ECETOC Staged Assessment Task Force

Framework for Classifying Chemicals for Repeat Dose Toxicity using New Approach Methodologies (NAMs)

ABSTRACT / BACKGROUND

As part of EPAA's 'NAM Designathon 2023' challenge for human toxicity we sought to identify a better classification systems based on the NAMs framework of Ball et al. 2022.

ECETOC's Approach: Chemicals in the challenge are assessed for bioavailability and bioactivity according to the EPAA classification matrix (Table 3). Initially all chemicals are assumed to be of High concern. Reassessments are based on accumulating evidence that can potentially move chemicals to Medium or Low concern.

Assessment integrates evidence from:

- *In silico* QSAR data.
- *In vitro* PBPK modelling data on bioavailability.
- *In vitro* data on bioactivity.

Bioavailability: 14-day PBPK simulation for standard oral dosing in humans, incorporating Clint and Fup, with plasma C_{max} as a metric to assess concern levels.

Bioactivity: Additional matrix incorporating dose response and assay implication to provide the concern level (H/M/L).

Overall Assessment: 12 chemicals placed in the EPAA matrix; Evidence appraised.

IN SILICO ASSESSMENT

For this analysis, several QSAR tools and models were run. Including both expert rule and statistical based QSAR prediction methodologies.

- **Models** (Run on default settings): Derek Nexus, Meteor Nexus, OPERA, Leadscope Model Applier, ACD/Percepta, T.E.S.T., VEGA, QSAR Toolbox and TIMES.
- **Endpoints:** Carcinogenicity, mutagenicity, reproductive and developmental toxicity, endocrine activity, neurotoxicity, acute oral, some organ specific and general toxicity.

A high concern was assigned to chemicals that showed positive predictions across multiple severe endpoints, demonstrated consistency across different models, fell within the applicability domain, and were deemed relevant by experts. No low category was assigned based on in silico as a lack of alert is not the same as a negative one.

BIOAVAILABILITY

- Accumulation concern levels were evaluated with simulated 14-day plasma C_{max} using a standard 0.1 mmol/kg dose with httk, PKSim and GastroPlus models.
- Dose measurement were expressed in Molar/kg units over mg/kg to ensure consistency with activity assessment metrics.
- Longer dosing periods of 28 days and 1 year did not have an observable effect on the C_{max} for 800 chemicals from the ToxCast database.

Table 1: Summary of Bioavailability data from 3 models. High >500µM (Red); Mid 500- 50µM (Orange); Low <50µM (Green).

Consolidated model results (Cmax in µM for 0.1 mmol/kg for 14 days)					
Substance	**Model inputs**	**httk**	**PK-sim**	**Gastroplus**	**Overall**
Nitrobenzene	*in vitro*	44	3.7	5.1	
Ouabain	*in silico*	13	0.013	18	
Benzoic acid	*in silico*	1011	810	1097	
Safrole	*in vitro*	232	40	117	
2,4,6-tri-tert-butylphenol	*in silico*	409	2.4	225	
Phenol	*in vitro*	40	4.0	62	
1-chloro-4-nitrobenzene	*in silico*	194	21	11	
Colchicine	*in vitro*	63	6.4	50	
4-nitrophenol	*in vitro*	86	8.4	125	
Diethylphthalate	*in vitro*	29	1.9	23	
Carbaryl	*in vitro*	18	0.19	16	
Chlorpropham	*in vitro*	36	0.9	25	

Cmax	**<50 µM**	**50-500 µM**	**>500 µM**
Category	L	M	H

Figure 2: Original Cmax boundaries for each category

BIOACTIVITY

- **Severity**: Assays are categorized as high, medium or low. E.g. oestrogenic receptor assays are rated High; while PPAR binding is rated Low.
- **Potency**: Dose-response curves are reviewed to ensure confidence in AC50 values. In vitro bioactivity data is primarily based on ToxCast, with the limitations it brings.

Potency	**<0.1 µM**	**0.1-10 µM**	**>10 µM**
Category	H	M	L

Figure 3: Potency categories determined by AC50

Chemical	**Colchicine**		Result:	H
	POT H	POT M	POT L	POT NO HIT
SEV H	**27**	**4**	**23**	**435**
SEV M	**18**	**5**	**3**	
SEV L	**74**	**56**	**7**	

Table 2: Original bioactivity matrix for Colchicine (High concern).

OVERALL ASSESSMENT

- Bioavailability and Bioactivity outcomes are placed first into the EPAA Matrix.
- The preliminary category is then reviewed using a weight of evidence approach.

