Modelling of the *in vitro* Biokinetics of Tebufenpyrad for *in vivo* Dose Estimation

Hiba Khalidi[1], Mahshid Alimohammadi[2], Barira Islam[1], Celine Brochot[1], Frederic Bois[1], Iain Gardner[1], Elias Zgheib[1], Marcel Leist[2]

(1) Certara, Simcyp® Division – Sheffield (UK)
(2) Universitat Konstanz, Konstanz (DE)

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Background

Neurotoxicity and neurodegeneration are of particular interest in the field of toxicology as they affect a large portion of the population. Here, we explore the applicability of AOP#3[1] (the generic pathway to mitochondrial toxicity) in predicting the key event (KE) cascade of the potent neurotoxicant, Tebufenpyrad (TEBU). We aim to build a QST model for neurodegeneration that is comprised of 3 modules as shown in Figure 1. Note that the mathematical model is ready but requiring some further calibration with time-dependant data.

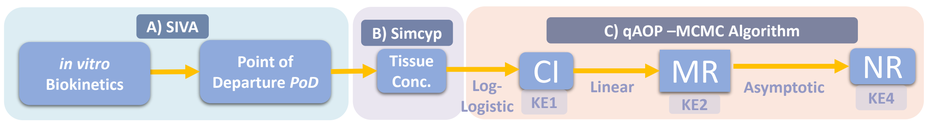


*Figure 1: **A)** The in vitro biokinetics model for PoD estimation, **B)** PBK modelling of TEBU, and **C)** the qAOP model based on AOP#3*

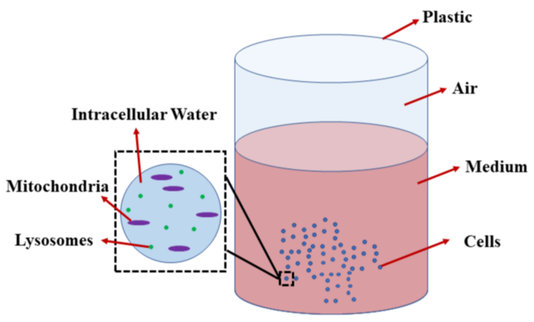


Figure 2: The compartments of an in vitro system as simulated by the VIVD model in SIVA

As a precursor to the PBK-qAOP linkage, we use Certara's SIVA software (which utilises the VIVD model[2]) to understand the *in vitro* biokinetics of TEBU-exposed LUHMES cells. The SIVA tool enables the prediction of a compound's partitioning into the air, plastic, medium, and cells (lysosomes, mitochondria and intracellular water) within an enclosed *in vitro* system at equilibrium. Therefore, an interesting byproduct of our simulations is the prediction of TEBU's mitochondrial concentration in LUHMES.

Methods

Experiments on the *in vitro* partitioning of TEBU within the medium, cell and plastic compartments have been reported in the EFSA documents by Alimohamadi *et al*[3]. We aim to mirror the biokinetics experiments of the EFSA document in this work using the VIVD model.

Parametrising the VIVD model

The *in vitro* biokinetic simulations were parametrized with appropriate physiological characteristics of dopaminergic LUHMES. The culture conditions of each of the experiments were accounted for:

Culture Temp. 25◦C | **Bovine Serum Albumin (BSA) or no BSA** | **Medium pH of 7.4**

Medium Surface Area in contact with Plastic Container

400µL of TEBU-containing medium were applied to the 3 types of containers which were used in the EFSA biokinetics experiments as shown in Figure 3.

