The state of the art in AgChem – an example using HPPD inhibitors

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ECETOC workshop on Quantitative Response-Response Relationships (qAOPs), 18 & 19 October 2022

Syngenta: What Do We Do?

- **Protecting crops**

- Weed control - Control weeds that compete with crops for light and nutrients
- Insect control - Control pests which reduce yields by damaging crops
- Disease control - Prevent and cure fungal disease

- **Improving seeds**

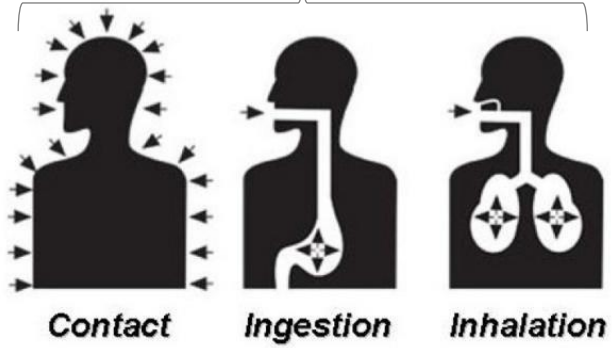
- Corn
- Soybean
- Sunflower
- Cereals
- Vegetables

Aggregate Exposure and Adverse Outcome Pathway Frameworks at Syngenta

Proposed uses → External dose → Internal exposure → Target site concentration

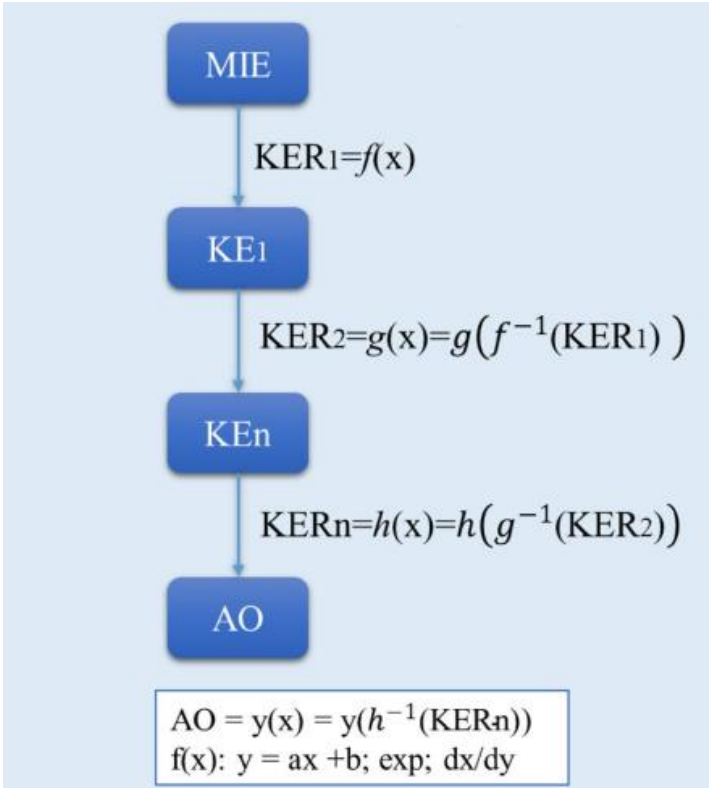
Exposure | ADME*

Multiple routes of exposure



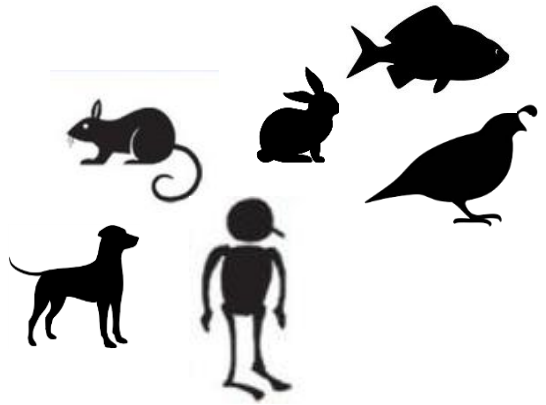
* Absorption, Distribution, Metabolism, Excretion

qAOP: Mechanistic Approach



Spinu, Cronin, Enoch, Madden, Worth. Archives of Toxicology (2020) 94:1497–1510. https://doi.org/10.1007/s00204-020-02774-7

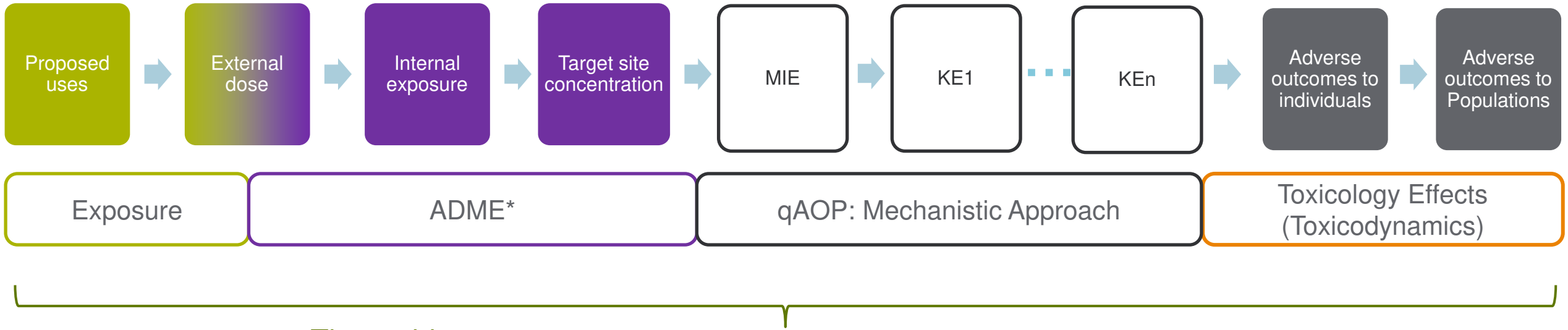
Multiple species



Adverse outcome to individuals → Adverse outcomes to populations

Toxicology Effects (Toxicodynamics)

More(!) challenges of Mechanistic qAOP Modelling



- The problems are:
  - Multiscale (spatially nm to m; temporally micro-seconds to years)
  - Nonlinear
  - Dynamic
- We need to predict exposure (target site, from different exposure routes) & toxicological effect
- We have data (some, but not from humans)

Our Mechanistic qAOP Approach: An Example Using HPPD Inhibitors

HPPD Herbicides

- Discovered for herbicidal use in 1980 (approx) (14 in class; 1 pharma)
- HPPD herbicides are one of the cornerstone herbicidal modes of action for weed control in corn and an essential component of the farmers toolbox
- HPPD herbicides bind tightly to both plant and mammalian HPPDs.
  - Results in elevated plasma tyrosine levels & a range of tyrosinemia associated toxicities.
- A critical success factor in Syngenta HPPD research projects is to **understand the tyrosinemia potential**.

