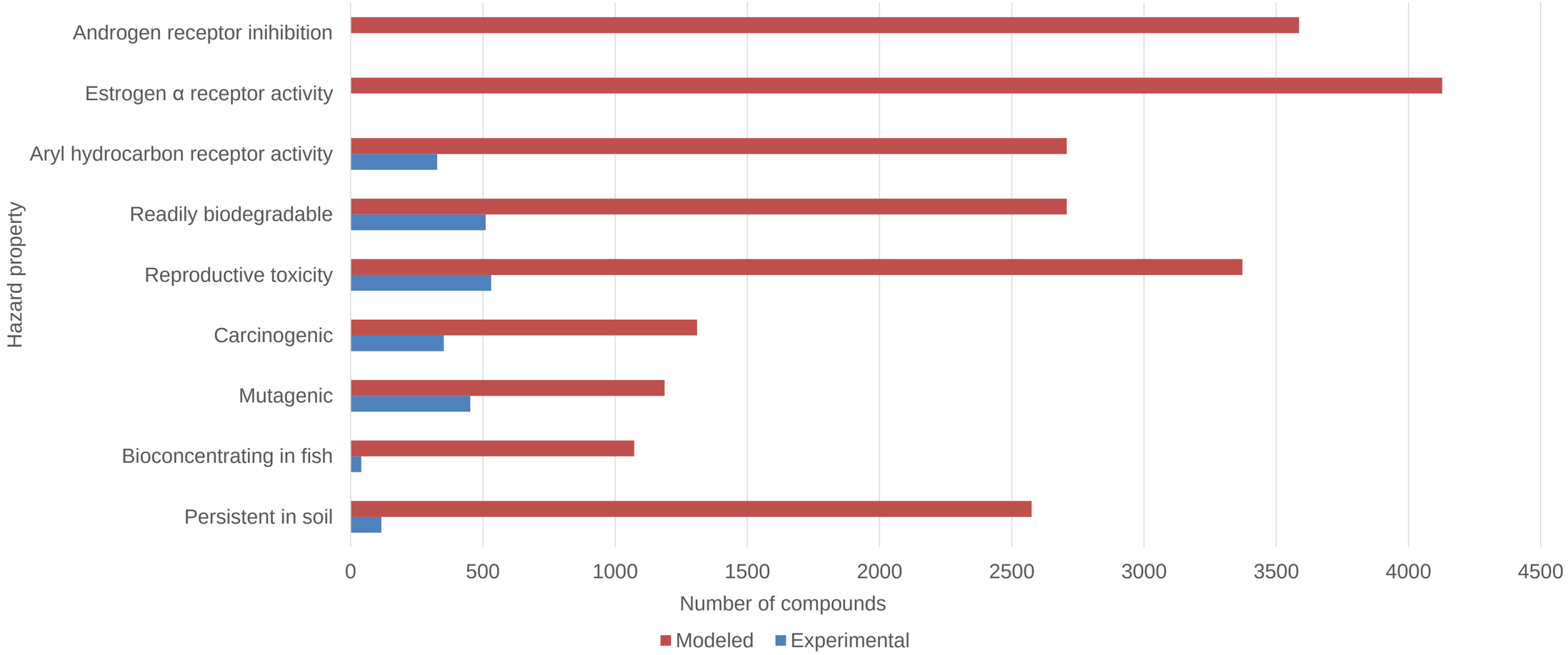
Validation

Testing of identified chemilas in the bioassays

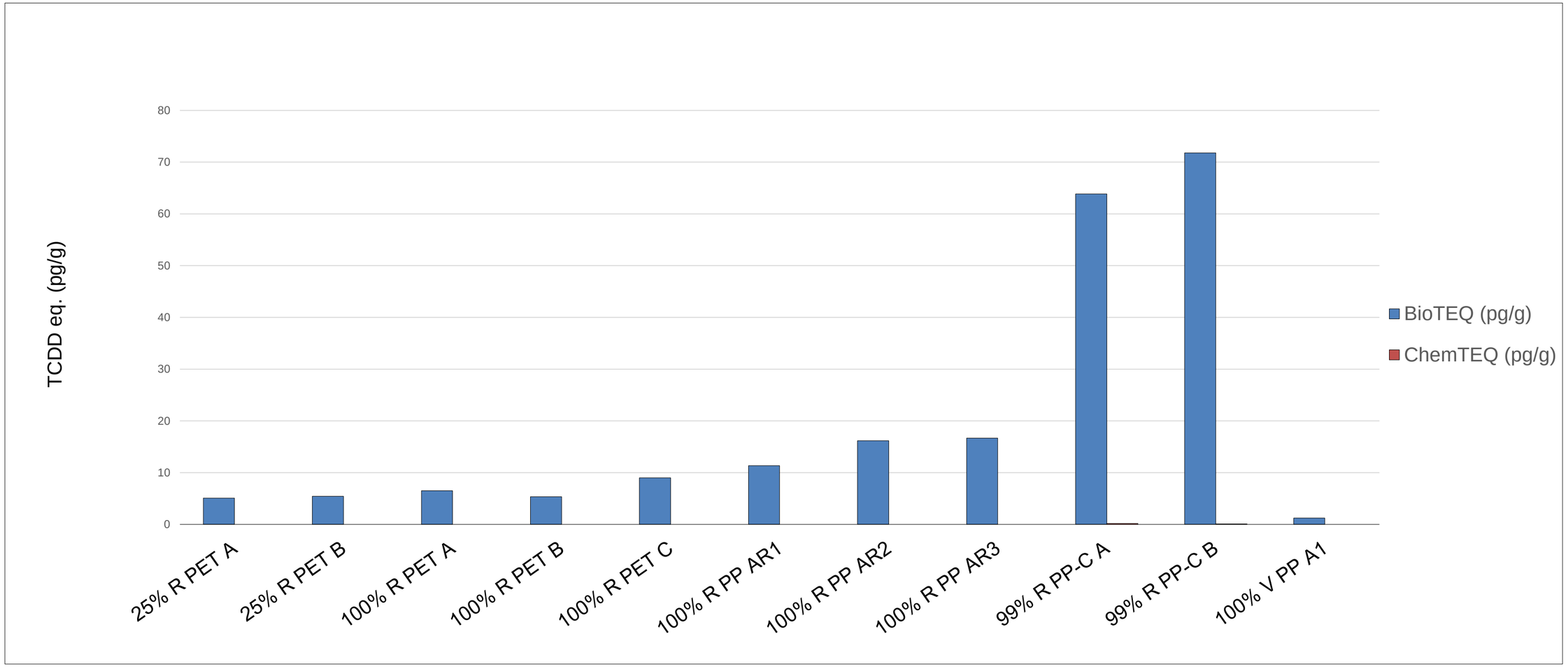


List of identified compounds

