ECETOC Staged Assessment Task Force

Framework for Classifying Chemicals for Repeat Dose Toxicity using NAMs

ABSTRACT / BACKGROUND

Principle: Initially all chemicals are of High concern. Reassessment is based on accumulating evidence to 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: Concern levels placed in the EPAA matrix; Evidence appraised.

IN SILICO ASSESSMENT

Unique Structure ID
- Unique structural identifiers were defined by CAS and converted into Canonical SMILES
- The SMILES structures were cleaned accordingly (removal of charge, inorganics and salts)

(Q)SAR Tools

All models were run under their respective default settings.
- Derek Nexus, Meteor Nexus, OPERA, Leadscope, ACD/Percepta, T.E.S.T., VEGA, QSAR Toolbox and TIMES
- Internally developed models

Endpoints
- Acute oral, Genotoxicity, Endocrine activity, Carcinogenicity, Reproductive and Developmental toxicity, Neurotoxicity, General toxicity

Integration of results
- Across endpoints
- Across tools
- Evaluate relevance and reliability of predictions

Range of in silico tools
- Helps ascertain if the range of activity assays is adequate
- Helps to determine if parent or metabolite to be assayed
- Introductory indication of a concern level and possible toxophores

Figure 1: In silico flow diagram

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 of 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.

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.

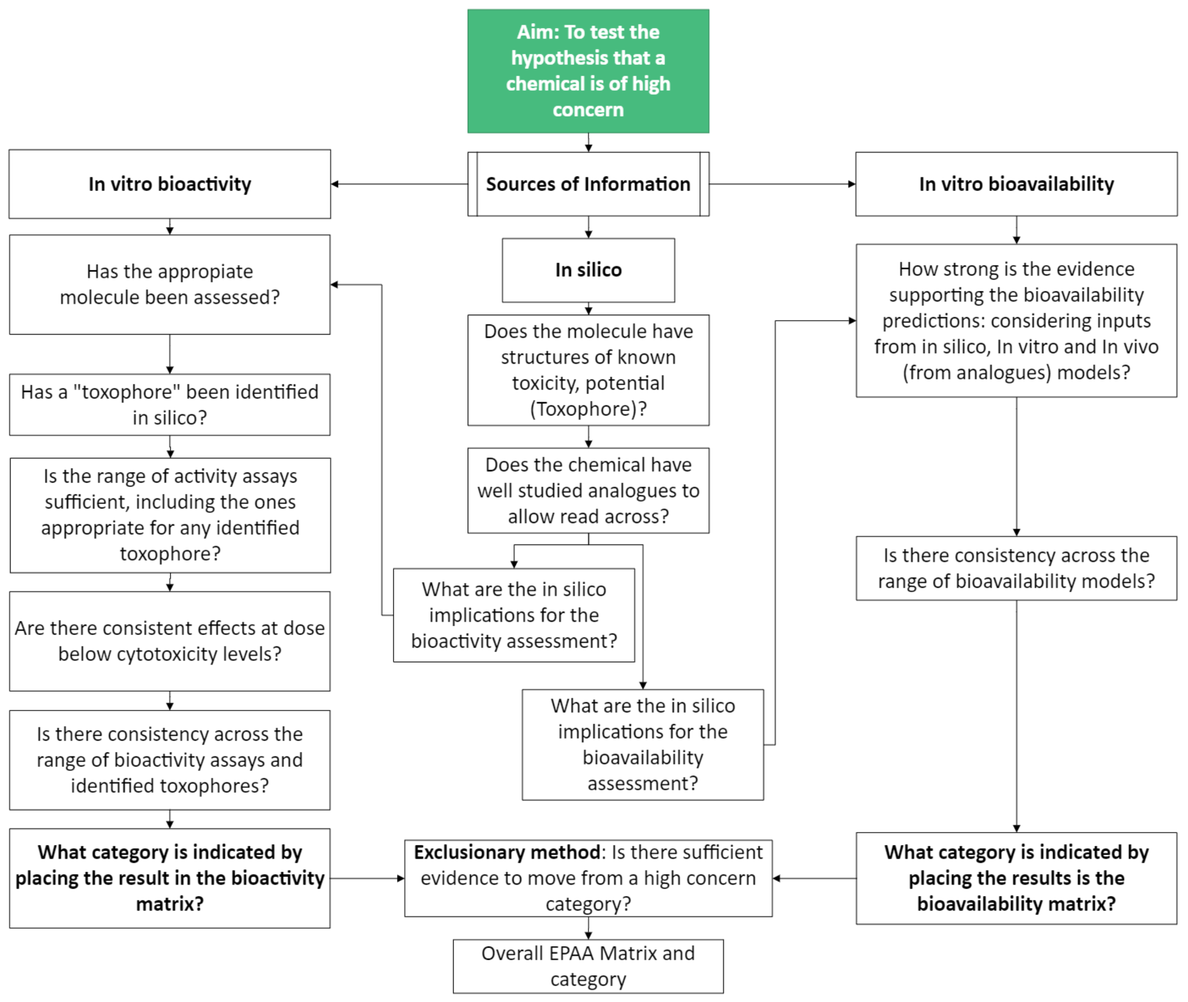
Chemical			
Safrole	Activity H	Activity M	Activity L
Availability H			
Availability M			**X**
Availability L			

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?	No 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 ran 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: Comparison of In silico, In vitro bioactivity and bioavailability against 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	M	M	M	M	L
4-nitrophenol	M	L	L	M	M	L
Diethylphthalate	M	L	L	L	L	L

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WE ARE THE CENTRE FOR CHEMICAL SAFETY ASSESSMENT

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