when these BERs related to Nrf2 can be considered as adverse

Safety, Environmental & Regulatory Science

Application of NAM-based systemic safety toolbox on case study of bioactive food component sulforaphane

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Introduction

Our NAM-based systemic safety toolbox (Fig. 1(a)) aims to protect human health by considering points of departure (PODs) from various sources, including a cell stress panel (CSP), in vitro pharmacological profiling (IPP), high-throughput transcriptomics (HTTr), and physiologically based kinetics (PBK) modelling to predict the maximum concentration (Cmax) of the target chemical in plasma. The bioactivity exposure ratio (BER) is calculated as the ratio between the minimal PODs and Cmax to illustrate potential risks under various exposure scenarios (Middleton et al., 2022). This approach may be conservative, especially for compounds like sulforaphane, found in cruciferous vegetables like broccoli.

Previous studies categorized Sulforaphane as uncertain risk with the NAM-based systemic toolbox, which contradicts the historical safety profile of broccoli (Middleton et al., 2022). This discrepancy may arise because PODs based on bioactivity do not necessarily translate into adversity.

To refine the NAM-based systemic safety toolbox, two aspects are considered. First, the PBK model is updated with in vitro data for metabolic clearance and fraction unbound in plasma, which can be considered more reliable than the in silico predictions for these parameters. Second, a comparison was made with BER of benchmark chemicals. From the bioactivity perspective, sulforaphane induces the Nrf2 pathway, regulating genes for cellular protection and antioxidant responses. To benchmark the activity of sulforaphane, BERs for different Nrf2-inducing benchmark (alpha lipoic acid, CDDO-ME, and andrographolide, Fig. 1(b)) were calculated, providing a means to evaluate the BER in relation to reference chemicals and the risk classification of these reference chemicals.

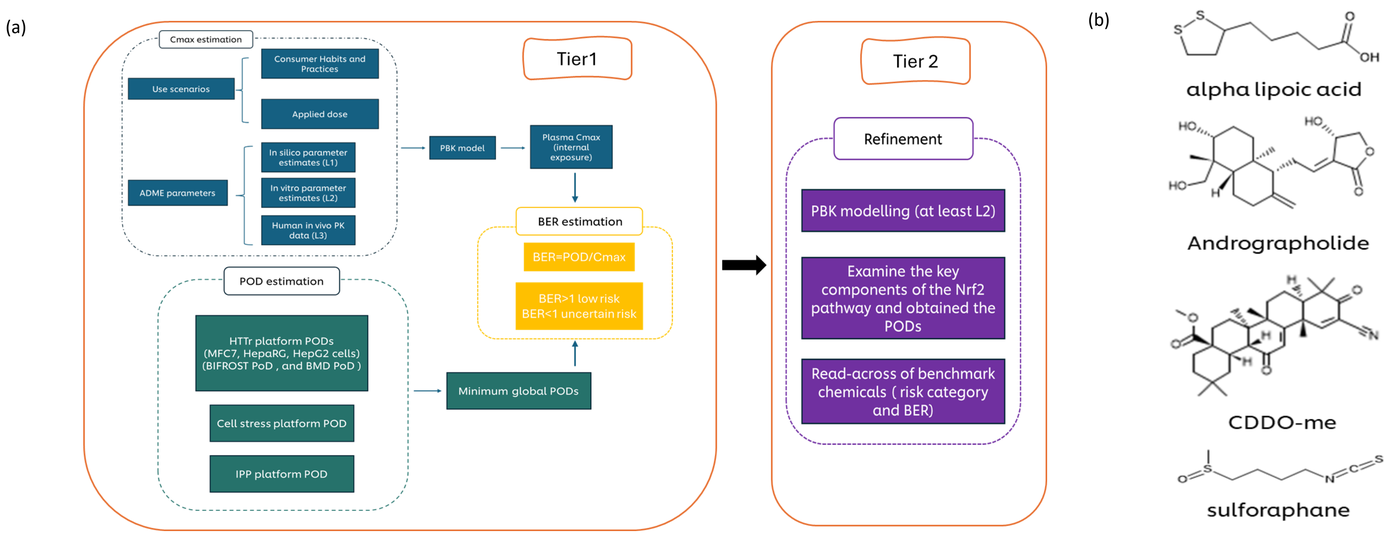

Figure 1. Schematic of the systemic safety toolbox and associated workflow (a), and chemical structures of Nrf2-inducing benchmark chemicals and sulforaphane (b)

Refinement of NAM-based systemic safety toolbox

Methods: Internal exposure (Cmax) estimation based on sulforaphane PBK modelling

- Level 1: *in silico* only: sulforaphane specific parameters are obtained using only *in silico* prediction.
- Level 2: *in silico* and *in vitro*: values for water solubility (5.41mg/mL vs 0.6 mg/mL), unbound fraction in plasma (74.7 vs 95), hepatic intrinsic clearance (12.3L/h vs 87.3 L/h), and intestinal absorption (7.8 cm/s *10^4 vs 4.4 cm/s *10^4) were updated from *in vitro* measurements.
- Model evaluation: the evaluation was conducted at two parameterization levels. Predicted Cmax and concentration-time curve patterns from PBK modelling were compared with clinical data.