Aim of the mechanistic approach:

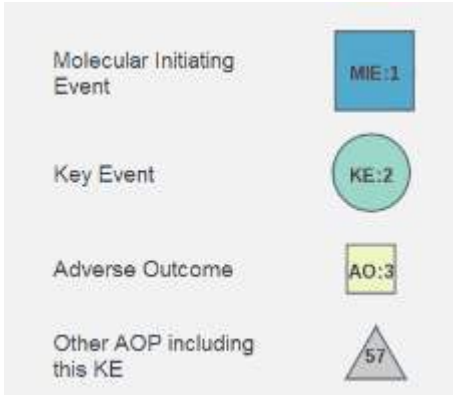
- To **aid compound selection, reduce the development time, reduce animal use** → allows early **mitigation of tyrosinaemia related toxicities**.

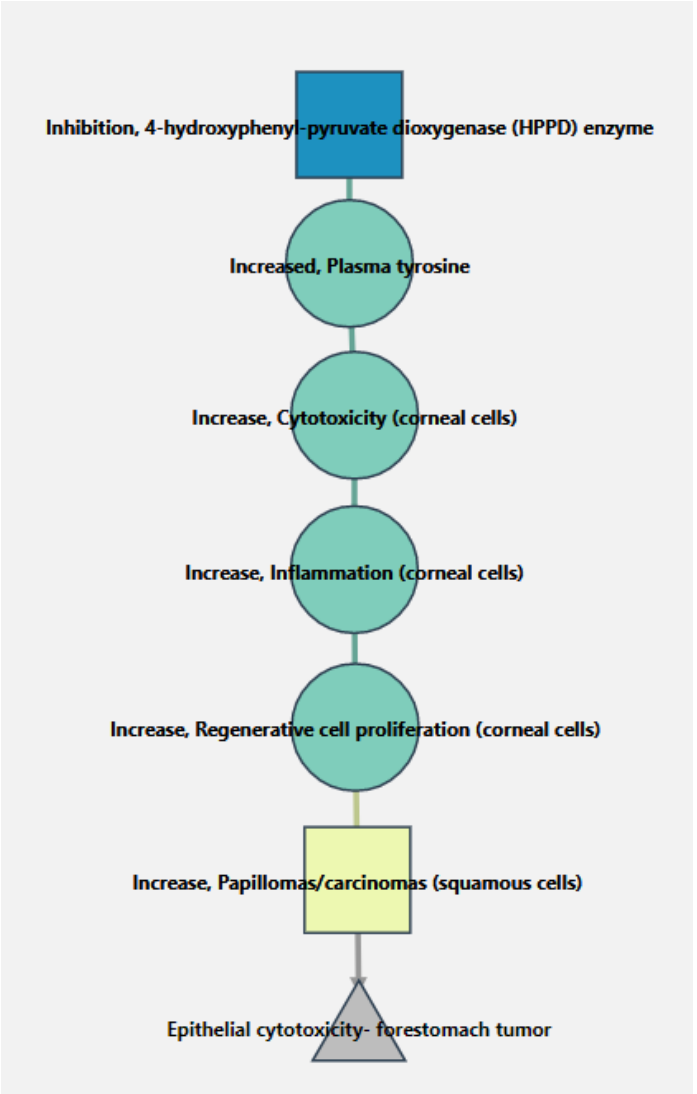
E.g. HPPD inhibition leading to corneal papillomas and carcinomas (in rat)

Under Development

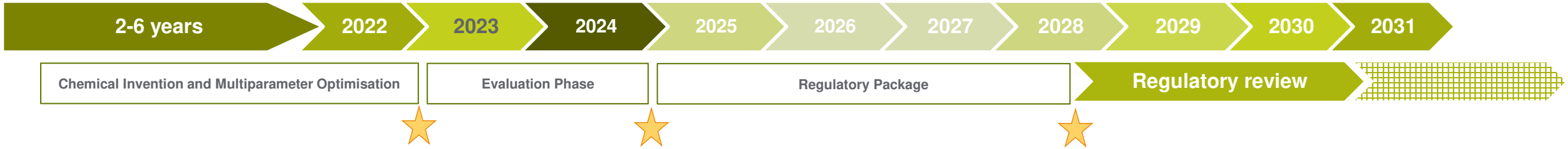
Aop: 114
https://aopwiki.org/aops/114

OECD project: 1.29





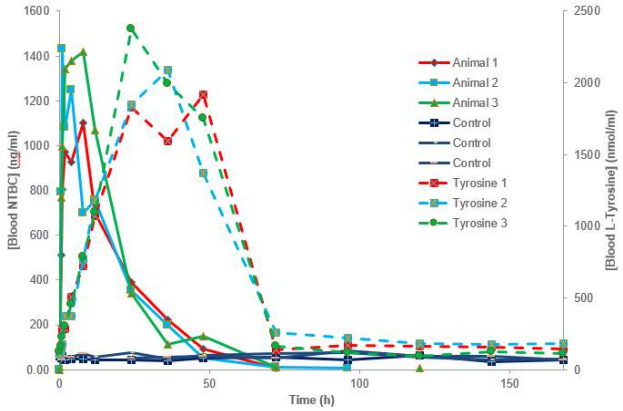
A Classical *In Vivo* Approach to HPPD Chemical Program Development



