A tiered in silico/in vitro/in vivo approach for PFAS categorization and testing

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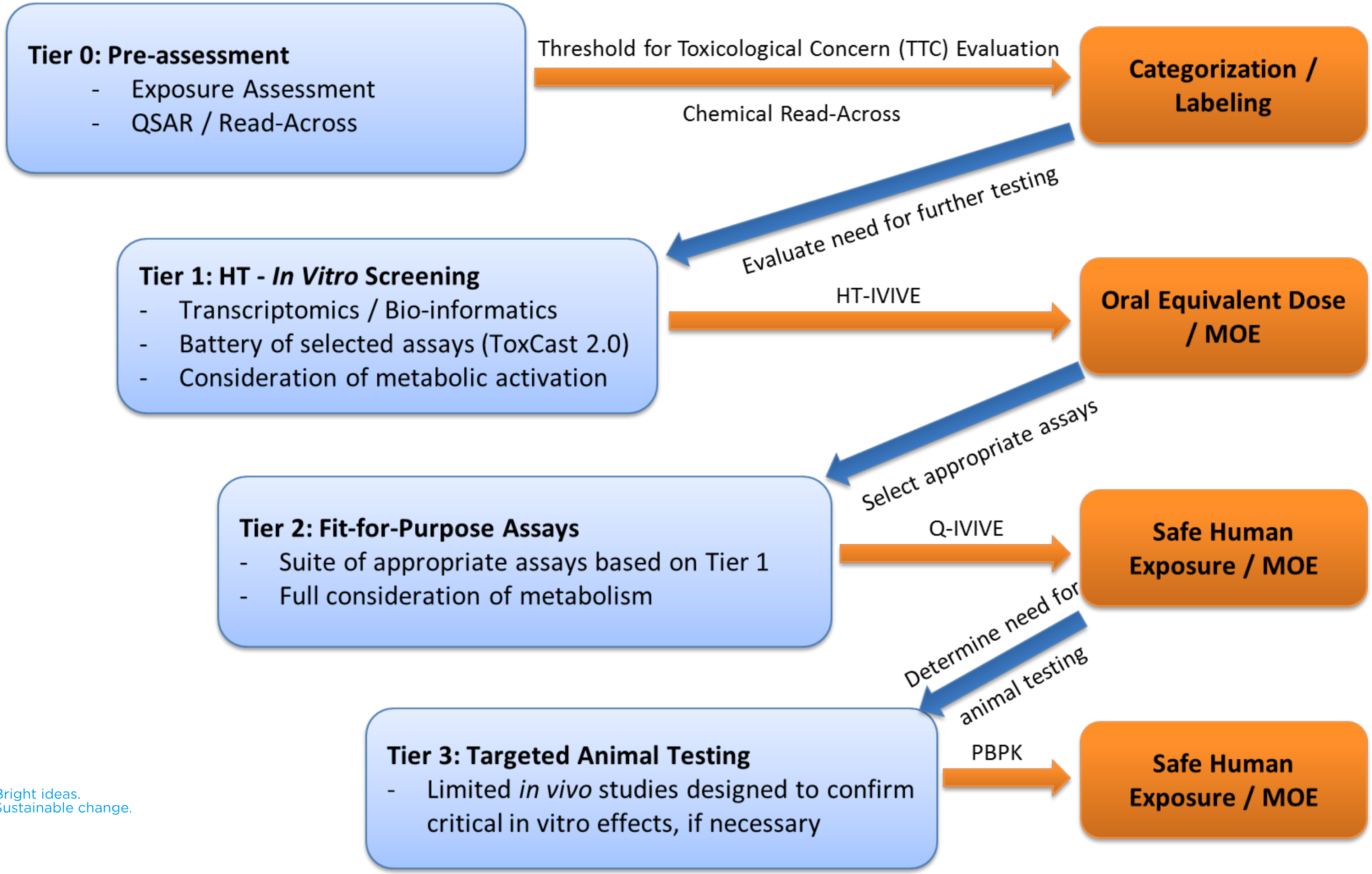
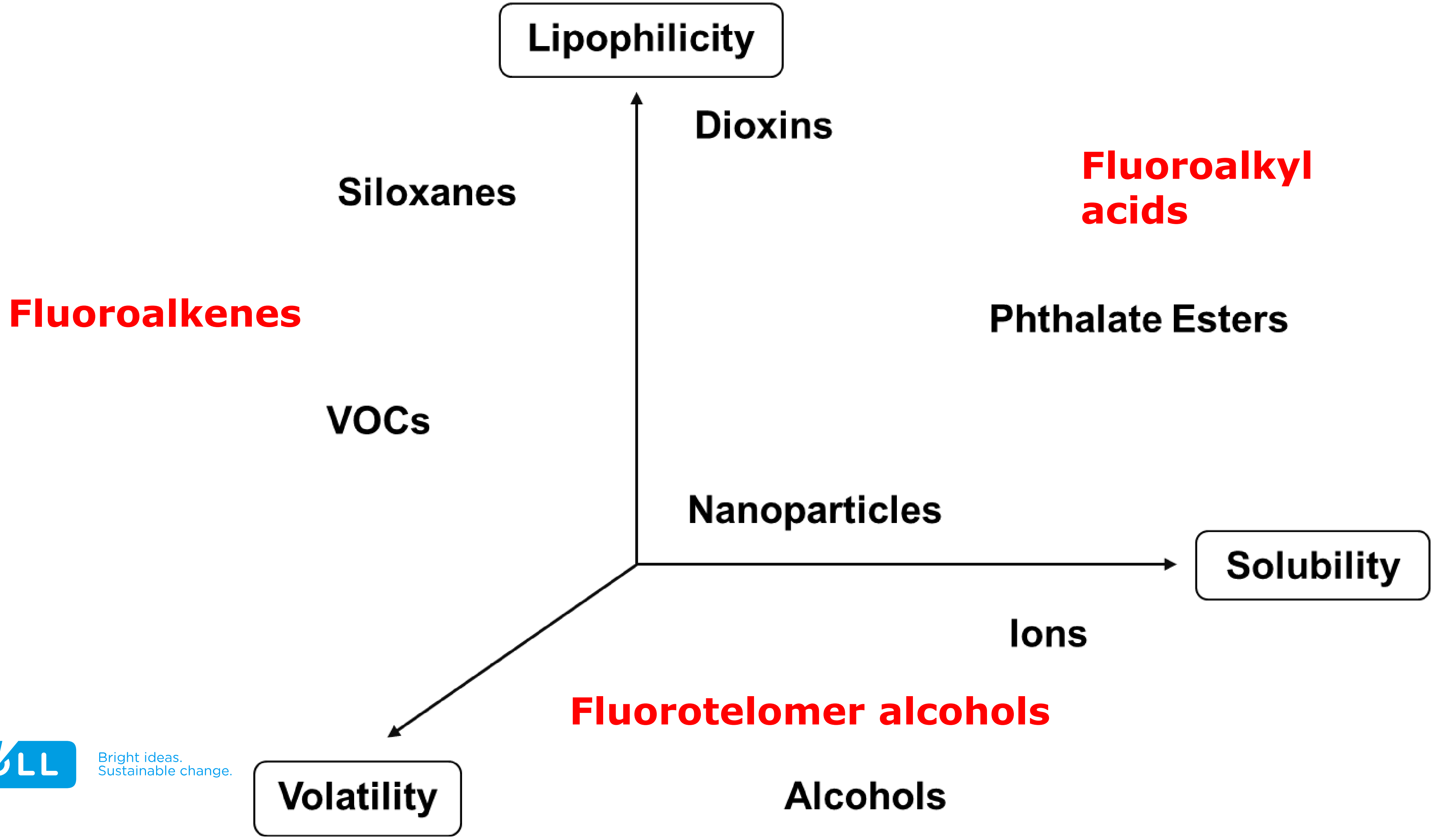
Bright ideas.
Sustainable change.

