

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

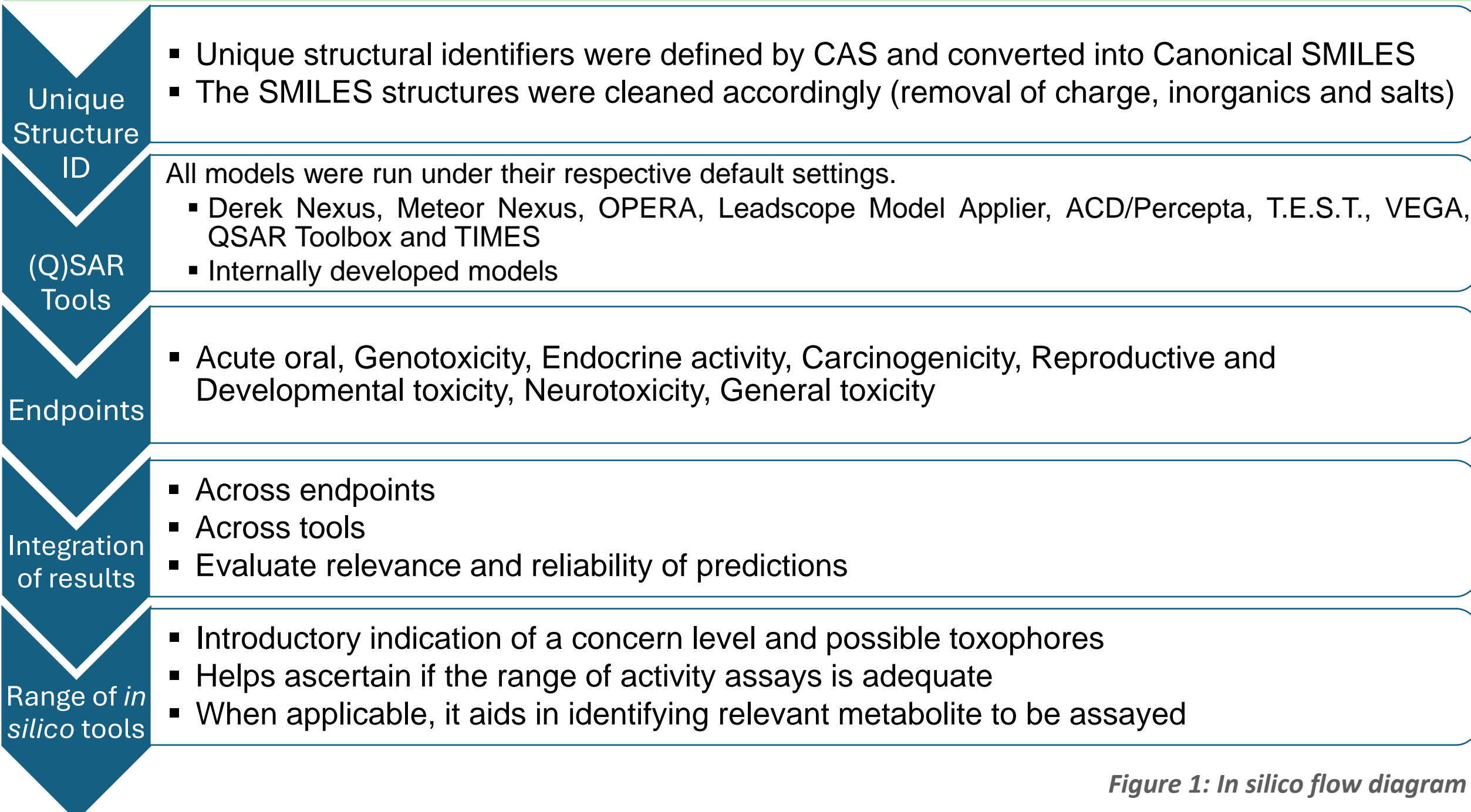


Figure 1: In silico flow diagram

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 >500 μ M (Red); Mid 500- 50 μ M (Orange); Low <50 μ M (Green).

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

Figure 2: Original C_{max} 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.

Figure 3: Potency categories determined by AC50

Potency Category	<0.1 μ M	0.1-10 μ M	>10 μ M
Category	H	M	L

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.