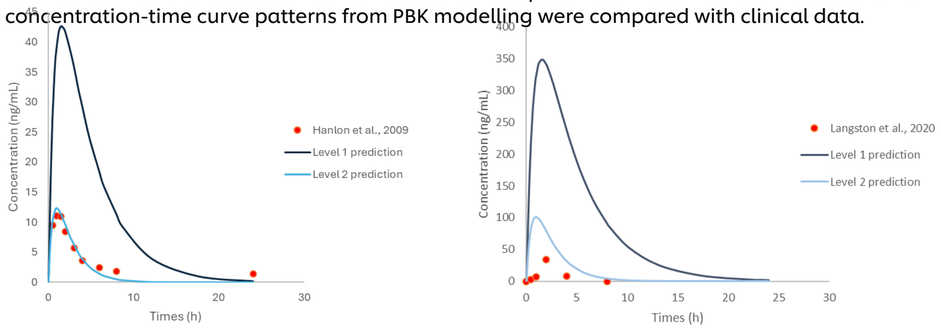

Fig. 1 Comparison of plasma concentration-time curves of sulforaphane among different levels of PBK modelling predictions with observed data. The observed data were derived from healthy subjects who were orally administered liquid broccoli containing 3.9 mg of sulforaphane (Hanlon et al., 2009) and capsules containing 32 mg of sulforaphane (Langston et al., 2020).

Results:

- PBK modelling predictions of sulforaphane were more accurate at level 2 compared to level 1.
- The predicted Cmax values were 11.83 ng/mL and 97.71 ng/mL following exposure to 3.9 mg and 32 mg of sulforaphane, respectively.
- These predictions closely matched the observed data, with a Cmax of 11.02 ng/mL from liquid broccoli (Hanlon et al., 2009) and were 2.8 times higher than the data derived from Langston et al. (2020) that was 34. 33 ng/mL from broccoli containing capsules.

Methods: PODs estimation based on in vitro bioactivity data

- 3 Nrf2-indicing benchmark chemicals as well as sulforaphane were tested by in IPP, HTTr, and CSP.
- Minimal PODs were selected from the lowest EC50 or IC50 values from IPP data, and the smallest global PODs derived from BIFROST and BMDExpress 2 analysis from HTTr and CSP data.

Table 1. Overview of platform PODs (in vitro pharmacological profiling-IPP, cell stress panel, and high-throughput transcriptomics-HTTr) obtained using the toolbox for each of Nrf2 induction chemicals. Hight-throughput transcriptomics data were generated for 3 cell lines (MCF7, HepaRG, HepG2) and analysed using 2 different methods (BMDexpress and BIFROST), resulting 6 transcriptomics platform PODs per chemical.

Compound	CSP Global PoD (μM)	IPP Global PoD (μM)	High-throughput transcriptions Global POD						Global PoD across all assays (μM)
			HepaRG BIFROST PoD (μM)	HepaRG BMD PoD (μM)	HepG2 BIFROST PoD (μM)	HepG2 BMD PoD (μM)	MCF7 BIFROST PoD (μM)	MCF7 BMD PoD (μM)	
Alpha lipoic acid	5.3	2.82	13	10.51	5.3	52.22	13	NA	2.82
Andrographolide	0.051	No hits	1.4	7.94	0.045	6.40	0.09	4.84	0.045
CDDO-Me	0.000066	1.2	0.008	0.08	0.00026	0.07	0.00043	0.00109405	0.000066
D,L - Sulforaphane	0.035	18.876	1.6	2.81	0.059	2.24	0.21	2.46	0.035

Results:

- PODs varied across datasets by 9 orders of magnitude, from picomolar to micromolar concentrations.
- The most potent chemical was CDDO-me with lowest PODs at 66pM. The least potent chemical was alpha lipoic acid with lowest PODs at 2.82 μM.
- For 2 out of 4 chemicals (CDDO-me, and sulforaphane), the smallest PODs tended to come from the cell stress panel where the compounds showed a lowering of GSH content in the cells (data not shown).

Methods: BER calculation

- Selection of clinical studies for benchmark chemicals: One clinical study was chosen for each benchmark chemical to collect the Cmax value for BER calculation. For alpha lipoic acid, two enantiomers were included. For sulforaphane, both Cmax values from clinical studies and PBK modelling predictions based on fresh broccoli consumption level were listed.
- BER for each chemical was calculated as the ratio between the minimal PODs from bioactivity assays and the Cmax from clinical studies. BER values were expressed in logarithmic form.

