P721 Estimation of No-Observed-Adverse-Effect Level by Read Across for Large Inventories of Structurally-Related Chemicals and Metabolites

James Rathman, Chihae Yang, Tomasz Magdziarz, João Vinícius Ribeiro, Aleksandra Mostrag, Bryan Hobocienski

[1]MN-AM, Nürnberg, Germany & Columbus OH USA; 2 Chemical & Biomolecular Eng, Ohio State University, Columbus, OH USA

1 MOTIVATION FOR HIGHTHROUGHPUT READ-ACROSS

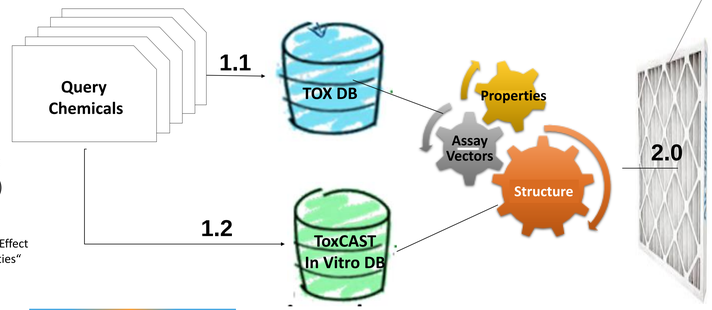
- Current read-across evaluation is mostly applied to a single compound manually, which can become unmanageable for large inventories.
- A high throughput read-across approach can be useful for a structurally related class of compound inventories, e.g., pesticide and its metabolites or compound classes of phthalates, parabens, etc..
- The published method for NOAEL bounds estimation was extended to apply via algorithm-driven process.
- A concept of assay vectors were developed to be include in the analogue quality. Examples can be Tox21/CAST HTS or AOP assays.

2 READ-ACROSS AND NOAEL BOUNDS ESTIMATION

- Read-across steps require the steps:
  - Chemical / biological similarity
  - Qualified study data for analogues
  - Endpoint-relevant information
  - Method to combine the evidence for an outcome (Calls or NOAELS, etc.) and uncertainty.
- NOAEL Bounds Estimation*
  - Chemoinformatics analysis of chemical space and NOAEL relationships
  - Design to address the needs in High Throughput Read-Across (HT-RAX) Approach

*Yang et al. *Chem. Res. Toxicol. 2021, 34, 616-633.* "Do Similar Structures Have Similar No Observed Adverse Effect Level (NOAEL) Values? Exploring Chemoinformatics Approaches for Estimating NOAEL Bounds and Uncertainties"

3 HIGH THROUGHPUT READ-ACROSS PROCESS



***SQ** : Study Quality Standardized 0 – 1.*

Algorithmic Workflow for High Throughput RAX

4 USE CASE-1: HT-RAX FOR A DIPHTHALATE GROUP

- Diphthalates are of interests in diverse industries including food, cosmetics, consumer products as well as pharmaceuticals.
  - Peroxisome proliferation (target organ of liver)
  - Endocrine disruption potential (reproductive/developmental findings)
- Over 100 phthalate containing substances were compiled from various data sources (ECHA, EFSA, SCCS, US EPA).
- Forty structures were found in ToxCAST invitro assay database
  - ER, AR, PR, PPAR assays results were vectorized to be used as descriptors

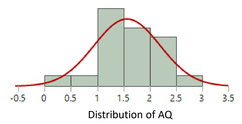
5 DIPHTHALATES – EXAMPLE OF DATA (IN STEP-2)

Structure Example	ToxPrint	Properties Skyline	Tox21 Skyline	AQ	SQ	NOAEL
CMS-6612	1.0	1.0 [Atoms, Bonds……ΔH_f][3]	1.0 [AR, ER, AhR, PR, PPAR][4]	1.0	NA	NO DATA
CMS-1777 (DBP)	0.875	0.970	0.906	0.916	0.85 (MED HIGH)	LOAEL= 1.5 - 3 mg/kg-bw/day (NOAEL=1mkd); Rat GD15- PND21 [EFSA, ECHA]
CMS-212 (BBP)	0.636	0.854	0.832	0.767	0.95 (HIGH)	NOAEL=50 mg/kg-bw/day; Rat 2-GEN [EFSA, SCCS, CHAP]
CMS-620 (DEHP)	0.636	0.819	0.893	0.775	0.9 (HIGH)	NOAEL=5 mg/kg-bw/day; Rat Multigen [EFSA, CHAP]
CMS-2404 (DINP)	0.636	0.818	0.862	0.765	0.9 (HIGH)	NOAEL=15 5 mg/kg-bw/day; Rat [EFSA]
CMS-4162 (DIDP)	0.636	0.803	0.862	0.761	0.95 (HIGH)	NOAEL=15 mg/kg-bw/day; Dog [EFSA, SCCS]