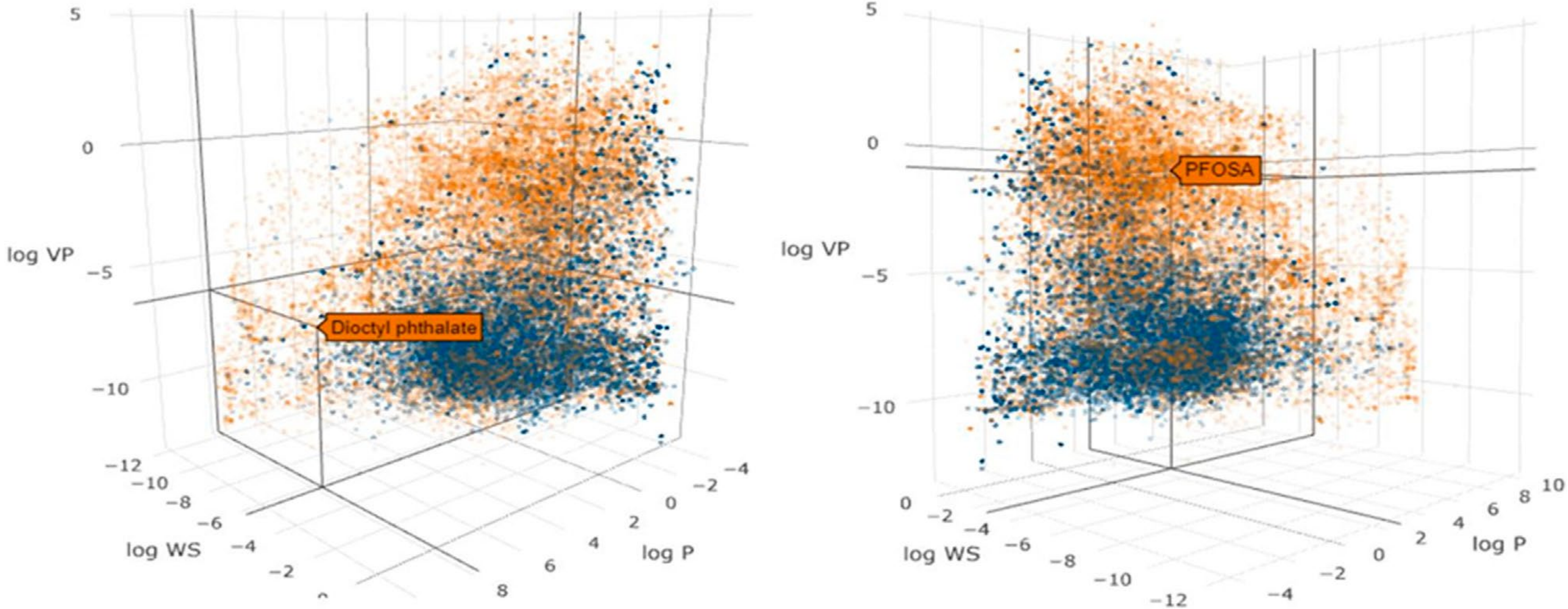
Tiered testing strategy



Tier 0:Property-Based Categorization



Example: Chemical Space Analysis



Scatter plot of the physicochemical properties of environmental chemicals (gold dots) and pharmaceuticals (blue dots) in the CERAPP database. The axes represent vapor pressure (log VP), water solubility (log WS) and lipophilicity (log P). Two different views are shown to illustrate the properties of dioctyl phthalate (left) and PFOSA (right).

(Moreau et al. 2022)

Other Tier 0 *In Silico* Analyses

- Estimation of physical properties like volatility, water solubility and lipophilicity (OPERA)
  - Recently updated to include more PFAS in training set (https://github.com/NIEHS/OPERA/releases/tag/v2.9.1)
- Estimation of PFAS half-lives
  - Exposure And Safety Estimation (EAS-E) Suite (www.eas-e-suite.com)
    - Trained on organic chemicals, some PFAS in training set
  - EPA Machine Learning Model (Dawson et al. 2023)
    - Mechanistically motivated descriptors for renal resorption
    - Based on existing in vivo measured t12 across four species (human, monkey, rat, mouse) and eleven PFAS
- Structural read-across from other PFAS

