Probabilistic Analysis & Quantitative Read-Across Assessment of 2-MCPD

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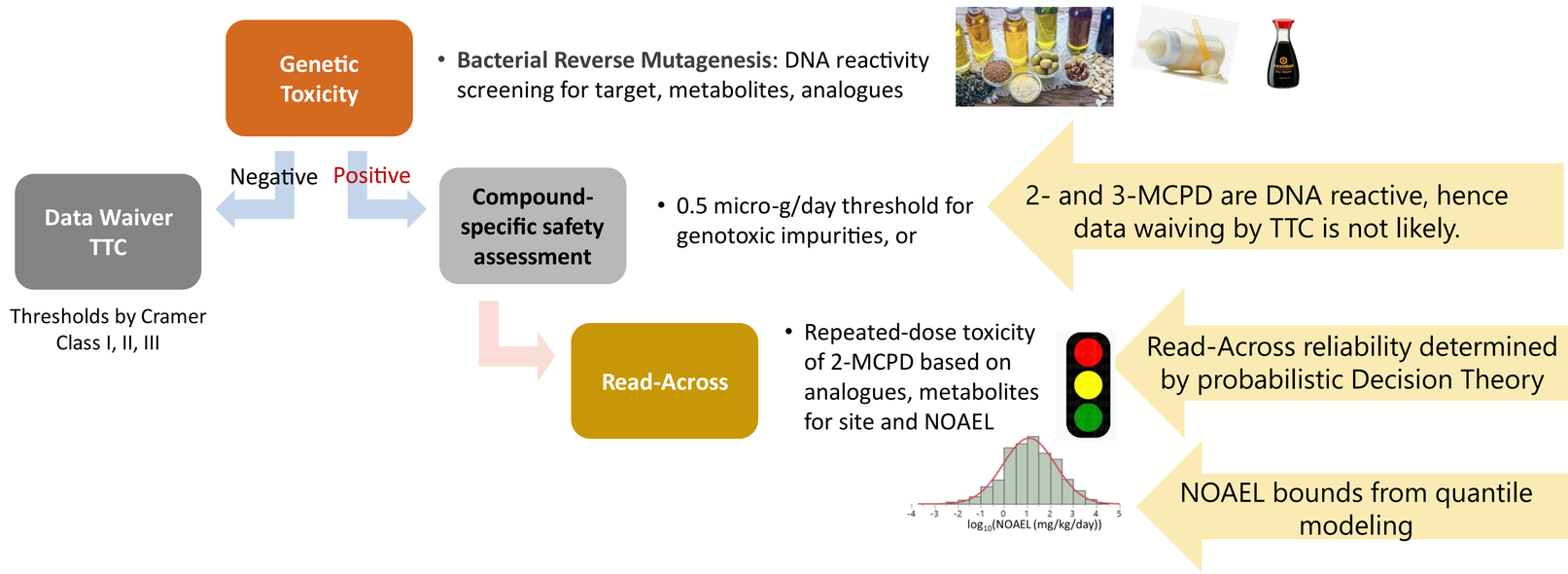
MOTIVATION

Use Case of A Knowledge Hub

- A use case is applied to thermal food degradants from foodstuff oils and fats: 2- and 3-monochloro-propanediol (MCPD)
- The Tier 0 assessment workflow for NGRA implemented in ChemTunes•ToxGPS® was employed for read-across feasibility and quantitative estimation of NOAEL bounds
- Components of the commercial ChemTunes•ToxGPS® platform can be accessed via the ONTOX Hub
- In silico models were provided by ONTOX partners and collaborators (*e.g.*, ProtoQSAR, VEGA, OPERA)

Tier 0 Approach of Next Generation Risk Assessment (NGRA)

