Description for a problem studied at the UK Mathematics-in-Medicine Study Group Reading 2011
< http://www.maths-in-medicine.org/uk/2011/hppd-inhibition/ >

HPPD inhibition case study

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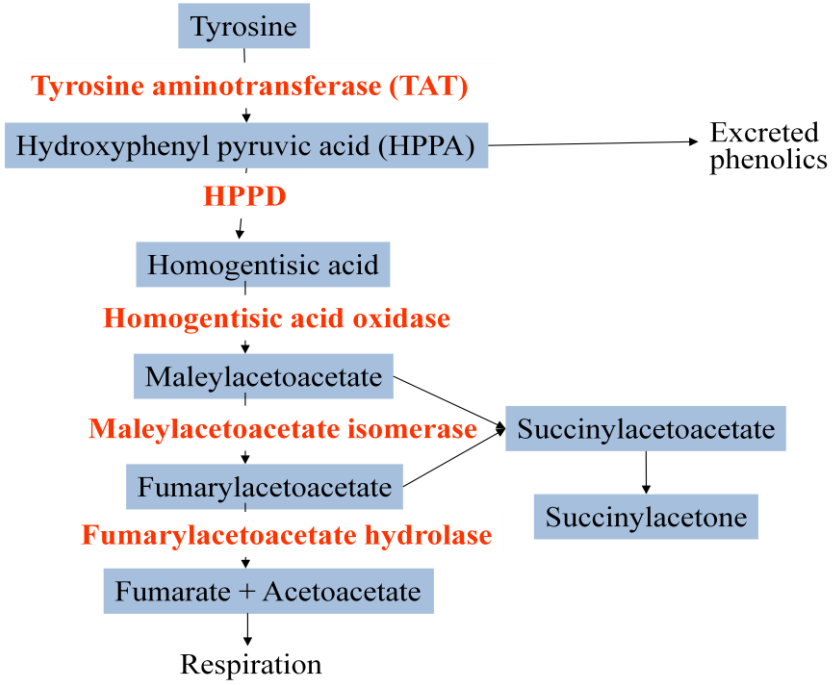
Summary

4-hydroxyphenylpyruvate dioxygenase (HPPD) is an enzyme involved in the breakdown of excess tyrosine, an amino acid, in the body of mammals. It is present mainly in the liver. One inhibitor of HPPD is used successfully as a pharmaceutical, where it acts by blocking the tyrosine breakdown pathway. However, the inhibition of HPPD can result in toxicities in some species, especially rats. In plants the same enzyme has a completely different role, being essential for the synthesis of components needed for photosynthesis. The inhibition of HPPD is the mode of action of a number of commercialised herbicides, as this inhibition causes bleaching and ultimately death of the plant. One of the challenges in discovering and developing new HPPD inhibiting herbicides is to ensure their safety to mammals. A variety of *in silico*, *in vitro* and *in vivo* information is now available. The challenge is to suggest ways of making better use of the existing database to assess the potency of new HPPD inhibitors. In particular, to explore the extent to which *in silico* and *in vitro* information can be used to predict *in vivo* outcomes.

Information available

The tyrosine catabolism pathways is shown below. This pathway breaks down excess tyrosine in the diet or resulting from the normal replacement of proteins in the body.



HPPD inhibitors can block this pathway, resulting in the accumulation of tyrosine in the body, and this tyrosine itself can cause a variety of toxicities (elevated plasma tyrosine is called tyrosinaemia). In conditions of HPPD inhibition the excretion of various phenolic compounds in urine places an upper limit on the concentration of tyrosine that accumulates, and this varies between species (and in the case of rats, sexes). These species differences are believed to be due to differences in the amount of TAT, the top enzyme in this pathway, though other factors may also be relevant.

One HPPD inhibitor, nitisinone (also called NTBC) has been used in man for many years to treat a rare metabolic disorder. This has resulted in the generation of human data on plasma concentrations of the drug and of tyrosine, as well as human safety data.

HPPD has a completely different role in plants, where it is essential for the synthesis of compounds needed for photosynthesis. A number of commercialised herbicides act by inhibiting HPPD (roughly 10 compounds), resulting in a characteristic bleaching of weeds. In the course of discovering and developing such herbicides, a range of different data and information has been generated, which is shown below.

Main information available (numbers of chemicals in red)

<table>
<tr><th>Species</th><th>in silico</th><th>in vitro</th><th>in vivo</th></tr>
<tr><td>Plants</td><td rowspan="4">Crystal structures

HPPD protein sequences and homology modelling</td><td>HPPD inhibition assay data (‘00s)</td><td>Herbicidal potency (‘000s)</td></tr>
<tr><td>Mouse</td><td></td><td>Assay (kinetic and tyrosine data, ‘00s)
Toxicity studies (‘0s)</td></tr>
<tr><td>Rat</td><td>HPPD inhibition assay data (‘0s)</td><td>Assay (kinetic and tyrosine data, ‘0s)
Toxicity studies (‘0s)</td></tr>
<tr><td>Human</td><td></td><td>Kinetic and tyrosine data and safety record (1)</td></tr>
</table>