Hazardous properties of chemicals in plastics

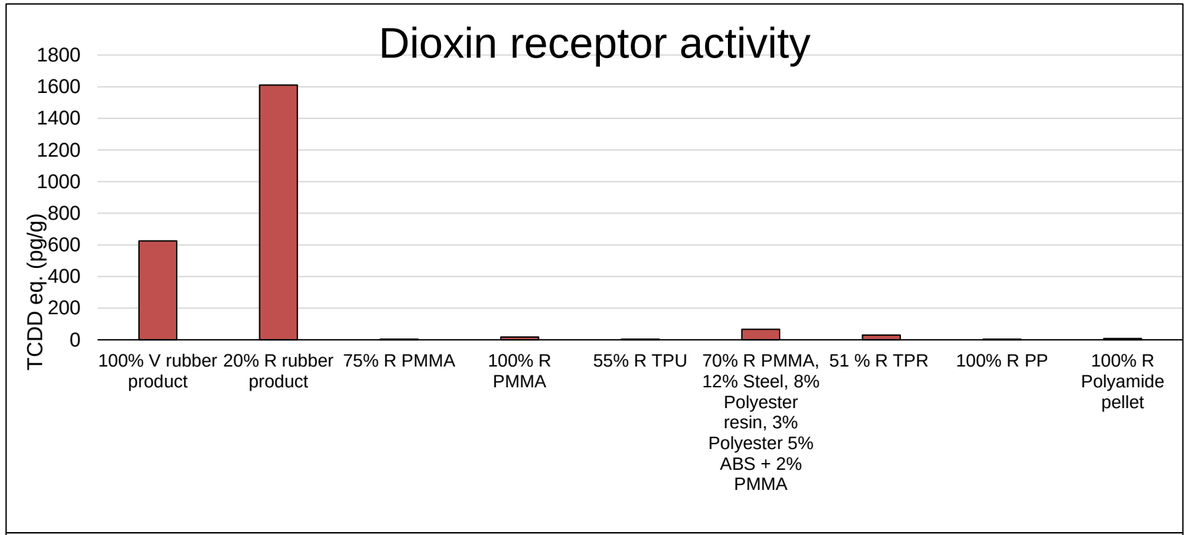


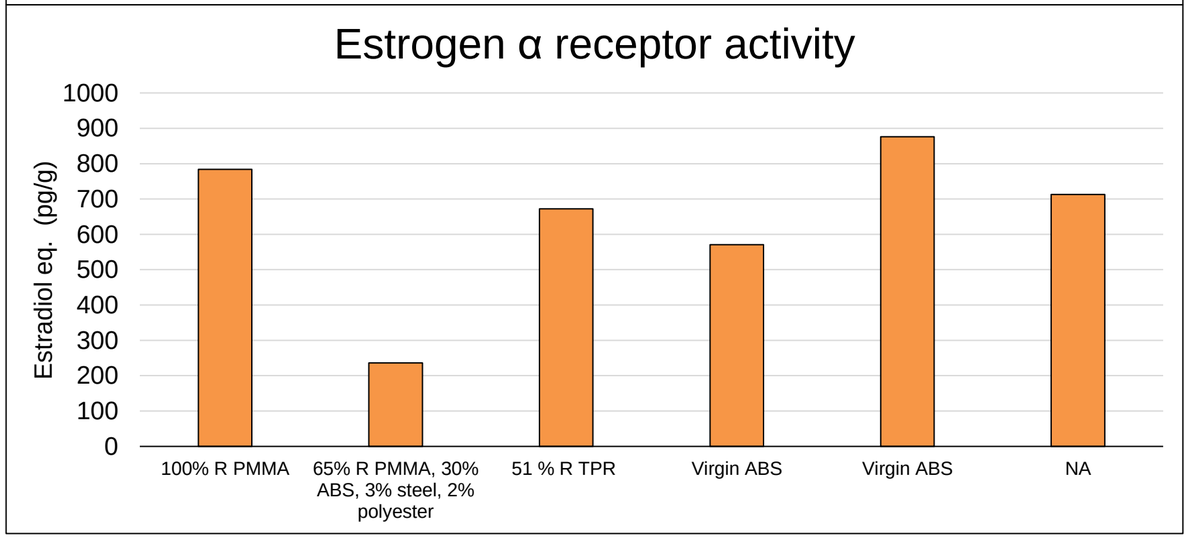
Dioxin receptor activity for PET, PP-C, and PP