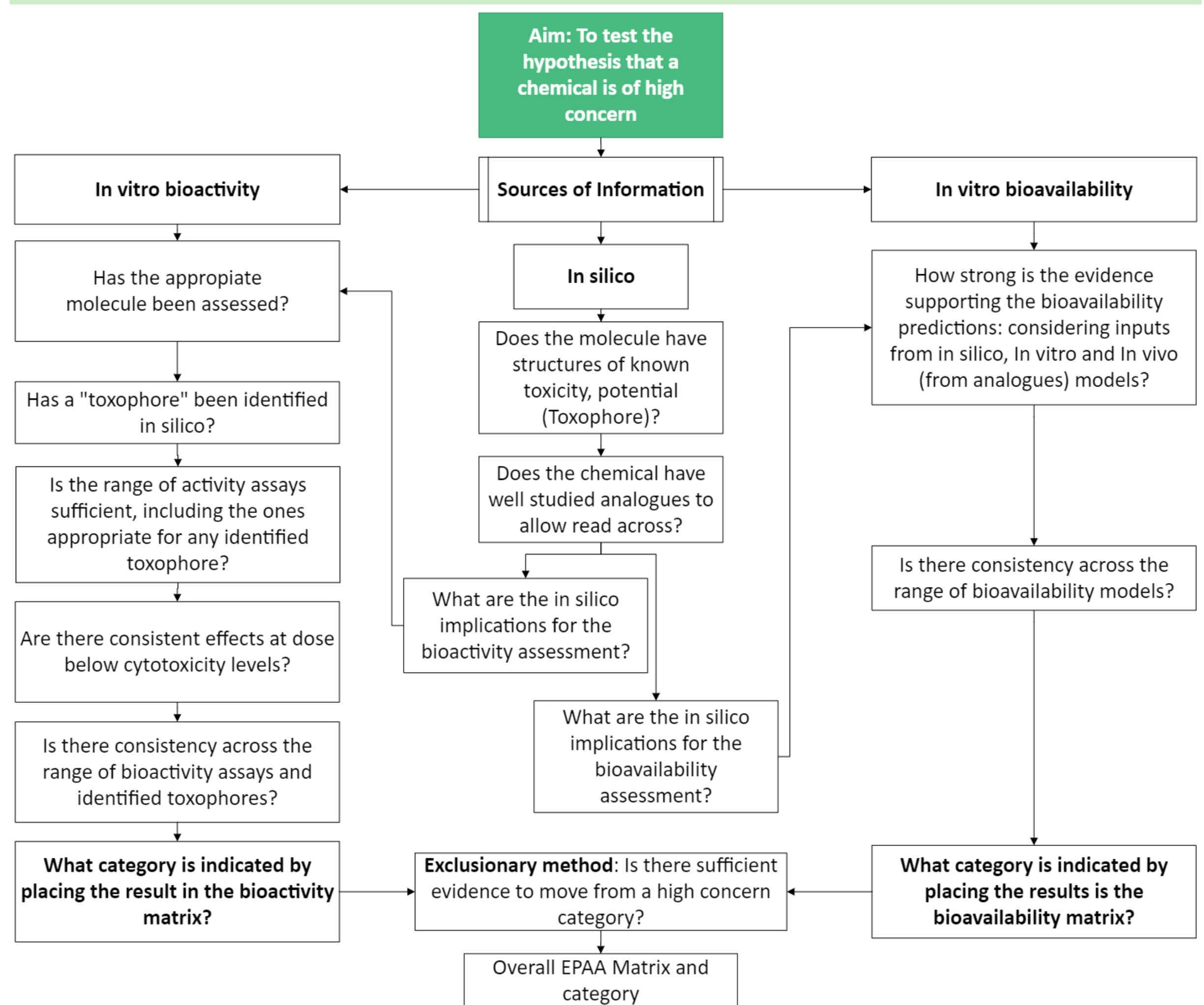
Chemical	Activity H	Activity M	Activity L
Safrole			
Availability H			
Availability M			X
Availability L			

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?	Some indications of concern from <i>in silico</i> ; 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	<i>In silico</i>	SEV/POT & 50-500 μ M	POT only & 50-500 μ M	SEV/POT & 10-100 μ M	POT only & 10-100 μ M	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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