Business development decision points

in vivo tyrosine screens to assess/ rank HPPD inhibitor potency

- ♂ rat; n=3/group; gavage
- Low throughput;
- Moderate cost



in vivo repeat dose studies to assess tyrosine dose response

- Diet & gavage
- Rat & mouse + other species
- Low throughput;
- High cost

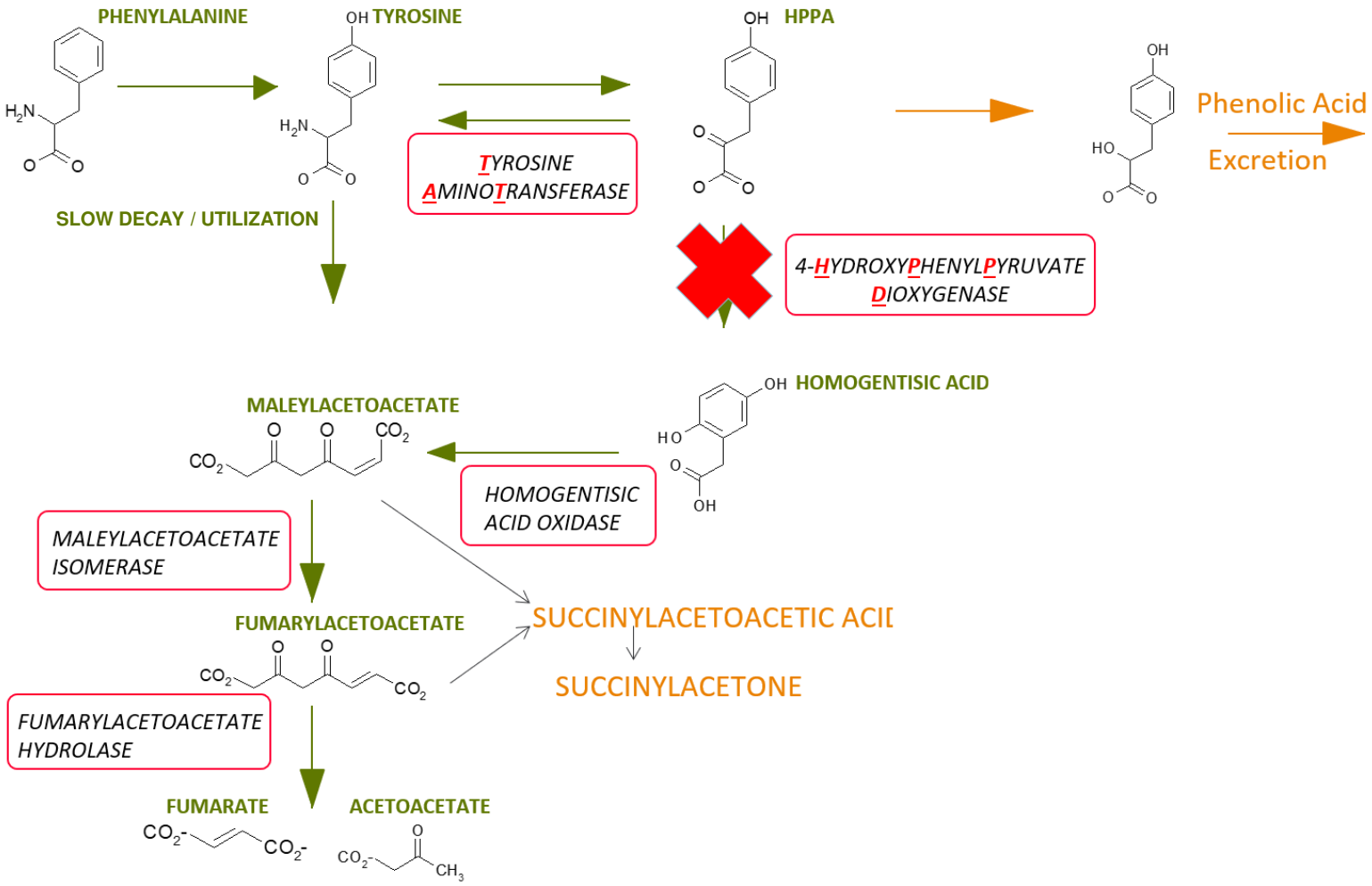
Studies to assess safety and regulatory endpoints

Limitations

- Reliance on *in vivo* animal studies to understand potency.
- Inability to truly develop Structural Activity Relationships/optimise chemistry (test system and throughput)
- Dose response and Points of Departure are not used in compound selection
- Difficult to assess species differences

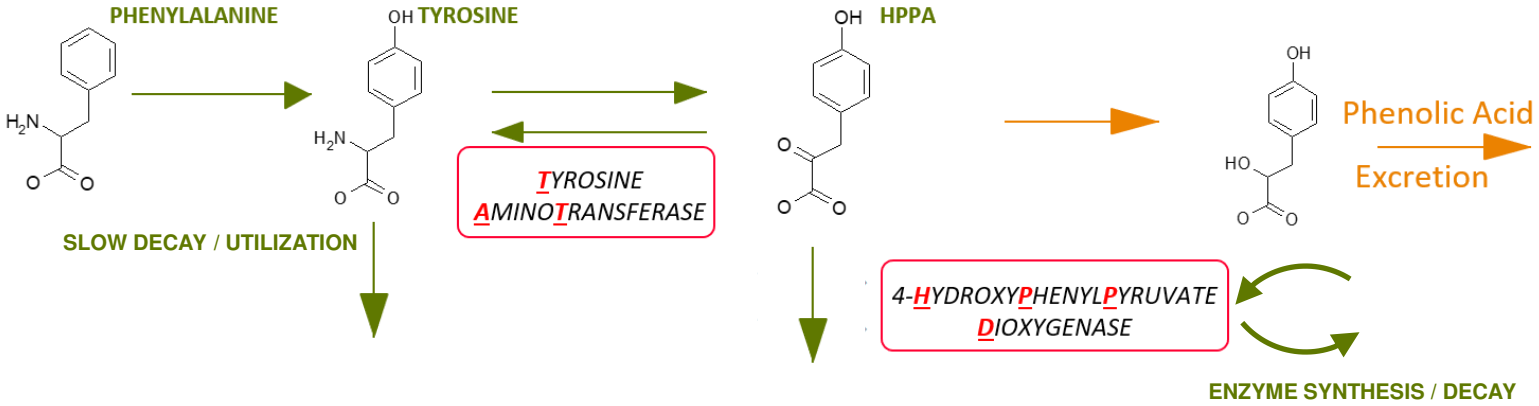
A qAOP:mechanistic Model of the Tyrosine Pathway and HPPD Inhibition