The structure and sequence of the HPPD enzyme is known in several instances. It is highly conserved in all species and very highly conserved within phyla (eg animals). *In vitro* plant HPPD inhibition data are available for many compounds, but high quality *in vitro* rat data have only recently been generated. The various steps in the process potentially resulting in toxicity are:

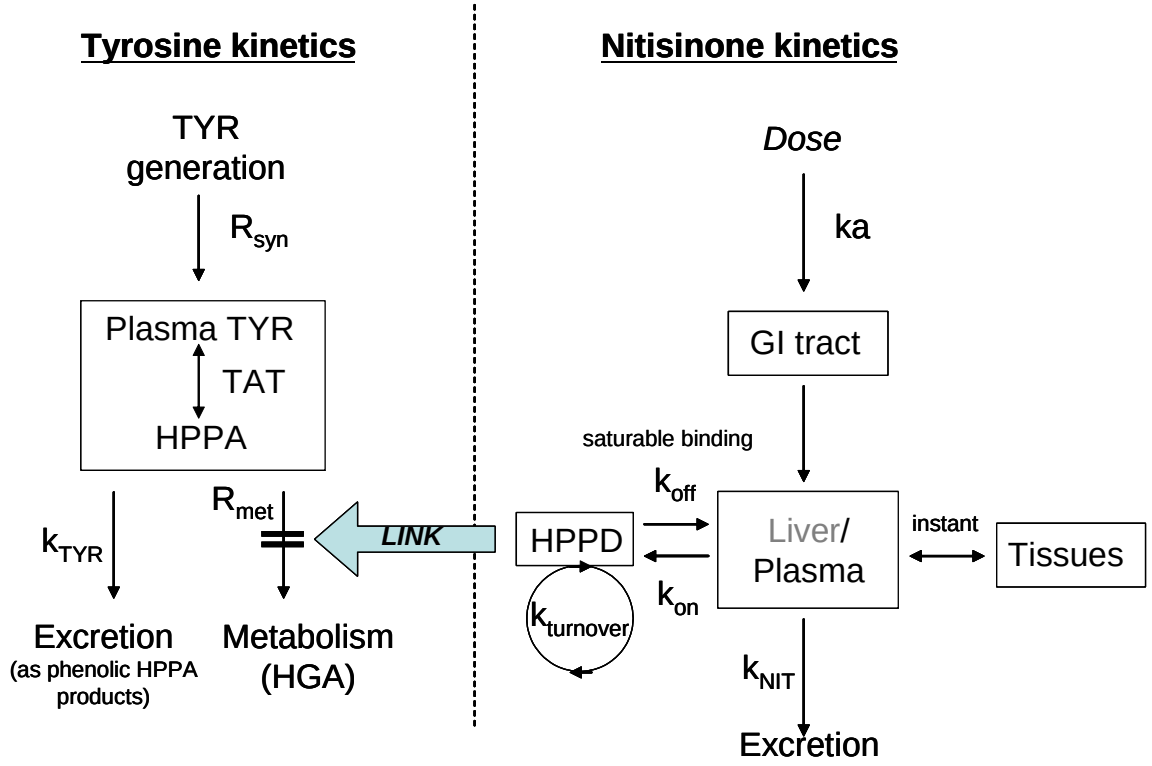
HPPD inhibitor absorbed ⇨ Inhibition of HPPD ⇨ Tyrosine elevation ⇨ Toxicity

A single dose assay in the mouse has long been used to measure concentrations of test compound (kinetic data) and tyrosine in plasma over time. The mouse has been used because it is the test species closest to man in its response to HPPD inhibitors, and because the screen requires minimal test compound. The regulatory view has shifted, and the rat is now the main tox species of concern - there is a similar assay in the rat for which there is data on a smaller range of test compounds. These assays produce no toxicity due to their short duration. Repeat dose toxicity studies in a range of species have been conducted for all marketed HPPD inhibitors.

Human data is mainly available for nitisinone. However, there are also human data for rare genetic defects of each enzyme in the tyrosine degradation pathway. There is also limited human kinetic and tyrosine data for one other HPPD inhibitor.

Some modelling work has also been done, both in the rat and in man (for example, see the figure below). The modelling has been based on the pharmaceutical nitisinone, which is somewhat extreme in its properties compared to many HPPD inhibitors and all commercial HPPD inhibiting herbicides. This

modelling has not significantly incorporated *in vitro* data, and has not been applied to a range of HPPD inhibitors.



The challenge is to suggest ways of making better use of the existing database to assess the potency of new HPPD inhibitors. In particular,

- Can existing information be used to better predict *in vivo* outcomes for novel HPPD inhibitors?
    - What insights are to be had on the relationship between *in vitro* and *in vivo* data?
    - Can we better understand the relationship between mouse, rat and human responses *in vivo*?
    - Can we use models to better address these questions?
- Can we use the data to better separate toxicity to plants and mammals
- What new data would increase the ability to make *in vivo* predictions based on *in vitro* data.