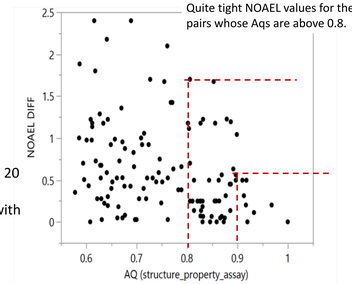
6 WORKFLOW STEP-2

- SAR Node to focus clusters in the HT-RAX Process
  - Structure Activity Relationship (SAR) filtering node can be included, e.g., compound category (scaffolds) or aliphatic chain size in this case.
  - Aliphatic chain, e.g.,
    - Length <3 (methyl, ethyl)
    - Length between 3-8: Repro/Dev effects
    - Length >8 : Liver effects

CHEMICAL SIMILARITY & NOAEL PROXIMITY



- Of the 99 phthalates list, 40 had ToxCast assay data and 20 had NOAEL data (EFSA, SCCS, ECHA, CHAP reports).
- NOAEL DIFF is calculated for each structure pair along with the AQ considering structure, properties, and ToxCAST assays.
- Over 450 pairs are plotted for AQ and NOAEL DIFF.



$$NOAEL_{Diff} = \left|\log_{10}(NOAEL_2) - \log_{10}(NOAEL_1)\right| = \left|\log_{10}\left(\frac{NOAEL_2}{NOAEL_1}\right)\right|$$

7 DECISIONS IN STEP-3

- For structures having sufficiently large (n>3) number of nearest neighbors with data, the algorithm applies the Ensemble Nearest Neighbor method.
  - The results are weighted using the study quality (SQ).
- For structures having insufficient number of nearest neighbors with data, the NOAEL is estimated using the NOAEL DIFF distribution.
  - Requires an analogue and its NOAEL value within known AQ bin.
  - The results are weighted using SQ.
- For structure with no analogs within the acceptable AQ, the system returns warning of "estimation not advisable".

8 Example of Results

- Using the method, we applied to the rest of the structures with no tox data.

Target Structures (examples)	Structure Group	Methods	Number of analogs or pairs	AQ BIN	NOAEL Bound 95%
CMS-6612 (DPP)	R =3	NOAEL DIFF	24 pairs	0.73 – 0.83	8.1 – 148 mg/kg-bw/day
CMS-1921 (DOP)	3 =< R <= 8	Ensemble NN	9 analogs	0.83 – 0.93	14 – 32 mg/kg-bw/day
CMS-2406 (DIBP)	3 =< R <= 8	Ensemble NN	10 analogs	0.62 - 0.72	16 – 60 mg/kg-bw/day
CMS-4239 (DUDP)	R >9	NOAEL DIFF	35 pairs	0.7 – 0.8	6.4 – 124 mg/kg-bw/day
CMS-395 (DAP)	R =3	Ensemble NN	9 analogs	0.60 - 0.75	10 – 194 mg/kg-bw/day

3. Properties Skyline (from Left to Right): Atoms, Rot Bonds, HBA, HBD, Lipinskiy rule violation, MW, Complexitiy, XlogP, Asphericity, Eccentricity, Diameter, Radius gyration, Span, Heats of Formation. 4. EDSP Assays: AR activities (6), ER/ERa/ERb activities (14), Aromatic hydrocarbon receptor (3), Progesterone(5), and PPAR α, δ, γ-activities (7)

9 USE CASE-2 HT-RAX FOR METABOLITES[1]

- Multiple metabolites for an active substance,
  - Dimethenamid-P/ Dimethenamid and 30 metabolites
    - Chloroacetamide herbicide
    - 10 metabolites needed RDT and genetic toxicity data
- RAX Results
  - Data gap filled for RDT and genetox[2]
  - SQ: assumed equal for all metabolites

Analogue Structures (examples)	Role	NOAEL (mg/kg-bw/day)
CMS-7136 Dimethenamid-P	Parent	5
M23	M	36
M31	M	100
M45	M	117
M62	M	10

Target Structures (examples)	# Analogues in analysis	95% CI NOAEL (mg/kg-bw/day)
M25	6	27.2 – 140.2
M30	7	13.8 – 163.1
M32	9	9.0 – 102.9
M53	7	12.2 – 152.7
M59	8	9.9 – 123.7

1 RDT and genetic toxicity data were taken from EFSA Journal 2018;16(4):5211; Ecological Risk Assessment of Dimethanamide. US EPA Environmental Fate and Effects Division. 2006. 2 Manuscripts are being prepared to described methodologies in detail. The genetic toxicity approach requires a separate database .

ACKNOWLEDGEMENT: Ann M Richard (US EPA) for discussions on using ToxCAST assay data.

Contact: james.rathman@mn-am.com