Pharmacological Inhibition of Tyrosine Catabolism In Mammalian Systems

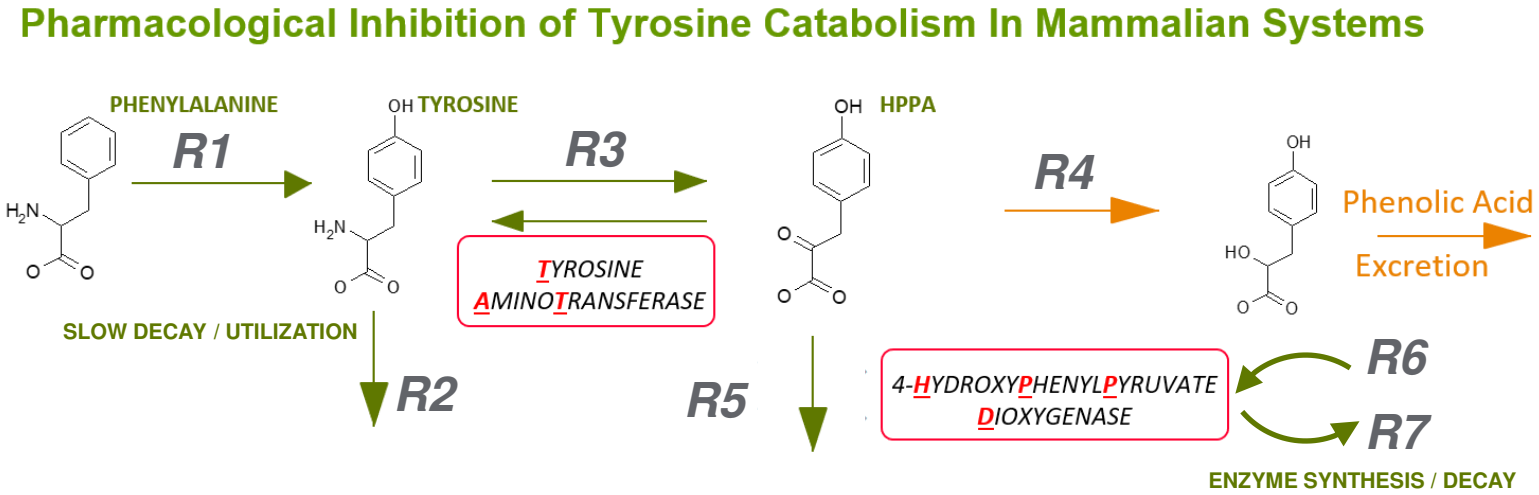


Identifying the relevant part of the pathway for mechanistic modelling

Pharmacological Inhibition of Tyrosine Catabolism In Mammalian Systems

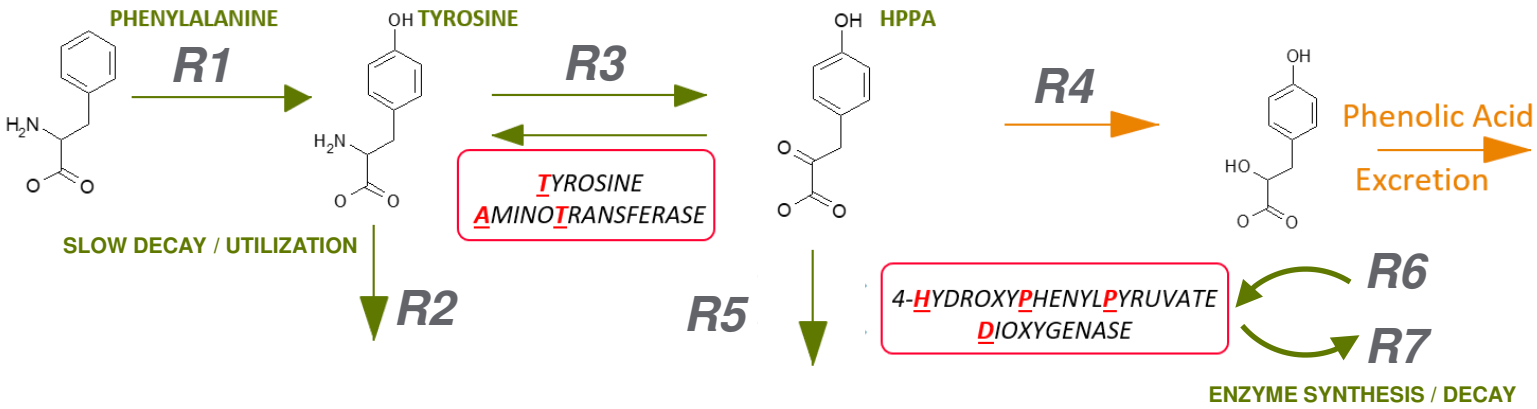


Characterising each step in the pathway (in a mechanistic manner)



