NAMASTE: NAM-based Assessment of Systemic Toxicity Effects
EPAA Designathon on NAM-based solutions

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BACKGROUND

The NAM DESIGNATHON is a research exercise aiming to explore a future hazard classification system for chemicals. The current goal is to assign a level of concern (high, medium and low) for systemic toxicity to each chemical based on "New Approach Methodology" (NAM) data related to their toxicodynamic and toxicokinetic properties. Here, we propose a simple, reproducible and transparent workflow using currently available open-access data and tools to classify the reference set of chemicals. The approach is a preliminary "screening-level" assessment which is, at the moment, neither sufficient to perform hazard classification of chemicals nor for any regulatory decision-making. However, the workflow can be used as a starting point for working towards a NAM-based solution in a future "Next Generation Risk Assessment" (NGRA) framework.

DATA & METHODS

Tool/Model	Description	Estimated Value
Integrated Chemical Environment (ICE) Tool (https://ice.ntp.niehs.nih.gov/)	NTP's ICE is a user-friendly platform for accessing curated ToxCast/Tox21 ($\mathbf{AC_{50}}$) NAM data and computational tools. The Pharmacokinetics (PK) tool predicts tissue-level concentrations resulting from *in vivo* doses ($\mathbf{C_{ss}}$).	**Hazard/potency estimate (TD)** The fifth percentile ($\mathbf{P_{05}}$) based on the distribution of all available $\mathbf{AC_{50}}$ values for assay endpoints relevant to systemic (repeated dose) toxicity. **Systemic bioavailability estimate (TK)** Modelled steady-state plasma concentrations ($\mathbf{C_{ss}}$).
OECD QSAR Toolbox (https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm)	The OECD toolbox incorporates information and tools from various sources into a logical workflow. **Oral absorption** profiler predicts oral absorption (high, medium and low).	**Systemic bioavailability estimate (TK)** Adjustment of $\mathbf{C_{ss}}$ (100 %, 50 % or 10 %) based on estimated **oral absorption** (high, medium or low).
Generalised Read-across Framework (GenRA) (https://www.epa.gov/comptox-tools/generalized-read-across-genra)	The generalised read-across framework (GenRA) as an algorithmic approach to read-across to predict analogues for chemicals (target) lacking *in vitro* data.	**Hazard/potency estimate (TD)** *In vitro* data from five analogues were used to get a read-across estimate of a **PoD** for the target chemicals lacking *in vitro* data (similarity weighted average).
QSAR *In Vivo* Points of Departure (PoD) Model (https://doi.org/10.1016/j.comtox.2020.100139)	Quantitative structure-activity relationship (QSAR) model to predict quantitative **points of departure** (PoD) for *in vivo* repeated dose toxicity.	**Hazard/potency estimate (TD)** Prediction for ***in vivo* PoDs** expressed as external oral dose values in mg/kg/day.

Table 1. A brief description of the tools and models employed in workflow and the relevant predictions obtained from each of them

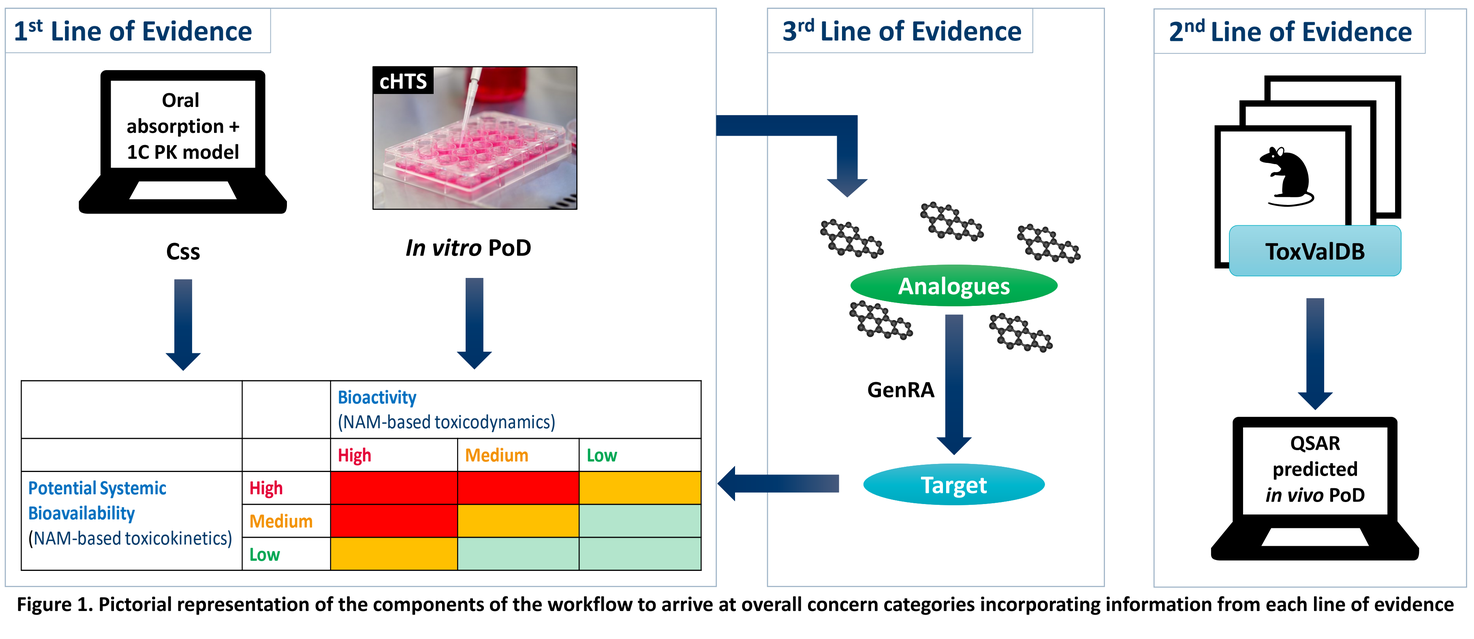

Figure 1. Pictorial representation of the components of the workflow to arrive at overall concern categories incorporating information from each line of evidence

