Exposure-Based Bioactivity Classification (ExpoClass)

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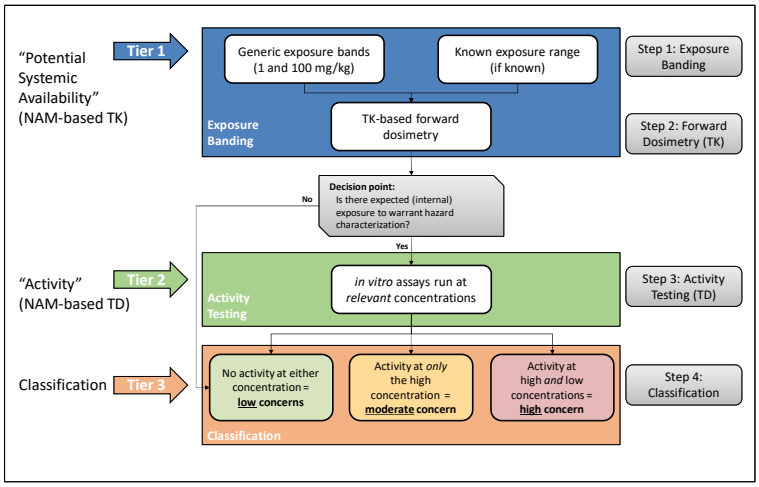
ExpoClass Framework

- A framework to utilize NAMs-based methods in chemical hazard classification
- Combines external exposure coupled with forward dosimetry to define relevant concentrations for NAMs-based assay testing
  - define generic bands of oral exposure or integrate realistic exposure bounds for toxicodynamic (TD) assays
  - ensure *in vitro* testing is appropriate for hazard identification by only testing at concentrations defined by the toxicokinetics (TK) models
  - sidesteps the need to link *in vitro* activity to adversity, as potential for non-biologically relevant activity is avoided
- *In vitro* activity is basis for chemical hazard classification
  - scientifically robust and sufficient to begin implementing as a NAMs-based hazard classification system



ExpoClass Methodological Details

Tier 1: Potential for Systemic Availability (NAM-based TK)

Step 1: Exposure Banding

- Unknown exposure:
  - Use generic (STOT-RE) classification bands of 1 and 100 mg/kg
- Partially or fully defined exposure, controlled exposure:
  - Use quantified exposure, i.e., lower and upper bound of measured environmental exposure
  - Use any existing QSAR-based exposure banding approach, such as the modelled/ predicted human exposure or a TTC exposure band based on chemical structure

Step 2: Forward Dosimetry

- Steady state acute and chronic kinetics in adult humans modelled using a generic TK model with generic physiological parameters:
  - HTTK, PKsim, Tkplate, Plethem, GastroPlus, or SimCyp
- ADME/phys-chem input parameter sources:
  - Tier 1 = measured experimentally
  - Tier 2 = QSAR (OPERA, VEGA, Gastroplus ADMET predictor)
  - Tier 3 = conduct additional studies to measure

Tier 2: Activity Tier (NAM-based TD)

Step 3: Activity Testing

- Determine the TD activity at the defined relevant exposure concentration
- Battery of *in vitro* assays with broad biological coverage
  - Assays will need to be explicitly defined using case studies
  - i.e., targeting transcriptomics, metabolomics, proteomics
- Characterize uncertainty/variability
  - Utilize modeling approaches that provide confidence intervals
  - Apply *in vitro* distribution models to calculate free *in vitro* concentrations vs nominal

Tier 3: Classification

Step 4: Classification

- Use biological activity (Step 3) at the internal dosimetry (Step 2) for final hazard classification
- Incorporate uncertainty – likely qualitative
  - No response at either concentration = low concern
  - Response at high but not at low concentration = medium concern
  - Response at low concentration = high concern

Discussion and Conclusions

Framework Benefits

- Incorporates realistic exposure into hazard classification ranking
  - provides upper and lower bounds on hazard based on human relevant exposure for *in vitro* testing assays
- TK and TD metrics are incorporated without an explicit integration step
  - integrates TK and TD into a single decision point
  - functionally simplifies the final classification criteria into a "yes" or "no" for activity at each tested concentration
- Decision point – defined by user as risk or hazard-based outcome
  - if concentration cannot be tested/achieved *in vitro*, or if below existing thresholds (i.e., iTTC), then the concern is low
- Flexibility to be tailored for fit-for-purpose, from screening level, prioritization, or definitive assessment by refinement of exposure band
  - screening level: outcomes other than a "low concern" could be used to trigger additional and more targeted testing
  - prioritization level: compounds with highest modeled exposure
- All testable chemicals are classifiable

Uncertainty and Domains of Applicability

- Generic concept does not incorporate an expression of variability. Variability or uncertainty can be incorporated in each Step
  - Step 1: confidence intervals or upper and lower measured or predicted exposure bounds
  - Step 2: QSAR models to predict ADME/phys-chem properties have confidence matrices to indicate high and low quality
  - Upper bound (e.g., 99% CI) internal concentration from highest predicted tissue/blood compartment as test concentration increases conservatism
  - Step 3: selection of *in vitro* model suite includes those with rigorous validation in terms of variability, reproducibility, and uncertainty
- Next steps - run case study chemicals through conceptual framework
  - selection of appropriate tools for Step 2 (TK model) and Step 3 (assays cover the intended biological activity, TD)
  - evaluate framework by comparison to existing hazard classification outcomes, using known biology/toxicity

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