Identifying the appropriate kinetic terms for each step

Pharmacological Inhibition of Tyrosine Catabolism In Mammalian Systems

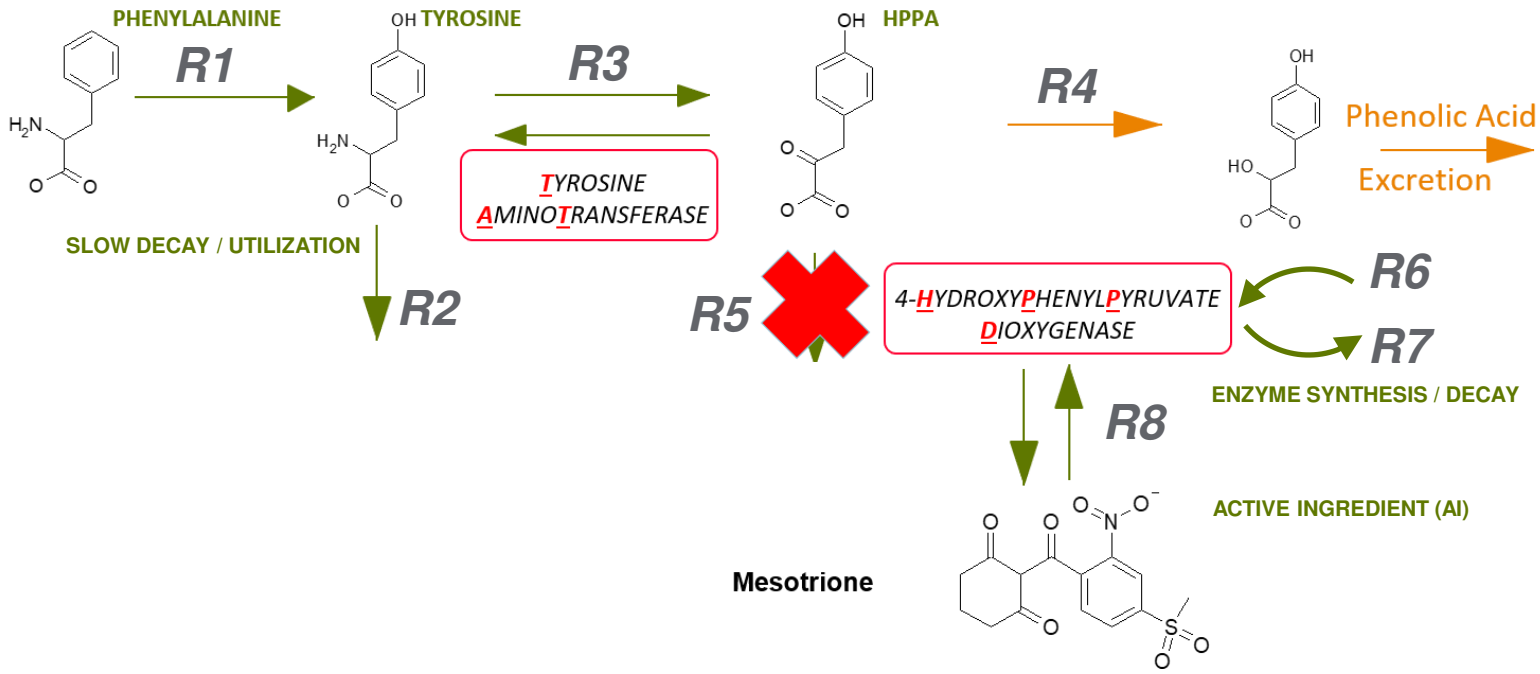


	Description	Rate law	Reaction
R1	Phenylalanine hydroxylated to tyrosine	Diurnal variation	R_{syn}
R2	Slow decay of tyrosine & thyroid hormone synthesis	First order (linear)	$K_{TYR}[\mathrm{TYROSINE}]$
R3	Tyrosine to HPPA via TAT (reversible reaction)	Reversible Michaelis-Menten	$\frac{V_{max}\left(\frac{[TYROSINE]}{K_T}-\frac{[HPPA]}{K_H}\right)}{1+\frac{[TYROSINE]}{K_T}+\frac{[HPPA]}{K_H}}$
R4	Excretion of HPPA as phenolic acids	First order	$K_{HPPA}[HPPA]$
R5	HPPA to HGTA via HPPD enzyme	Michaelis-Menten	$\frac{K_{cat}[HPPD]\times[HPPA]}{[HPPA]+K_{HPPD}}$
R6	HPPD enzyme synthesis	Zero order (constant)	$k_{deg}[HPPD]_0$
R7	HPPD enzyme decay	First order (linear)	k_{deg}

[ ]=concentration (nmol/ml)

Modelling the inhibition of HPPD

Pharmacological Inhibition of Tyrosine Catabolism In Mammalian Systems

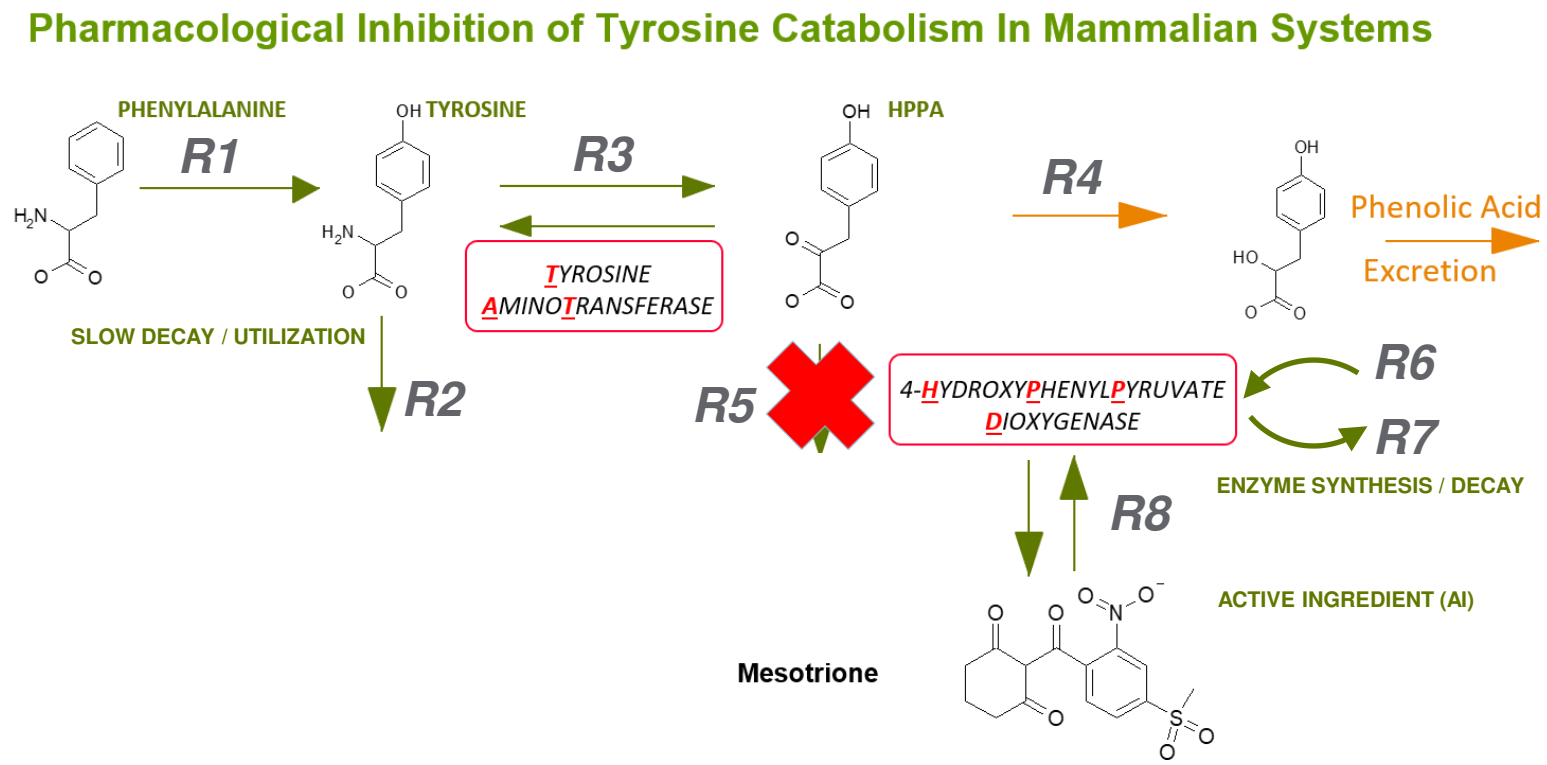


	Description	Rate law	Reaction
R8	Inhibition of HPPD enzyme by active ingredient (AI) (tight/slow binding)	Mass action	$k_{on}[HPPD] \times [AI] - k_{off}[HPPD{:}AI]$

[ ]=concentration (nmol/ml)

$HPPD{:}AI$=HPPD:AI complex

Combining kinetic terms to create the full model



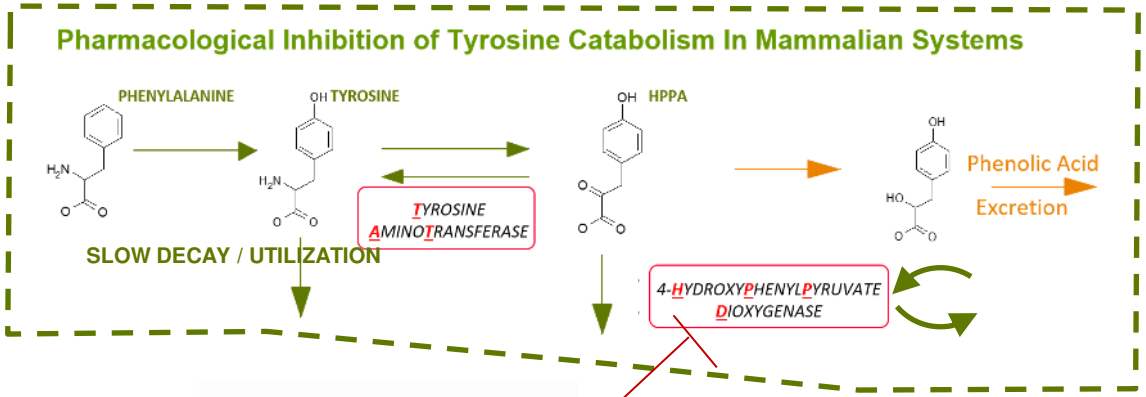
$$\frac{d[Tyrosine]}{dt} = \text{Rate at which } \textbf{Tyrosine} \text{ changes over time} = +\boldsymbol{R1} - \boldsymbol{R2} - \boldsymbol{R3}$$

