Combining *In Vivo*, *In Vitro* And Toxicokinetics Data In Read-across: A Case Study Using Caffeine

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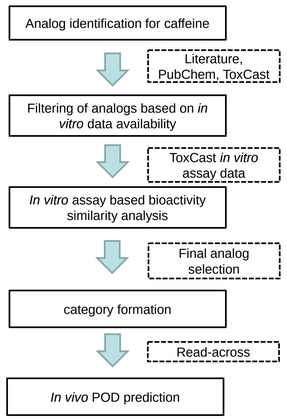
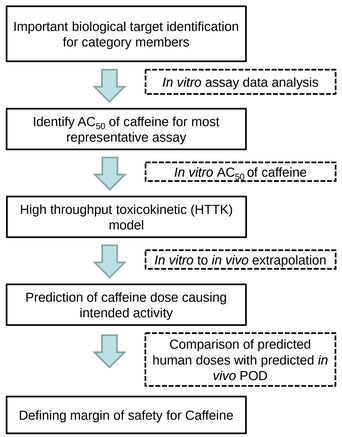
Introduction

- US-EPA and Unilever are collaborating to develop new, more efficient approaches for chemical safety assessment.
- In this case study, we combined *in vivo*, *in vitro* and toxicokinetics data for the purpose of assessing chemical safety using read-across.
- Read-across can be used to fill data gaps in chemical safety assessments.
- The read-across workflow comprises several steps starting with the identification and evaluation of source analogs.
- Analog evaluation includes assessing the physicochemical and mechanistic similarity of source analogs.
- Different data inputs (*in vivo*, *in vitro, in silico)* characterize the similarity context to justify read-across predictions.
- **The main goal of this study was to construct a quantitative read-across prediction of point of departure (POD) using caffeine as a target chemical.**
- To provide a more complete profile of caffeine, we have predicted receptor mediated bioactivity, genotoxicity and possible metabolites.
- Finally, we performed an *in vitro* to *in vivo* extrapolation (IVIVE) directly on caffeine using *in vitro* assay activity (AC_{50}) and high-throughput Toxicokinetics (HTTK) data to derive intended bioactivity dose.

Methods



Scheme-1: Read-across POD prediction



Scheme-2: IVIVE margin of safety prediction

Results: scheme 1

Thirteen analogs were identified for caffeine based on structural similarity. Among those only three were found to have ToxCast *in vitro* bioassay data.

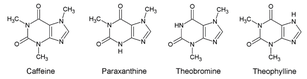
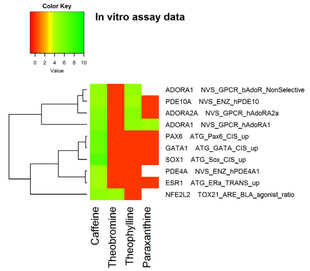


Figure 1: Structures of target (caffeine) and its identified analogs (paraxanthine, theobromine and theophylline).



The *in vitro* assay data confirmed the blockade of adenosine receptor by caffeine as well as its 2 analogs i.e. theophylline and paraxanthine.
Moreover, caffeine and theophylline have shown activity in three of the assays that are related to adenosine binding such as nonselective binding to adenosine A1 receptor, and selective binding to adenosine A1 receptor and adenosine A2a receptor.

Figure 2: Analysis of *in vitro* assay data to identify biological similarity between the target and its analogs.

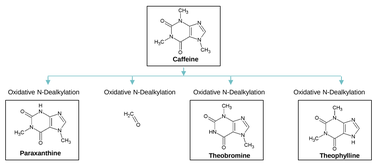


Figure 3: Predicted metabolites of caffeine in the *in vivo* rat TIMES[#] metabolism simulator show that the three analogs are likely metabolites, further supporting their use as read-across analogs. (Complete predicted metabolic pathway not shown).

[#]Reference: Curr. Pharm. Des., 10, 2004, pp. 1273-1293

Scheme 1: Read-across prediction of POD

Table 1: Prediction of LEL for caffeine: the most conservative LEL value among analogs was chosen as predicted LEL for caffeine. A cup of coffee is equivalent to a dose of 1-2 mg/kg/day.

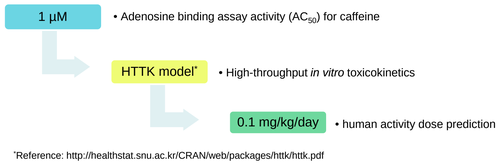
	Target	Analog-1	Analog-2
	Caffeine	Theophylline	Theobromine
Sub-chronic toxicity (species = rat)	100 mg/kg/day	75 mg/kg/day	250 mg/kg/day
Chronic toxicity (species = rat)	49 mg/kg/day	7.5 mg/kg/day	250 mg/kg/day
Predicted POD (species = rat)	**7.5 mg/kg/day**		

Human equivalent dose (HED) = rat dose / 6.2
HED = 1.2 mg/kg/day

Results: scheme 2

Scheme 2: IVIVE prediction of *in vivo* potency for caffeine for its intended target

Figure 4: *In vitro* to *in vivo* extrapolation to predict human doses.



*Reference: http://healthstat.snu.ac.kr/CRAN/web/packages/httk/httk.pdf

Predicted margin of safety = 1.2 / 0.1 = 12

Supporting information for caffeine's toxicity profile

In addition to POD and human dose we have also predicted other possible toxicities of caffeine using TIMES model.

Table 2: Predictions for caffeine using TIMES Models that account for metabolism, compared with experimental data when available.

Source	Alert	Result	Source	Experimental Information
TIMES	*In vivo* Comet Genotoxicity	Positive	-	-
TIMES	*In vivo* liver Clastogenicity	Positive	HSDB	Not clastogenic in *in vivo* mouse model
TIMES	*In vivo* TGR Mutagenicity	Negative	-	-
TIMES	*In vivo* Micronucleus	Positive	CCRIS	Negative in *in vivo* micronucleus test in mouse
TIMES	*In vitro* Ames Mutagenicity	Negative	-	-
TIMES	*In vitro* Chromosomal aberration	Positive	HSDB	Did not increase CA in *in vitro* CHO assay
TIMES	Estrogen binding affinity s9 activated	Inactive	-	-
TIMES	Skin sensitization with autoxidation	Non-sensitizer	-	-

Conclusions

- The study demonstrates that the point of departure (POD) for caffeine can be predicted by quantitative read-across using physicochemical, *in vitro* and *in vivo* data.
- Caffeine and its 2 analogs were active in three assays related to blockade of adenosine receptor A1 and A2a, demonstrating mechanistic similarity among target and analogs. The analogs are also direct metabolites of caffeine.
- The *in vitro* assay concentration at which target compound was blocking adenosine receptor was chosen as input to predict the human bioactivity dose prediction, combined with HTTK data.
- This IVIVE study yielded a dose for target bioactivity of 0.1 mg/kg/day, significantly lower than the *in vivo* read-across POD (1.2 mg/kg/day).
- The read-across prediction derived for caffeine provide a proof of principle of this approach for using structure, physicochemical and *in vitro* data to help select read-across analogs.