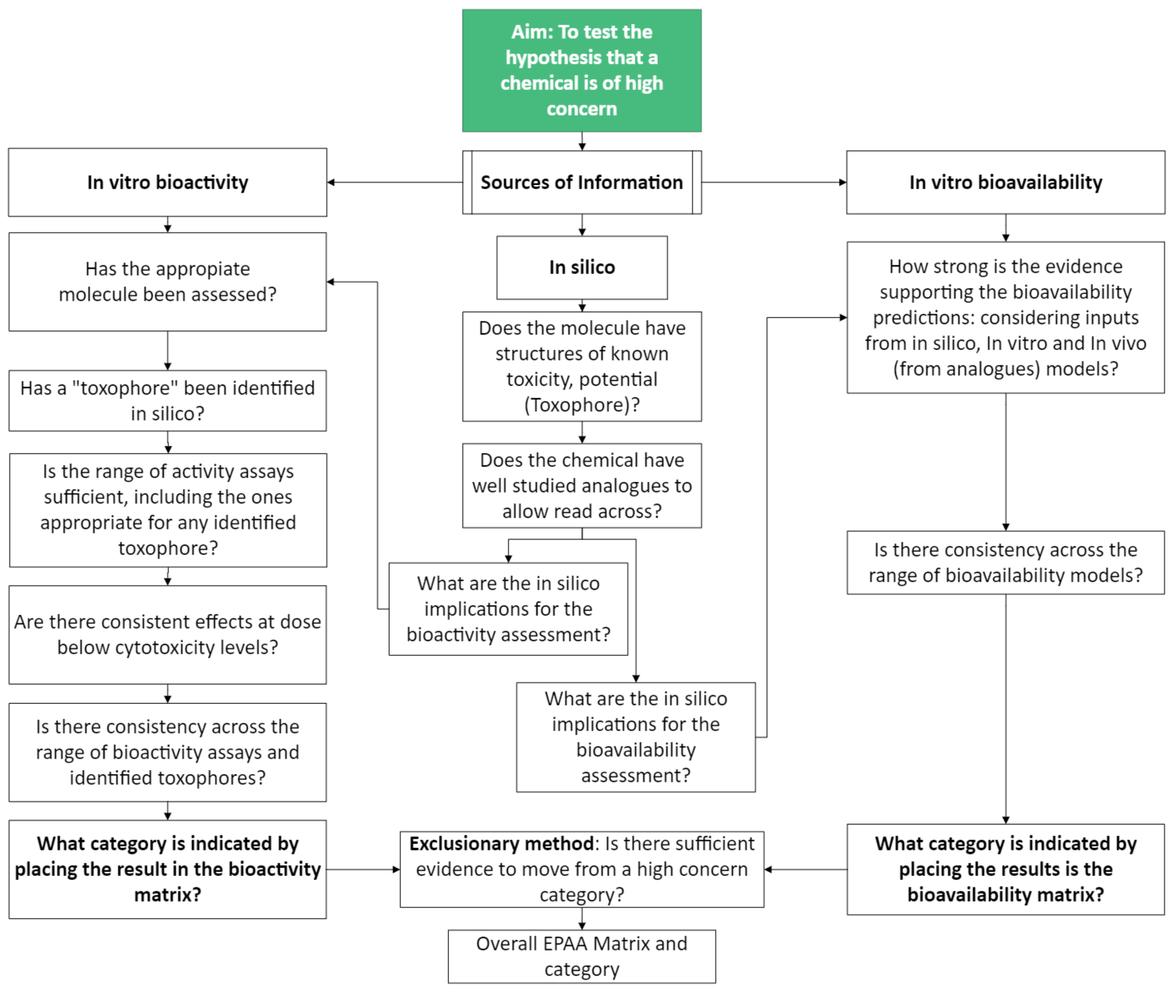
Chemical			
Safrole	Activity H	Activity M	Activity L
Availability H			
Availability M			✗
Availability L			

Table 3: Examples of the overall concern matrix for Safrole (Low concern).

Figure 3: Examples of one of the weight of evidence question for Safrole (Low concern).

Question	Answer	Conclusion
Is there sufficient evidence to move from High concern category?	Some indications of concern from *in silico*; No consistent indications from Bioactivity; Mid Bioavailability; Matrix indicates Low level of concern	**Low**

FIGURE 4: FRAMEWORK FLOWCHART



REVIEW OF THE RESULTS SO FAR

12 chemicals have been assessed through the framework and compared with the reference Level of Concern (LoC) derived from open literature review considering potency and severity in repeat dose studies (not using STOT RE criteria specifically).

The framework **initially** had **a trend towards classifying chemicals in lower categories of concern than the reference levels**.

A sensitivity analysis was conducted varying the criteria for bioactivity (using only potency) **and bioavailability** (reducing the boundaries by a factor of 5). These changes are displayed below and further "calibration" of the framework is possible.

The basic concept put forward by the EPAA has been shown to be workable, but **the process is highly dependent on having an "adequate" range of *in vitro* assays**. How to define "adequate" remains a major question.

Table 5: In silico output and overall assessment results of the framework with varying criteria of in vitro bioactivity and in vitro bioavailability compared to the reference level of concern (LoC).

Chemical	*In silico*	SEV/POT & 50-500 µM	POT only & 50-500 µM	SEV/POT & 10-100 µM	POT only & 10-100 µM	Reference LoC
Nitrobenzene	**H**	**M**	**M**	**H**	**H**	**H**
Ouabain	**H**	**M**	**M**	**M**	**H**	**H**
1-chloro-4-nitrobenzene	**H**	**H**	**H**	**H**	**H**	**H**
Colchicine	**H**	**H**	**H**	**H**	**H**	**H**
Phenol	**H**	**L**	**L**	**L**	**M**	**M**
Tri Tertiary Phenol	**M**	**M**	**M**	**H**	**H**	**M**
Carbaryl	**H**	**L**	**L**	**M**	**M**	**M**
Chlorpropham	**M**	**L**	**L**	**M**	**M**	**M**
Safrole	**H**	**L**	**M**	**M**	**H**	**L**
Benzoic Acid	**M**	**H**	**H**	**H**	**H**	**L**
4-nitrophenol	**M**	**L**	**L**	**M**	**M**	**L**
Diethylphthalate	**M**	**L**	**L**	**L**	**L**	**L**

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