$$\frac{d[HPPD]}{dt} = \text{Rate at which } \textbf{HPPD} \text{ changes over time} = +\boldsymbol{R6} - \boldsymbol{R7} - \boldsymbol{R8}$$

$$\frac{d[HPPA]}{dt} = \text{Rate at which } \textbf{HPPA} \text{ changes over time} = +\boldsymbol{R3} - \boldsymbol{R4} - \boldsymbol{R5}$$

$$\frac{d[HPPD:AI]}{dt} = \text{Rate at which } \textbf{HPPD:AI} \text{ changes over time} = +\boldsymbol{R8}$$

HPPD mechanistic model: A Combined PharmacoKinetic/PharmacoDynamic Model



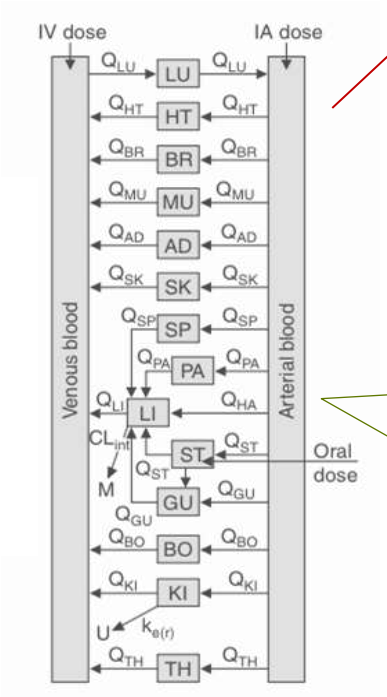
Tyrosine (TYR) dynamics

Parameterised via literature and in house data

HPPDi I (slow/tight binding, k_{on}, k_{off})

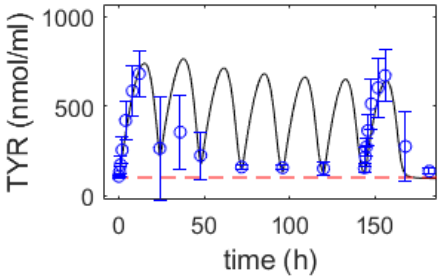
Link

In vitro measured values

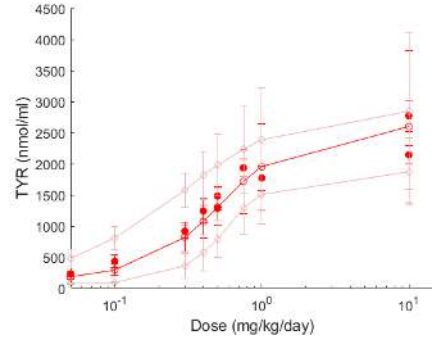


Physiologically Based Kinetic modelling – to predict internal exposure in the rat

7 day repeat gavage



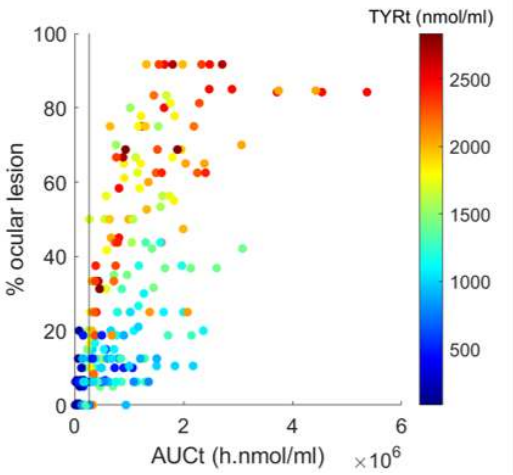
90 day dietary dosing



Prediction of extended *in vivo* tyrosinemia responses

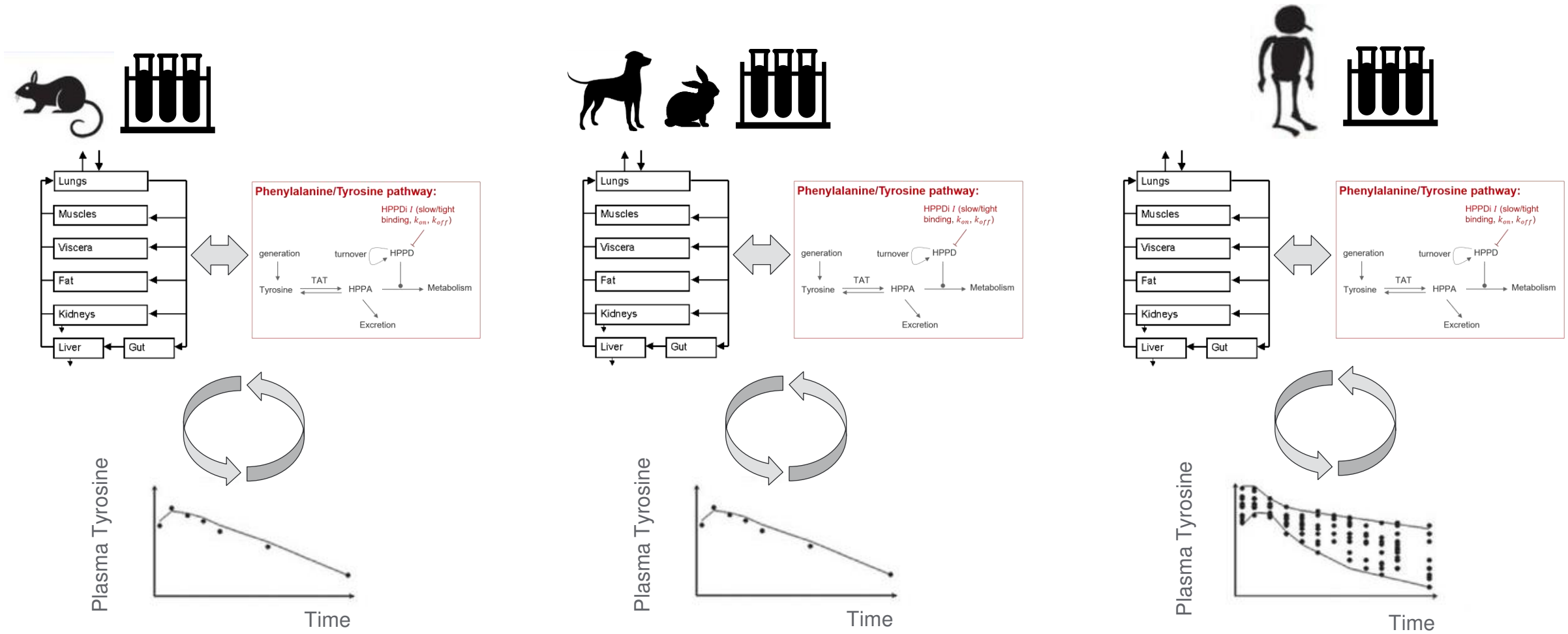
Prediction of ocular lesion risk

Plasma TYR and ocular TYR concentrations were measured in vivo as well as **incidences of corneal lesions** after oral administration of HPPD Inhibitors (gavage and diet):