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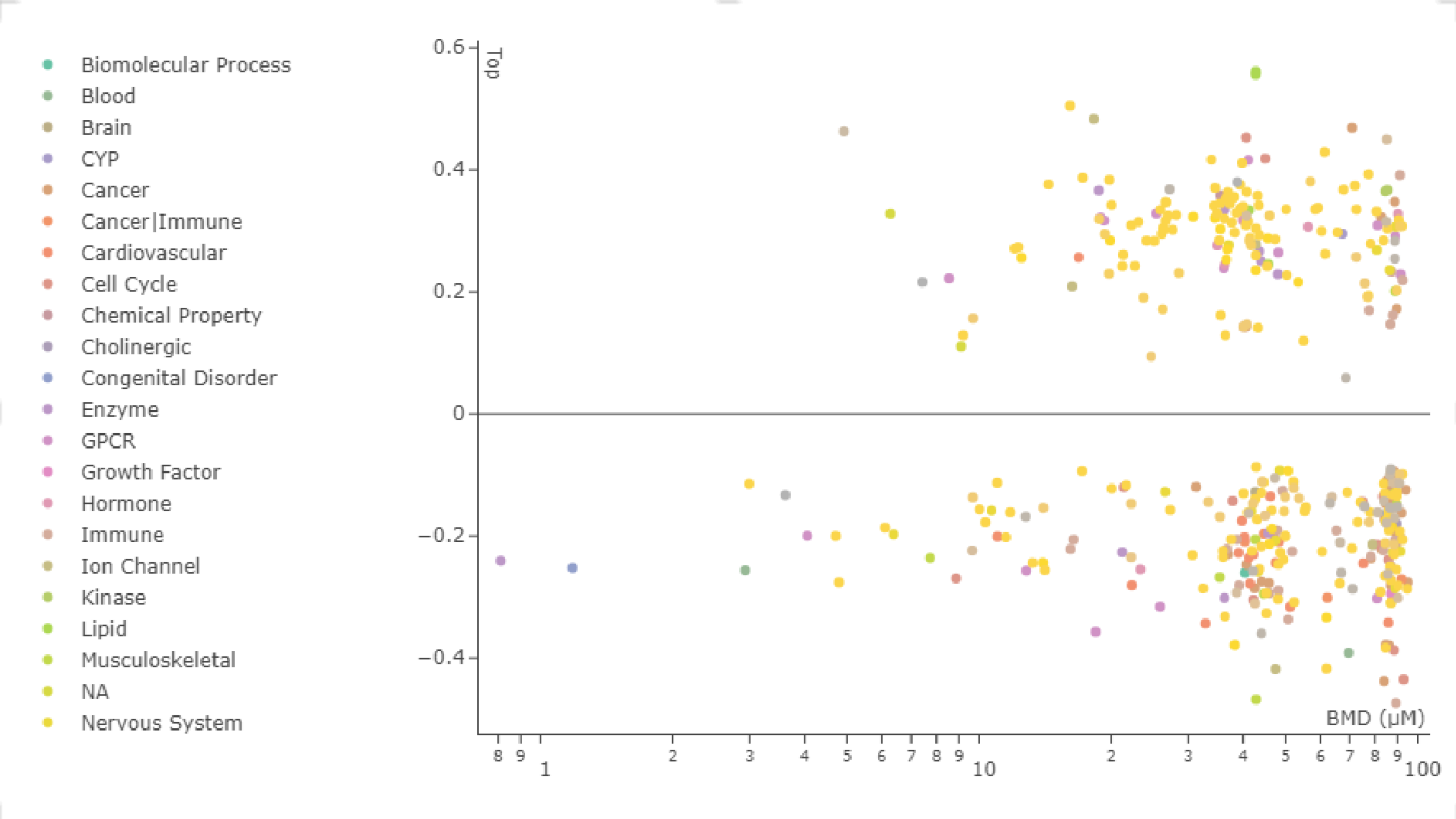
Tier 1 *In Vitro* Analyses

- ToxCast Assays
  - To support categorization through read-across from PFAS compounds with similar profiles
  - Example of PFAS with different ToxCast profiles:

	Lowest AC50	Largest Scaled Response
PFOA	CYP1A1 (metabolism) CD69 (lymphocyte activation) transthyretin (T4 displacement) PPP1CA (protein phosphatase) THBD (anticoagulant)	PPAR assays ESR1/2 assays
GenX	Serpine1 (viral immunity) CSF1 (inflammation) IL1A (inflammation)	CXCL8 (inflammation) PPAR assays