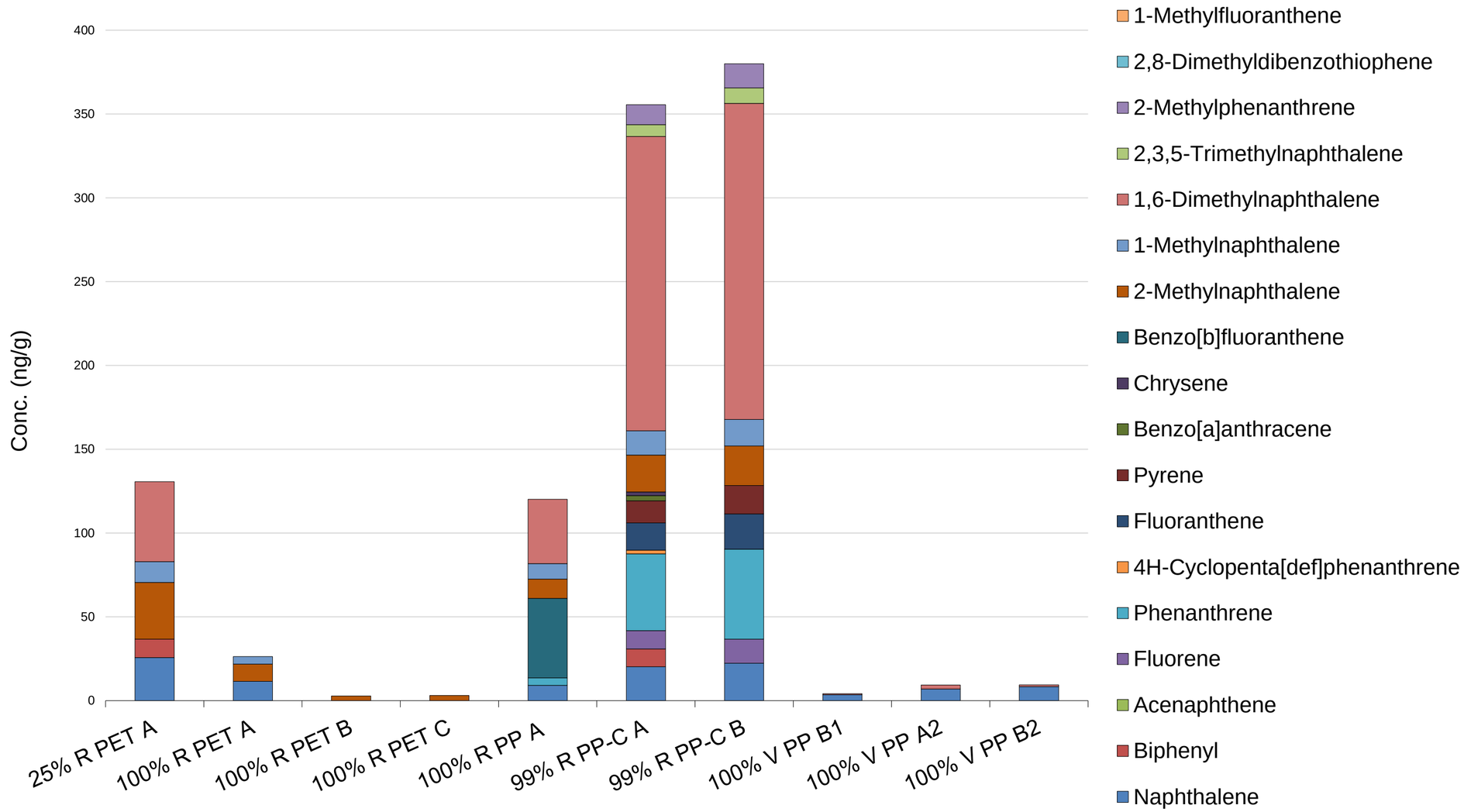
Estrogen α and dioxin receptor activity

- Rubber (only 2 analyzed!) show much higher dioxin receptor activity when compared to plastics
- Estrogen α receptor activity observed