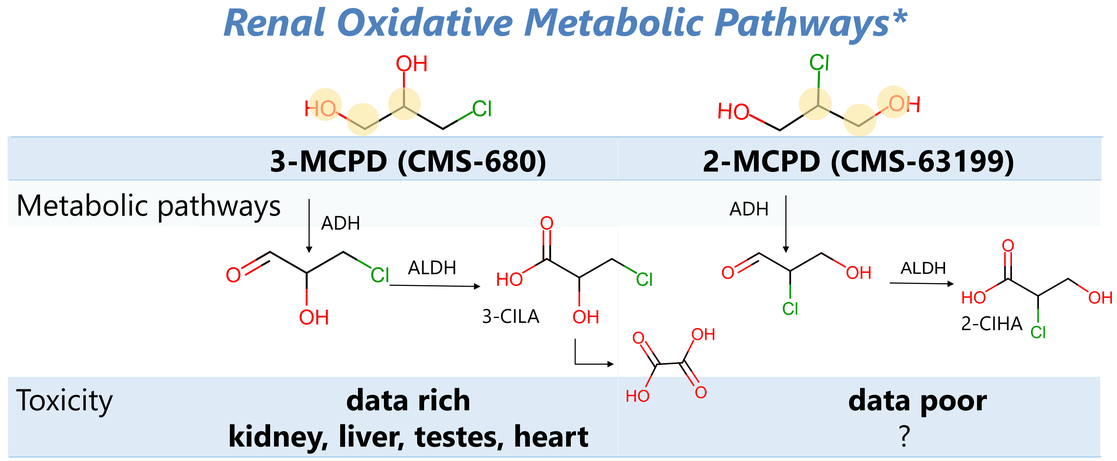


METHODOLOGIES USED IN THIS ANALYSIS

Compile Relevant Information

- **Metabolic reactivity**
  - Primary alcohol oxidation
  - Dehalogenation
- **Human metabolism**
  - Renal oxidative pathways
    - Alcohol -> aldehyde -> acid
  - 3-MCPD capable of forming oxalic acid, hence kidney effects
- **ADME data**
  - **Bioavailability**
    - FUB, HIA, Caco-2 cell permeability, Skin permeability, BBB,
    - **Hepatic and Renal Clearance**
    - **Transporters** – OCT2 and OCT1 (ProtoQSAR)

*Renal Oxidative Metabolic Pathways**



*Bergau N, Zhao Z, Abraham K, Monien BH. *Mol. Nutr. Food Res.* **2021**, *65*, 2000736.
*Lynch BS *et al. Int. J. Toxicology* **1998**, *17*, 47-76.
*EFSA Journal DOI: 10.2903/j.efsa.2016.4426.
‡ Burhke *et al. Food and Chemical Toxicology* **2017**, *106*, 6-46.
† Schutrich *et al. Arch. Toxicol.* **2017**, *91*, 3145-3155.

Compile Relevant Information

- **Toxicity study data**
  - 3-MCPD is a known genotoxic carcinogen (IARC Group 2B)
  - RDT findings of kidney, liver, testes, and heart
- **Transcriptomics studies**
  - Microarray analysis of transcriptomics responses from 28-day rat study at 10 mg/kg/day
    - Liver, kidney, testes‡
    - Heart†
  - Different molecular mechanisms between 2- and 3-MCPD are expected