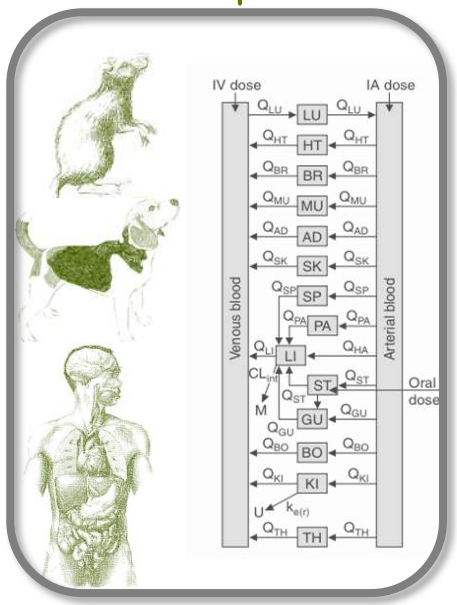
Species extrapolation of toxicity risk

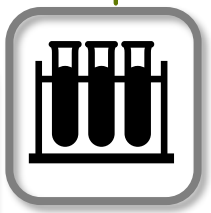
IVIVE PK → Species extrapolation → **Human population risk**

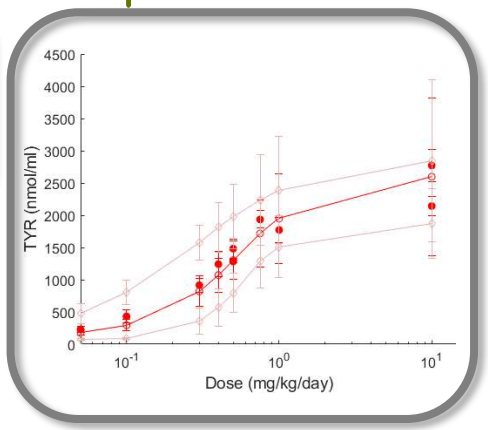


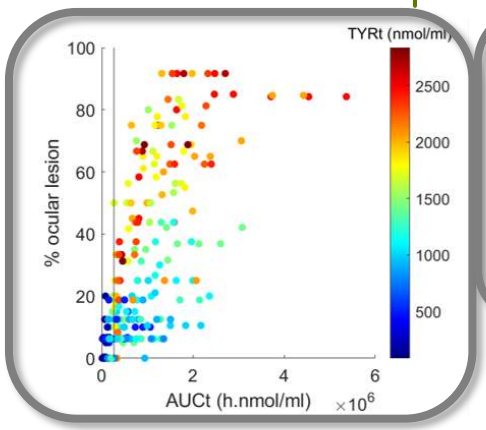
A qAOP:Mechanistic Model For HPPD Inhibition

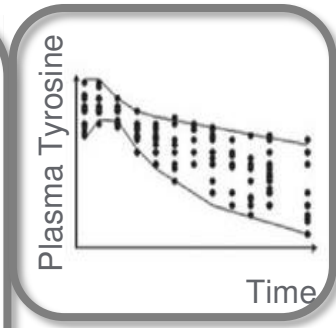
Proposed uses → External dose → Internal exposure → Target site concentration → MIE (Inhibition of HPPD enzyme) → KE1 (increased Plasma Tyrosine) → KE2 (Cytotoxicity (corneal cells)) → KE3 (Inflammation (corneal cells)) → KE4 (Regenerative cell prolif (corneal cells)) → Adverse outcomes to individuals (papillomas/ carcinomas (squamous cells)) → Adverse outcomes to Populations











Probabilistic Aggregate Exposure Models | **Multiple Species PBK** | ***In Vitro*** | ***In Silico* (Mechanistic Mathematical Model)** | ***In Vivo Data / Statistical Methods*** | ***popPBK / Monte Carlo sampling***

The *in vivo* burden required to build this qAOP:Mechanistic Model

Proposed uses → External dose → Internal exposure → Target site concentration → MIE (Inhibition of HPPD enzyme) → KE1 (increased Plasma Tyrosine) → KE2 (Cytotoxicity (corneal cells)) → KE3 (Inflammation (corneal cells)) → KE4 (Regenerative cell prolif (corneal cells)) → Adverse outcomes to individuals (papillomas/ carcinomas (squamous cells)) → Adverse outcomes to Populations

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National Health and Nutrition Examination Survey
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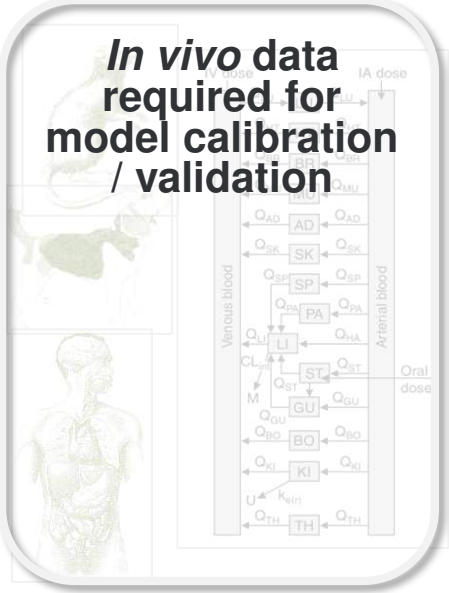
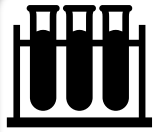
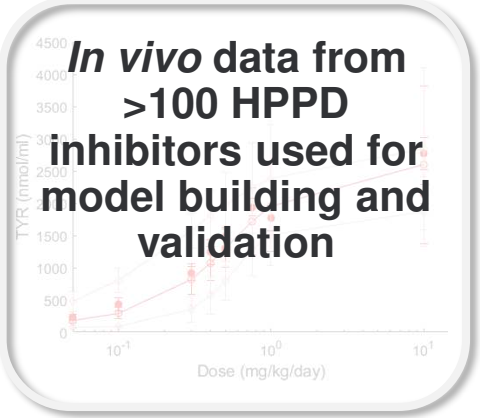
***In vivo* data required for model calibration / validation**