- To note: only the positive results are presented – some samples showed no activity in one or more receptor



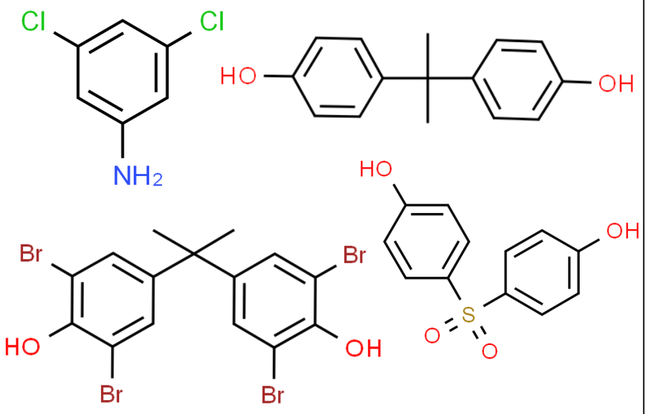
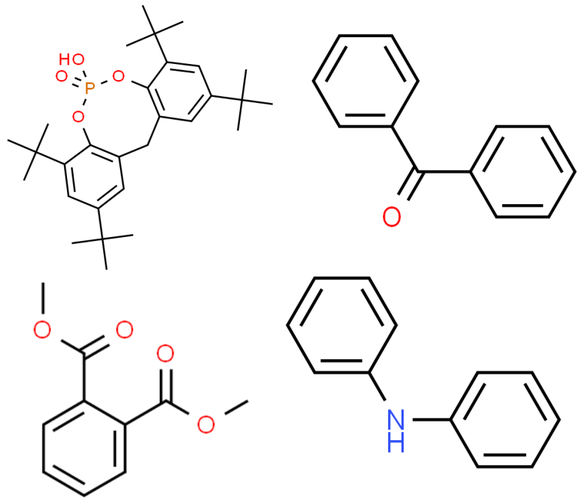
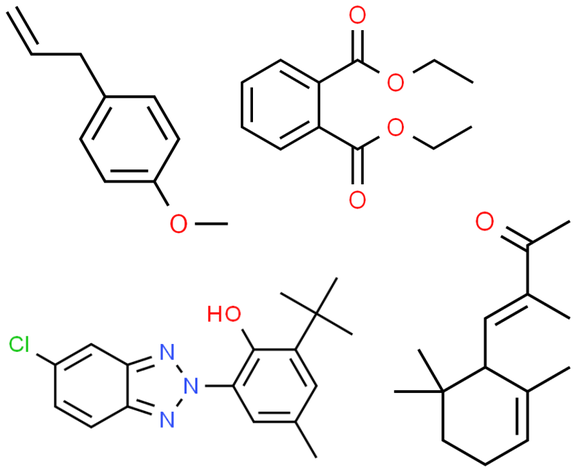
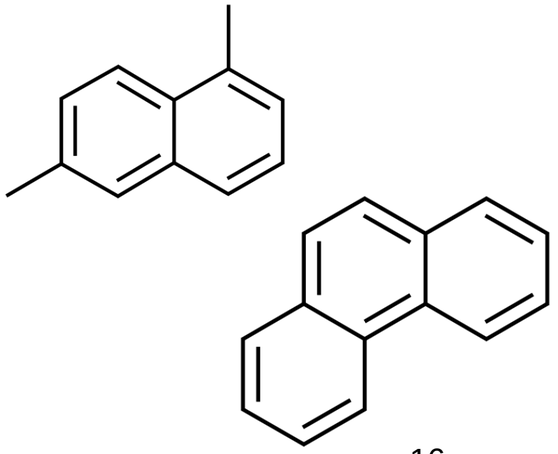