ToxCast High-Throughput Transciptomics Summary fior PFOA



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Tier 1/2 *In Vitro* Analyses

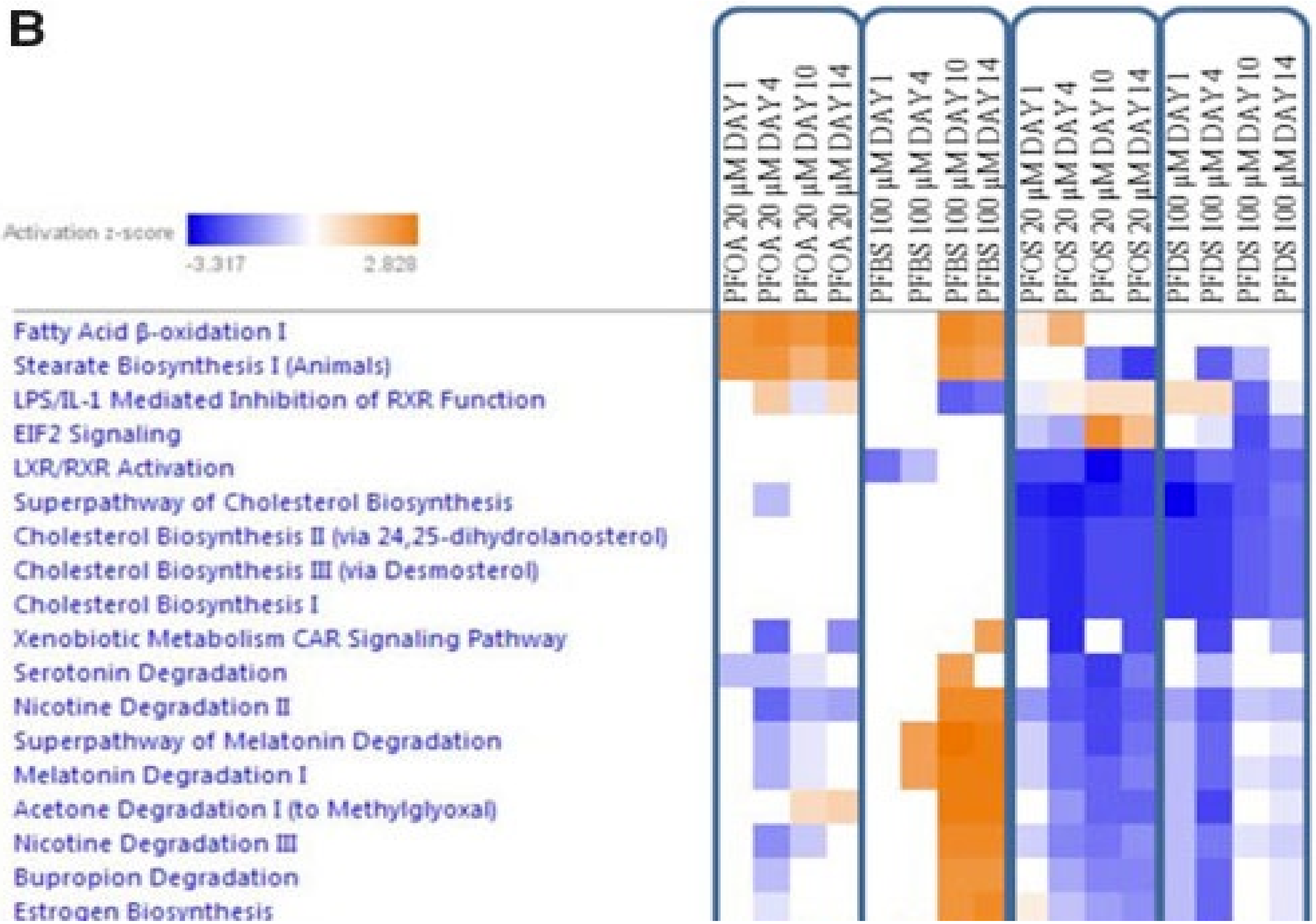
- *In vitro* transcriptomic studies
  - To support read-across from PFAS compounds known to activate key receptors for lipid transport/metabolism and steroidogenesis (e.g., PPAR-α,β,γ,δ, CAR, PXR, FXR, LXR)
  - Similar approach to previous studies (Rowan-Carroll et al. 2021).
  - *In vitro* assays for activation of key receptors (Takacs and Abbott 2007)
  - *In vitro* assays for membrane incorporation (e.g., Kasten-Jolly and Lawrence 2022)

Gene Expression changes from different PFAAs

Studies conducted with various PFAS using human primary hepatocyte spheroids.