Loss Assumptions

Here, loss refers to ratio of TEBU concentration recovered from cells, medium and plastic to the nominal TEBU concentration. To account for that in the VIVD model, we apply the nominal TEBU percentage that is irrecoverable as estimated in the EFSA document:

- **60%-82% irrecoverable** in BSA-containing medium
- **75%-91% irrecoverable** in BSA-free medium

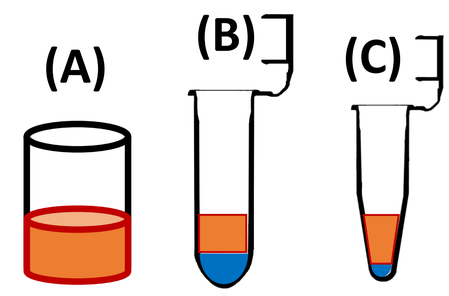


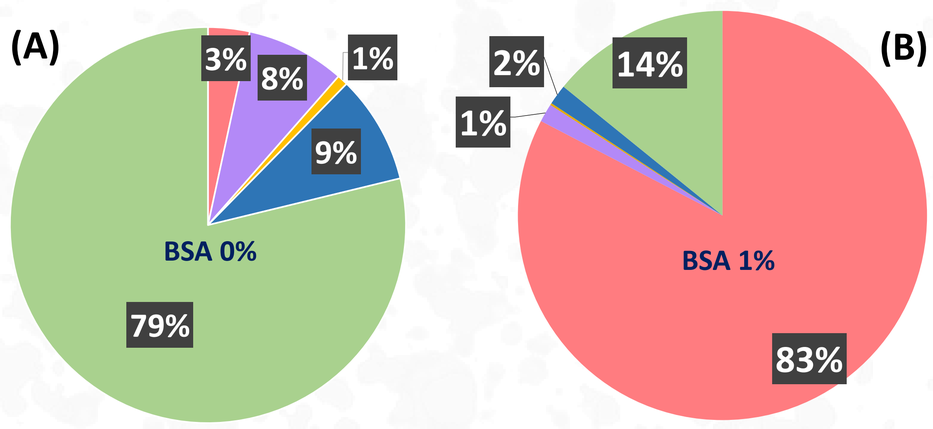
*Figure 3: **(A)** A single well from the 24-well culture plate; surface area (SA) 287mm². **(B)** A 2mL Eppendorf tube; SA = 224mm². **(C)** A 1.5mL Eppendorf tube; SA = 280mm².*

Results

↓ ***Table 1:** SIVA predictions of TEBU's in vitro biokinetics (**applying loss assumptions outlined in the Methods**) compared with experimental measurements of TEBU's partitioning in a 1.5mL Eppendorf tube with 5E6 cells.*

	BSA	Nominal Conc.	Medium Recovery	Cell Recovery
	(%)	(M)	(%)	(%)
SIVA (predicted)	0	1,0E-06	0,6%	15,6%
	0	3,3E-07	0,37%	9,6%
	1	1,0E-06	33,0%	6,4%
Experim.	0	1,0E-06	1,1%	16,5%
	0	3,3E-07	0,3%	10,5%
	1	1,0E-06	30,0%	10,0%

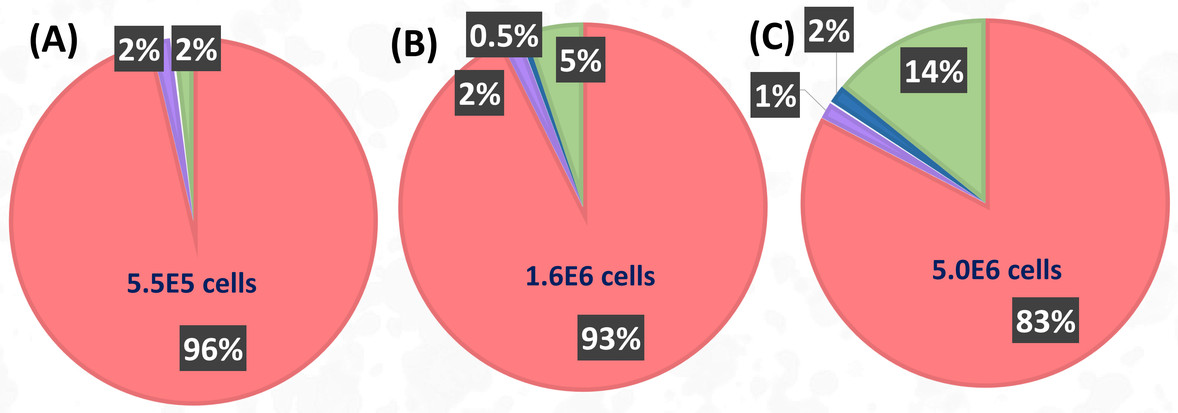
→ **Figure 4:** SIVA predictions of TEBU partitioning in: **(A)** 0% BSA medium and **(B)** 1% BSA medium, **assuming no loss, and that nominal TEBU is 100% recoverable.**