Polyaromatic compounds in PET, PP-C, and PP

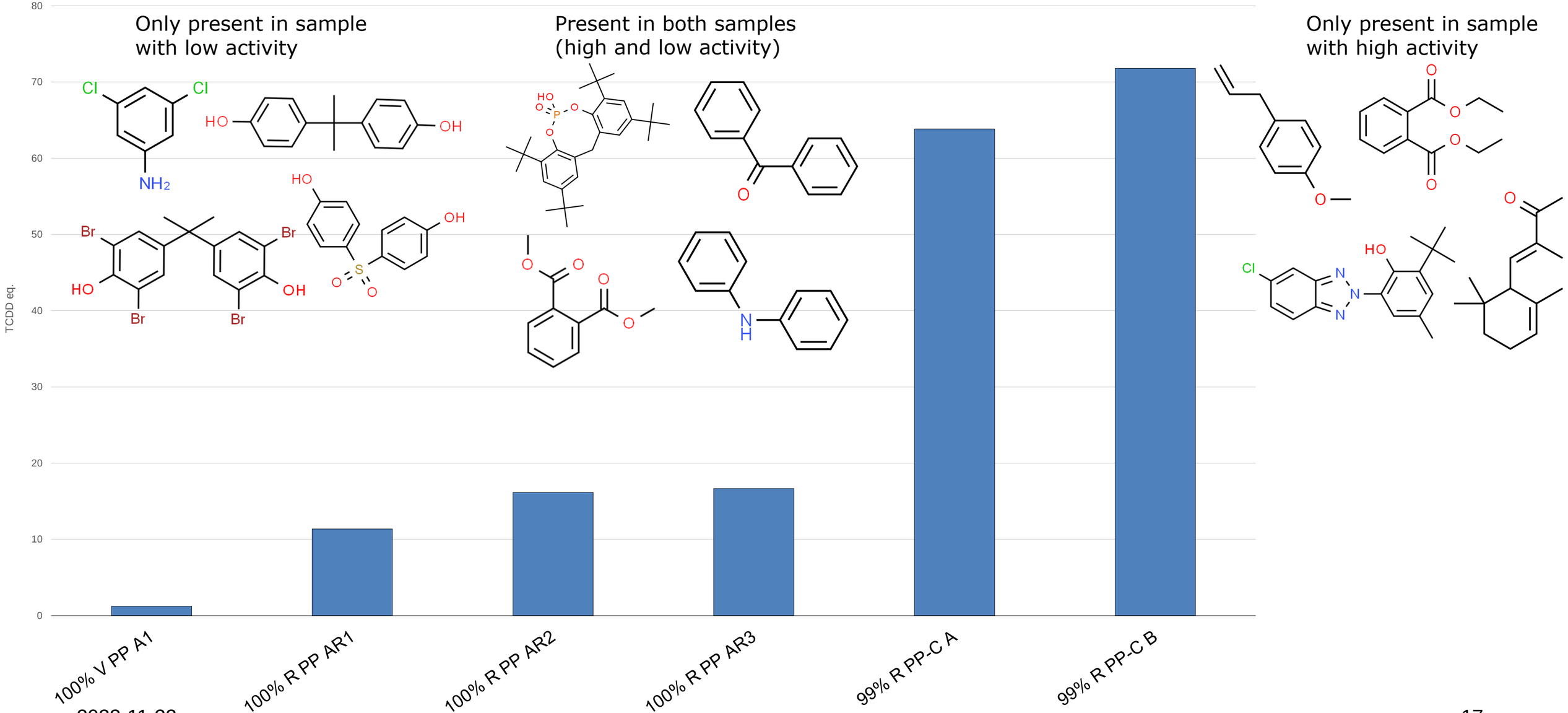


Gas chromatography high resolution mass spectrometry analysis – electron impact ionization