RAX RESULTS

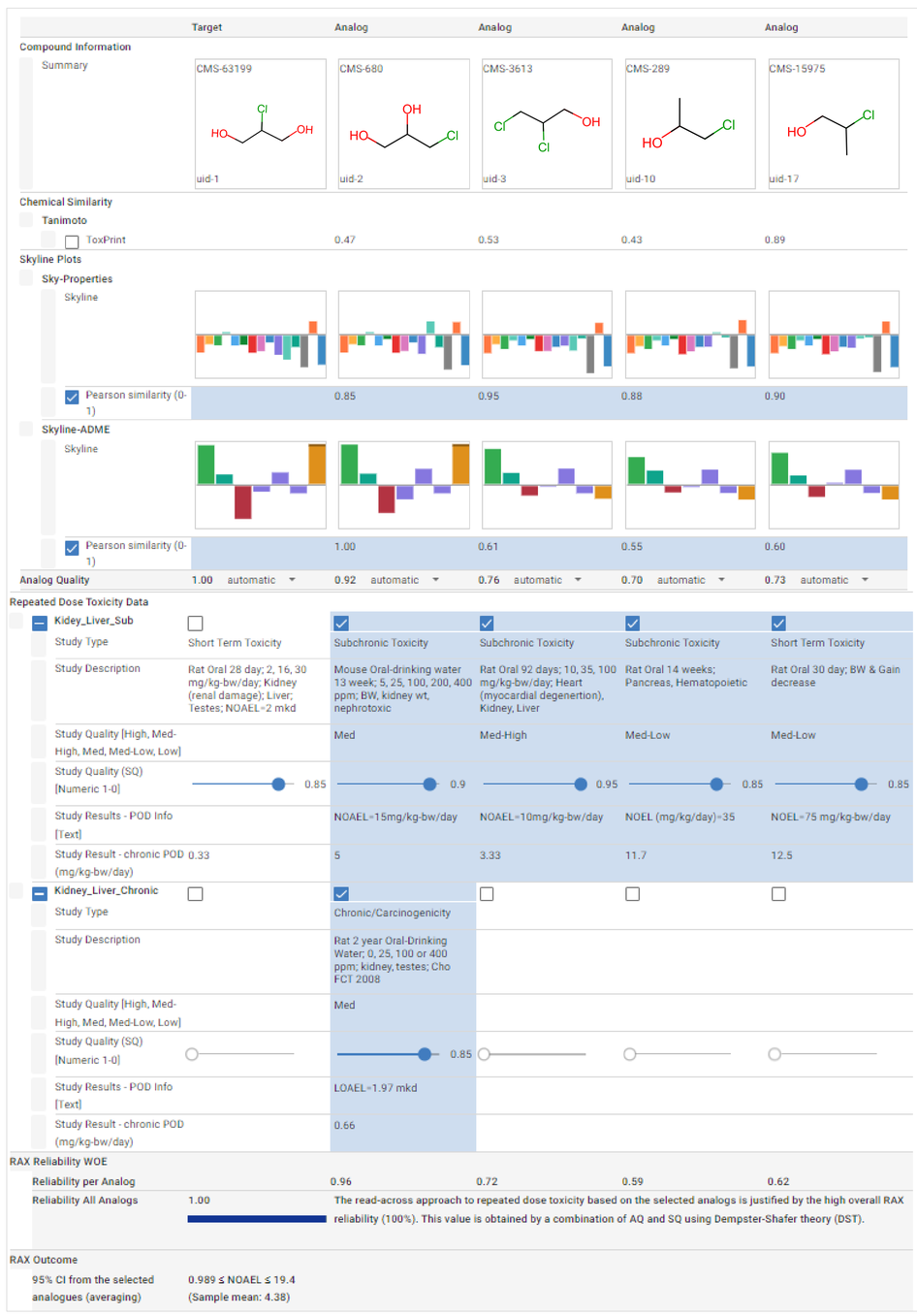
Evidence Profile

- Structural Similarity
  - ToxPrint fingerprints were used to calculate structural similarity
  - Due to small size, Tanimoto coefficients are low (0.47) between 2- and 3-MCPD
  - Not used in this analysis
- Molecular Property Similarity
  - Pearson similarities are compared*
  - Properties are quite similar between the two (0.85)

  Atoms; Rot Bond; HBA; HBD; Rule5; Stereo; MW; Complex; TPSA ; LogP; Aspheric; HeatF; HOMO; LUMO; H-L Gap
- ADME Similarity
  - Pearson similarities are compared*
  - Properties are very similar between the two (1.0)

  FUB; Caco-2 (logPap); LogKp; LogBB; HIA hepClint; RenClint
- Analogue Quality (AQ)*
  - Geometric mean of the similarity measures used
- Study Quality (SQ)*
  - Similar to Klimisch score for study reliability – Klimisch 1 (High), Klimisch 2 (Med-High, Med), Klimisch 3 or 4 (Med-Low, Low)
- RAX Reliability (RR)*
  - Probabilistic measure for the reliability of the read-across process
  - RR= AQ x SQ

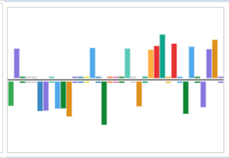
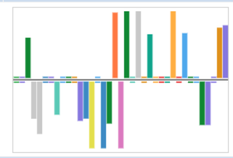
Read-Across (RAX) Assessment Table & NOAEL Bounds



- NOAEL Bounds Estimation (Liver, Kidney, Testes)
  - Based on five RDT studies from the four analogues, 2-MCPD was estimated to have a chronic NOAEL value of 1.0 - 19 mg/kg-bw/day at 95 CI

*Yang *et al. Computational Toxicology* **2023**, *26*, 100272.
*Yang *et al. Chem. Res. Toxicol.* **2021**, *34*, 616−633.

Tgm2; Cyp24a1; Rnf125; Gstp1; Akr1b10; Ugt2b4; Car15; Pla2g7; Cry1; Gabrb3; Mettl7a; Pcp4; Coch; Angptl7; Banp; Rcan1; LOC102554816; LOC691546; Snora15; LOC102556346; Nr1d2; Nr1d1; Slc7a7; Rab38; Gck; Pfkfb1; Slpi; Snord22; LOC102551949; Rn5s; Snord33; Meox2; Prf1; Nms; Znhit2; LOC102548247; Xk;

	2-MCPD	3-MCPD
Pearson SIM Properties		0.85
Pearson SIM ADME		1.00
Pearson SIM Genes		
Analogue Quality		0.79

RAX Outcome
95% CI from the selected analogues (averaging): 0.0308 ≤ NOAEL ≤ 34.4 (Sample mean: 1.03)

- Differentiation by Transcriptomics
  - Gene expression levels from the microarray data further assisted in differentiating 2-MCPD and 3-MCPD
  - Due to the differentiating profile from gene expressions in liver and kidneys, Analogue Quality (AQ) is lowered (from 0.92 to 0.79)
  - Chronic NOAEL for liver and kidney was estimated to be 0.04 - 34 mg/kg-bw/day at 95 CI
  - Chronic NOAEL for heart (Myocarditis) was estimated to be 0.84 - 86 mg/kg-bw/day at 95% CI (analysis not shown)

CONCLUSION

- Understanding human metabolic pathways along with ADME data was important in evidence compilation.
- ADME properties of many analogues are not available, hence access to reliable ADME models as well as transporter models was important.
- Probabilistic decision theories were used to combine various pieces of evidence to reach a decision with associated estimation of reliability of the read-across analysis.
- POD values (*e.g.*, chronic NOAEL) were estimated from studies whose associated study qualities were evaluated within this study.

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