Confidence Level	Criteria
1 (High)	If the concern categories based on 1st and 2nd line of evidence agree
If the concern categories between 1st and 2nd line of evidence disagree, or if data from both lines of evidence was not available, the level of confidence was based on the number of available *in vitro* bioassay results and, therefore, on the size of toxicity space covered	
2 (Medium)	1st line of evidence is based on the results of more than 10 *in vitro* bioassays.
3 (Low)	1st line of evidence is based on the results of less than 10 *in vitro* bioassays

Table 2. Criteria for the assignment of a confidence level in overall concern categories for each substance in the reference set of chemicals

RESULTS

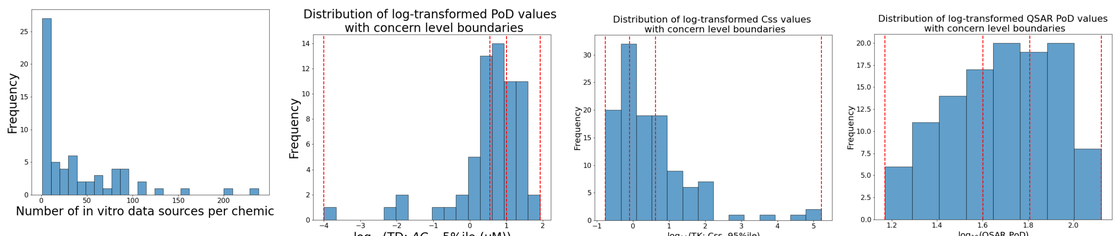

Figure 2. Frequency distribution of data availability (number of *in vitro* assay data sources) for all reference chemicals

Figure 3. Assuming an equal number of reference chemicals in each concern category the distribution of the calculated hazard values was used to set the range for potency (cut-off values: 33rd percentile, 66th percentile). (a) *In vitro* $\mathbf{AC_{50}}$ values, (b) $\mathbf{C_{ss}}$ values, and (c) *in vivo* (QSAR) PoD values

SUMMARY

Assessments / Categorisation	Count (Percentage)
Total No. of chemicals assessed	119/150 (79 %)
No. of chemicals assessed in the 1st line of evidence (*in vitro* bioactivity and PK)	60/150 (40 %)
No. of chemicals assessed in the 2nd line of evidence (*in silico* modelling)	115/150 (77 %)
No. of chemicals assessed in the 3rd line of evidence, example assessment (GenRA and PK)	1/150 (1 %)
No. of chemicals assessed in both, 1st and 2nd lines of evidence	57/150 (38 %)
No. of chemicals assessed for TK	117/150 (78 %)
No. of chemicals categorised as high concern	44/119 (37 %)
No. of chemicals categorised as medium concern	41/119 (34 %)
No. of chemicals categorised as low concern	34/119 (29 %)
Concern categories agree between the 1st and the 2nd line of evidence	Yes: 21/60 (35 %) No: 39/60 (65 %)
Concern category matches with current C&L classification	Yes: 40/117 (34 %) No: 77/117 (66 %)
Concern category less or more protective	Less: 38/117 (36 %) Same: 40/117 (37 %) More: 39/117 (37 %)

Table 3. Summary of the total number of substances categorised using the NAMASTE workflow

LIMITATIONS AND NEXT STEPS

Method	Problem	Possible Solution
	Toxicodynamic properties	
In vitro bioactivity data	• Insufficient coverage of toxicological space related to human systemic toxicity • Uncertainty related to the individual assay results with respect to human relevance	• Establishing an AOP network to identify central key events or key characteristics and corresponding *in vitro* bioactivity tests • Developing a standard test battery (IATA/DA) for systemic toxicity as a first-tier testing strategy
	• Insufficient coverage for metabolites	• Using metabolically competent primary human cell culture
	• Concern categories do not provide information on the MoA/toxicological effect/target organ	• Reporting mechanistic information in addition to the potency assessment to allow further assessments, e.g. mixtures
	• Limited toxicity/potency spectrum covered	• Including more substances to broaden the toxicity spectrum and refine the categorization
QSAR *in vivo* PoD prediction	• Variability in underlying animal data • Chemical predictions limited by the relevant chemical descriptors	• Developing new models on better-curated data
GenRA	• Lack of relevant analogues • Limited data availability for relevant analogues	
	Toxicokinetic properties	
PK model	• 1C model may not be equally suitable for all chemicals	• Applying more advanced PBK models
	• Limited coverage of chemical space (chemical-specific (e.g. fu, CLint) information not available for all reference substances)	• Adding *in vitro* TK tests to the standard first-tier testing strategy • Extending the applicability domain of the in silico prediction
Oral absorption profiler	• Limited coverage of chemical space (chemical properties are outside of the threshold values for parametric boundaries; lack of data)	• Improving the prediction (adding more data?)
	• Not all routes of exposure covered	• Including *in vitro/in silico* barrier models (GIT, skin, respiratory tract) in the testing strategy
Overall	• Low regulatory acceptance	• Validating TD and TK method components • Establishing human relevance • Conducting more case studies and uncertainty assessment

Table 4. A brief description of key general and workflow related limitations along with possible solutions