- 30 hits with in-house high-resolution library
- ~100 hits from NIST14 (dot and R-dot >800)
- Compare activity (dioxin receptor) results with detected compounds (PP & PP-C)
- No quantitative chemical analysis as of yet!

Only present in samples with low activity	Present in both samples (high and low activity)	Only present in samples with high activity	Only present in samples with high activity, but has reference value for the given endpoint
			

Dioxin receptor activity compared to detected chemicals PP and PP-C



Liquid chromatography high resolution mass spectrometry analysis – negative mode

QTOF raw data
1,000-10,000 features

→ **Suspect list**
~2,500 compounds

→ **Suspect list matches**
100-600 features/sample

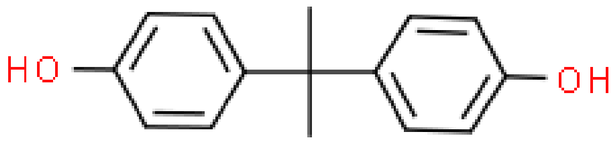
↓

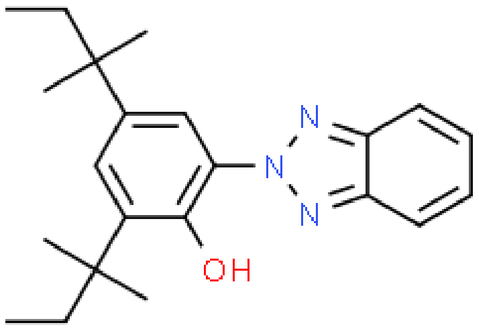
In-silico MS2 analysis
185 total tentative hits

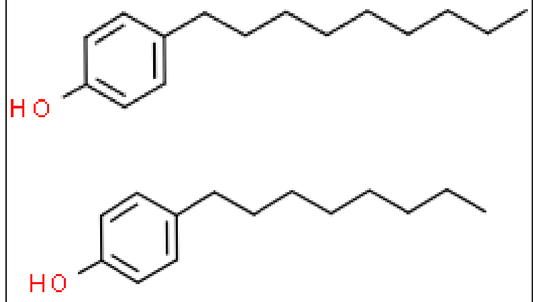
← ***Standards acquired***
~40 compounds

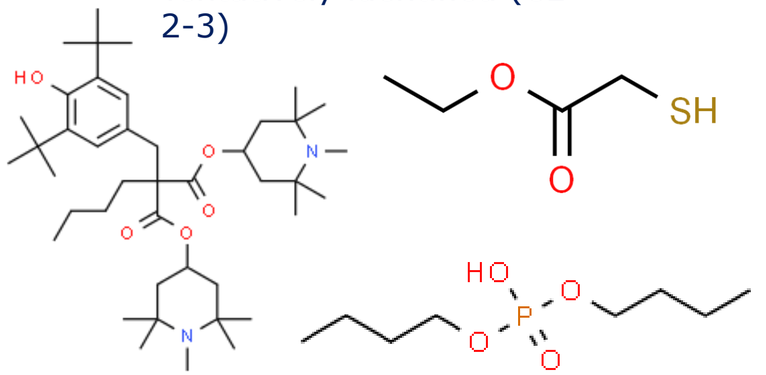
← **Confirmation**
~20 compounds

Bisphenols	Benzotriazoles	Alkylphenols	Other
3 confirmed	5 confirmed	Tentatively identified (CL 2-3)	Tentatively identified (CL 2-3)









Future work

- All results presented are preliminary and the identified compounds tentative!!
- Recycled/virgin pellets comparison
- Chemical analysis of very polar compounds – missed by current chemical analysis (~1/5 of chemicals associated with plastics)
- Fractionate and apply effect-directed analysis workflow on samples of interest (high activity, low explanation degree)
- Categorize hazardous chemicals according to polymer type and recycled/virgin