Table 2. Overview of the clinical pharmacokinetics studies of Nrf2 induction chemicals

Compounds	Exposure route	Vehicle/formulation	Dose (mg)	Cmax (ng/mL)	Cmax (μM)	Tmax (h)	BER	Reference
R-alpha lipoic acid	oral	tablet	200	440	2.13	0.9	1.32	Hermann et al., 1996
S-alpha lipoic acid	oral	tablet	200	280	1.36	0.9	2.07	Hermann et al., 1996
Andrographolide	oral	capsule	120	112	0.32	0.8	0.14	Songvut et al., 2023
CDDO-me	oral	amorphous spray-dried dispersion	20	6.09	0.01	3	0.01	Teuscher et al., 2013
Sulforaphane	oral	solution	3.9	11.02	0.06	1.2	0.58	Hanlon et al., 2009
Sulforaphane	oral	capsule	32	34.33	0.19	2	0.18	Langston et al., 2020
Sulforaphane	Oral	Broccoli	11	34.54	0.19	1.1	0.18	PBK modelling prediction based on concentration of sulforaphane in 100g of fresh broccoli (Wu & Pehrsson, 2021)

Results:

- For dietary exposure, using fresh weight of broccoli is conservative estimation of the bioavailable sulforaphane as different cooking styles influence the sulforaphane content.
- The log BER are less than 0 for 3 out of 4 chemicals (andrographolide, CDDO-me and sulforaphane), indicating that under the current exposure scenarios, the concentration level of chemicals in the plasma could induce bioactivity.
- Comparing the log BER values, the potential risk of sulforaphane could be lower than CDDO-me but in the same range of andrographolide.
- More studies need to be done to explore 1. to what extent the bioactivity related to the PODs are linked to the Nrf2 pathway; 2. Determine risk categories of benchmark chemicals.

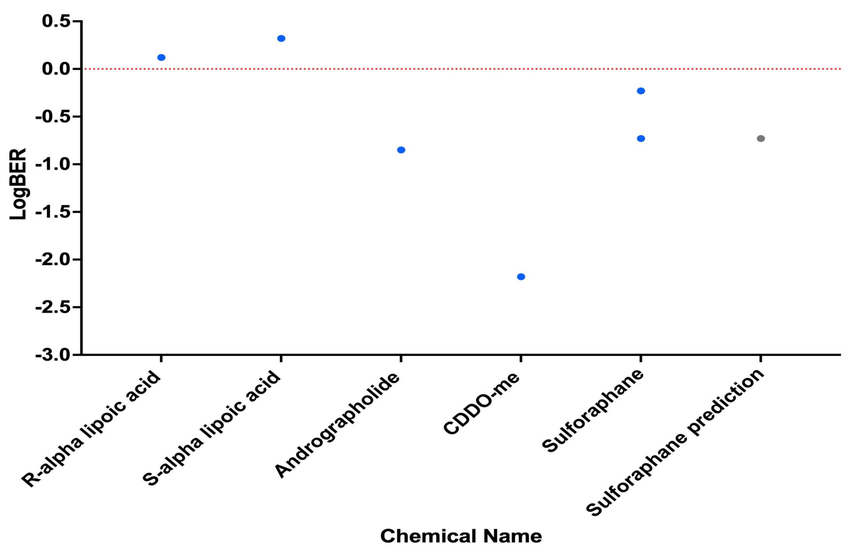

Fig2. Distribution of the bioactivity exposure ratio (BER) of Nrf2-inducing chemicals, calculated by determining the ratio between the Cmax obtained from clinical studies and the minimal points of departure (PODs) across all bioassays. The blue dots represent the Cmax from clinical studies and grey dots represent the Cmax from PBK modelling prediction

Conclusions

- The NAM-based systemic safety toolbox was refined by updating PBK modelling parameters with in vitro studies, improving the accuracy of predictions.
- For most chemicals, the lowest PODs were associated with GSH depletion, an indirect measurement of the Nrf2 pathway.
- Three out of four chemicals demonstrated a BER of less than 1 under exposure scenarios indicating that the risk of sulforaphane was still uncertain.. Further refinement is needed to refine exposure scenarios, identify the risk categories of benchmark chemicals at POD levels to determine when these BERs related to Nrf2 can be considered as adverse.
- Read-across from benchmark chemicals to sulforaphane provides a better indication of the risk level of sulforaphane by considering BER values calculated from Nrf2-specific PODs and the potential risks of benchmark chemicals
- This PBK refinement and benchmark data will be combined with other Tier 2 methods (including pathway analysis and live cell imaging) to provide a weight of evidence approach that integrates several lines of evidence to enable safety decision making.

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References

Middleton et al., 2022, Toxicological Sciences, 189(1), pp.124-147
Hanlon et al., 2009, Cancer Letters, 284(1), pp.15-20
Langston-Cox et al., 2020, Molecules, 25(4), p.829
Songvut et al., 2023, Frontiers in Pharmacology, 14, p.1230401
Teuscher et al., 2014. Clinical Pharmacology in Drug Development, 3(4), pp.314-320.
Wu & Pehrsson, 2021. Current Development in Nutrition, 5(8).