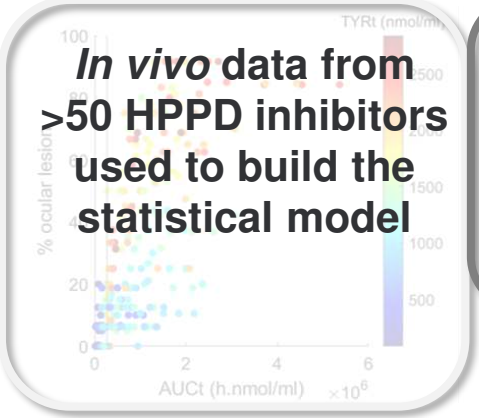


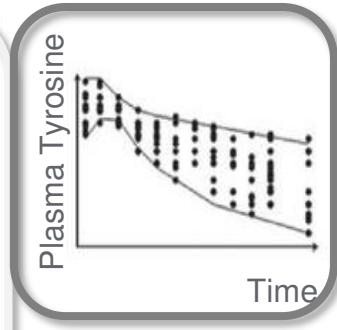


***In vivo* data from >100 HPPD inhibitors used for model building and validation**



***In vivo* data from >50 HPPD inhibitors used to build the statistical model**





Probabilistic aggregate exposure models | **Multiple species PBK** | ***In Vitro*** | ***In Silico* (Mechanistic Mathematical Model)** | ***In Vivo Data / Statistical Methods*** | ***popPBK / Monte Carlo sampling***

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Use of NAMs to alleviate this burden for future model building

Proposed uses → External dose → Internal exposure → Target site concentration → MIE (Inhibition of HPPD enzyme) → KE1 (increased Plasma Tyrosine) → KE2 (Cytotoxicity (corneal cells)) → KE3 (Inflammation (corneal cells)) → KE4 (Regenerative cell prolif (corneal cells)) → Adverse outcomes to individuals (papillomas/ carcinomas (squamous cells)) → Adverse outcomes to Populations

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In vitro **ADME parameters & read across approach to identify PBK analogues** (Madden, Paini, Penson, Thompson, Webb, Tan)

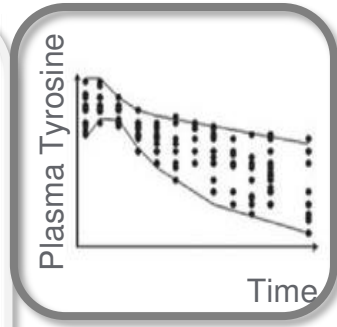
Uncertainty!

In vitro **data to quantify kinetics in the pathway + some in silico models are available**

Uncertainty!

In vitro **data (and possibly in silico models) to quantify kinetics in the toxicity pathway**

Uncertainty!



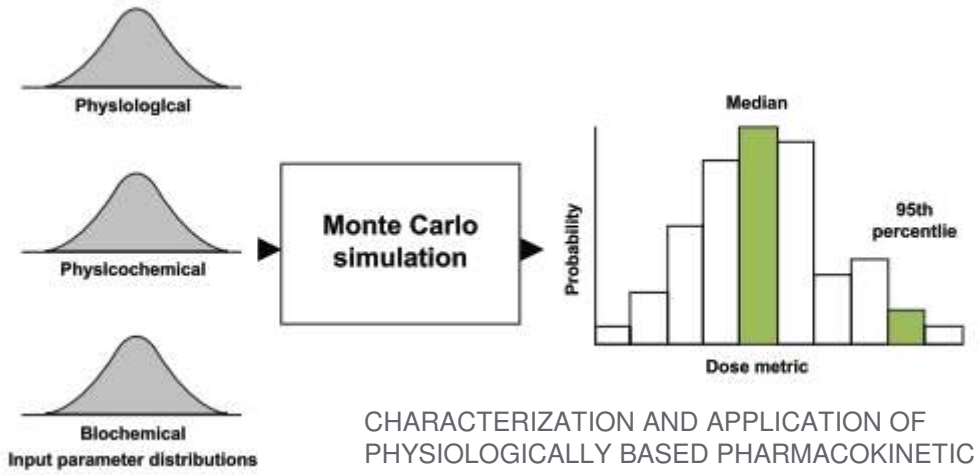
Probabilistic aggregate exposure models	Multiple species PBK	*In Vitro*	*In Silico* (Mechanistic Mathematical Model)	*In Vivo Data / Statistical Methods*	*popPBK / Monte Carlo sampling*

Uncertainty in *in silico* predictions

- ***There are In silico* methods to characterise and quantify uncertainty** → allows characterization of the level of confidence in the *in silico* model.

- **Issues with utilizing existing *in silico* models in a qAOP context:**
  - Insufficient information in publications (missing equations, code, parameter values)
  - Errors in the models (incorrect units, unrealistic assumptions)
  - Unable to reproduce key outputs
  - But, repositories of curated mathematical models of biological and biomedical systems do exist, e.g.

BioModels



CHARACTERIZATION AND APPLICATION OF PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS IN RISK ASSESSMENT, WHO (2010)

Blood exposure	Uncertainty			
Sensitivity		High	Medium	Low
	High		• Bodyweight • Lung flow rate • Liver flow rate	
	Medium			• Gut flow rate • Kidney flow rate • Colon transit time • Colon absorption rate
	Low			• Muscle flow rate • Skeletal flow rate • Kidney volume • Renal excretion rate

Our thoughts to go into Day 2 regarding mechanistic qAOP approaches…

- We developed a **quantitative multi-scale *in silico* model (*mechanistic qAOP*)** of mammalian Hydroxyphenylpyruvate dioxygenase (HPPD) inhibition to predict thresholds for toxicity and allow quantitative cross-species extrapolation.
- In this example, ***the gap*** is how to define the quantitative KE response-response relationship between tyrosine and the adverse effects in eyes etc using only NAMs.
  - Is it possible to derive this using only NAMs? Do we need to? (i.e. can we identify appropriate human health protective thresholds using clinical data?)
- **Mechanistic models based around biochemical perturbations have to be translated across spatiotemporal scales:**
  - So KE (or KER) "skipping" will often be needed (due to difficulties in quantifying some KEs (or KERs), i.e. data gaps)
  - Significant *in vivo* data was used for model developmental and validation (but this does give confidence in the model predictions! – required for regulatory acceptance)
  - A fully NAM approach is possible (but there are associated uncertainties!)
  - Generically, **the issue is how to infer the quantitative key event response-response relationship between biochemical changes and the adverse effects, using only human relevant NAMs**.

Bringing plant potential to life

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