↓ **Table 2:** SIVA predictions (**applying loss assumptions outlined in the Methods**) compared with experimental measurements of TEBU's partitioning in a 1.5mL Eppendorf tube with 1% BSA.

	Cell	Nominal Conc.	Medium+Cell Recovery
	number	(M)	(%)
SIVA (predicted)	5,5E+05	1E-06	17,7%
	1,6E+06	1E-06	17,2%
	5,0E+06	1E-06	24,6%
	5,5E+05	3E-07	19,7%
Experim. (measured)	5,5E+05	1E-06	18,0%
	1,6E+06	1E-06	17,5%
	5,0E+06	1E-06	25,0%
	5,5E+05	3E-07	20,0%

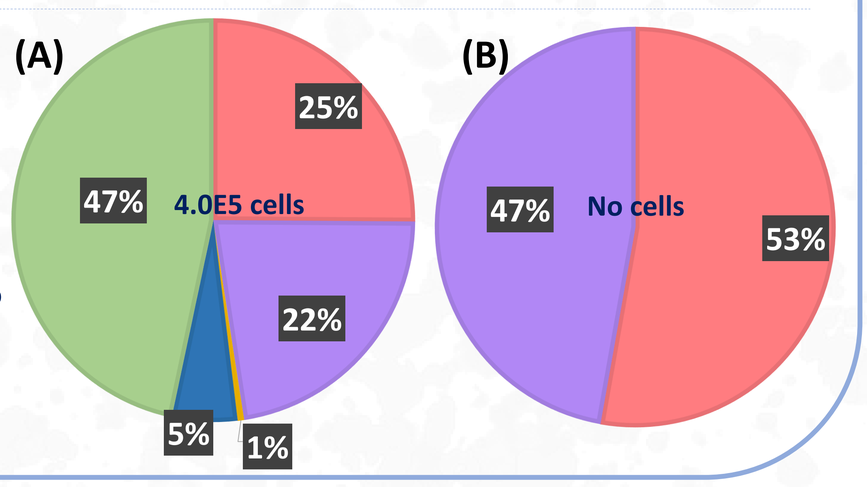
→ **Figure 5:** SIVA predictions of TEBU partitioning in medium with 1% BSA and **A)** 5.5E%, **B)** 1.6E6 , **C)** 5E6 cells, **assuming no loss , and that nominal TEBU is 100% recoverable.**



↓ **Table 3:** SIVA predictions (**applying loss assumptions outlined in the Methods**) compared with experimental measurements of TEBU's partitioning in a 24-well plate without BSA.

	Cell	Nominal Conc.	Medium Recovery	Cell+Plastic Recovery
	number	(M)	(%)	(%)
SIVA (predicted)	4,0E+05	1E-06	3,6%	10,9%
	4,0E+05	3E-07	2,2%	6,7%
	0	1E-06	7,4%	6,6%
Experim. (measured)	4,0E+05	1E-06	10,5%	4,00%
	4,0E+05	3E-07	7,2%	1,80%
	0	1E-06	11,0%	3,00%

→ **Figure 6:** SIVA predictions of TEBU partitioning in medium with 0% BSA with: **(A)** 4E5 cells, and **(B)** no cells, **assuming no loss , and that nominal TEBU is 100% recoverable.**



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