Ingenuity pathway enrichment showed qualitative differences between <PFOA and >PFOS.

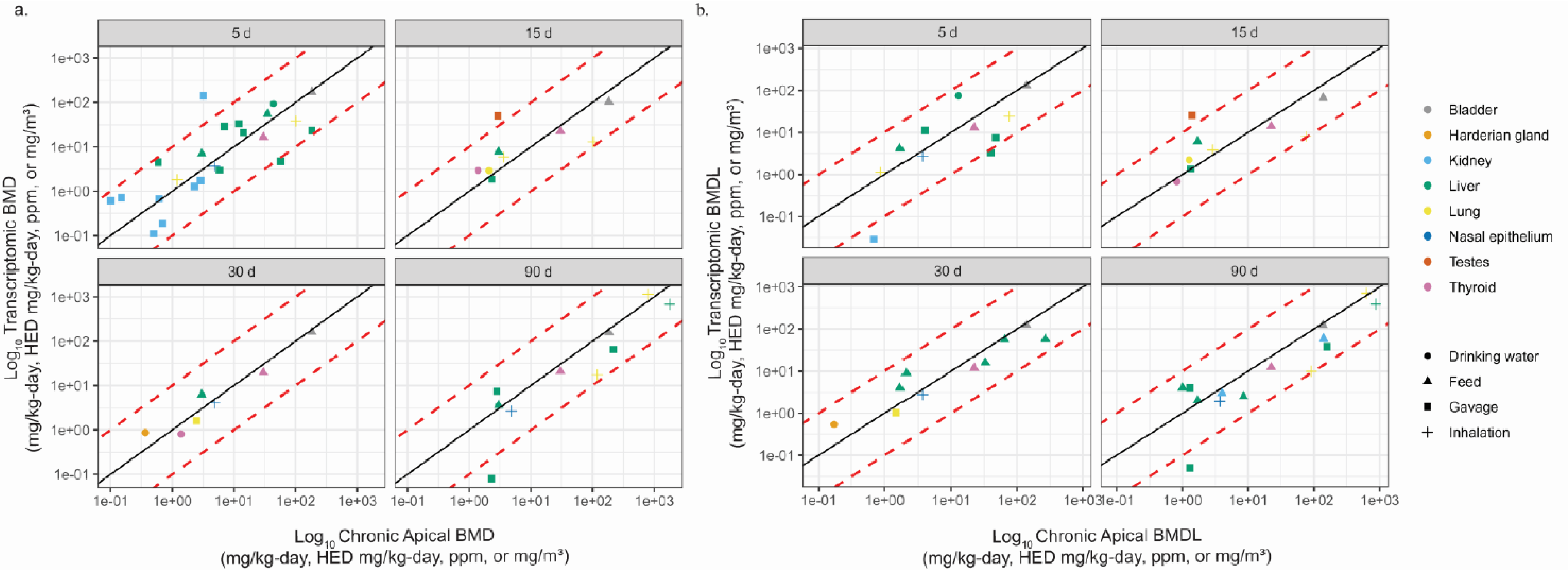
(Rowan-Carroll et al. 2021)



Tier 3 *In Vivo* Analyses

- Short-term *in vivo* transcriptomic dose-response studies
  - Example: EPA Transcriptional Assessment Product
    - 5-day rodent study
    - Transcriptomic BMDs for multiple tissues
    - predicts PODs from 2-year bioassays
    - Transcriptomic data publicly available for mode of action evaluation
  - Similar data could be derived from *in vitro* studies using organotypic tissues

EPA Transcriptional Assessment Product



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Short-Term Transcriptomic BMDs predict 2-year bioassay PODs

References

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