Development of a web-based toolbox to support quantitative in vitro-to-in vivo extrapolations (QIVIVE) within non-animal testing strategies.

Ans Punt †*, Nicole Pinckaers †, Ad Peijnenburg †, Jochem Louisse †

† Wageningen Food Safety Research
Akkermaalsbos 2
6708 WB Wageningen
The Netherlands

*Corresponding author address
E-mail: ans.punt@wur.nl
Tel: +31 317 481025

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LC-MS analysis

Samples from the metabolic clearance experiments with HLS9 and RLS9 were analysed on a Waters Acquity UPLC (Milford, USA) system. The system was equipped with a Waters Acquity UPLC BEH C18 (100 x 2.1 mm, 1.7 μm) column for analysis of MPB. The column heater was kept at 60°C and the temperature of the autosampler was kept at 10 °C. In case of PG, OG and DG the system was equipped with a HSS T3 C18 (2.1 x 100 mm, 1.8 μm) column in a column heater kept at 35 °C. MPB was eluted from the column using a gradient of 0.05% ammonia in water (A) and 0.05% ammonia in acetonitrile/water (90:10 v/v %) (B). The gradient was initiated at 0% B, kept at 0% B for 2 minutes, followed by a linear increase to 50% B in 1 minute and kept at this condition for 1 minute. Then, a linear increase was initiated to 100% B in 2 minutes and was kept at 100% B for 2 minutes to return to the initial condition of 0% B in 0.5 minutes at which it was kept for 2.5 minutes, before the next injection.

For propyl gallate, octyl gallate and dodecyl gallate the mobile phase A consisted of water (A) and 95% methanol (B), both containing 1 mM ammonium formate and 0.1% formic acid. The gradient started at 0% B, was kept at 0% B for 2 minutes, was then linearly increased to 100% B in 1 minute. This mobile phase composition was kept for 3 minutes, after which the gradient linearly decreased to 0% B in 4 minutes. The gradient was kept at 0% B for 1 minute before the next sample injection started.

Mass spectrometric detection was performed with a Micromass Quattro Ultima mass spectrometer (Waters, Milford, USA), which was equipped with an electrospray ionization interface (ESI). All samples were analysed in negative ion mode. A capillary voltage of 2.50 kV, a source temperature of 120 °C, a desolvation temperature of 350 °C, a cone gas flow of 194 L/h and a desolvation gas flow of 564 L/h was used. Argon was used as collision-induced dissociation gas. Cone voltage and collision energy were optimized by direct infusion for each compound. Specific dwell time, cone voltage, collision energy, ion mode and MRM transitions for each compound are described in Table 2 of Appendix A. Calibration curves were prepared in 100 mM potassium phosphate buffer with 5 mM magnesium chloride containing the same methanol:buffer ratio and liver S9 concentration as used in the incubations. S9 was inactivated in these samples by adding methanol before S9 to the samples.
Toolbox workflow

1. The toolbox can be accessed at [www.qivetools.wur.nl](http://www.qivetools.wur.nl)

Open tab panel to start with the PBK workflow
2. Physicochemical parameters tab

Add the pKa(s), logP and MW of your compound.

Fup is automatically calculated based on the provided physchem based on a method of Lobell and Sivarajah ([https://doi.org/10.1023/B:MODI.000006562.93049.36](https://doi.org/10.1023/B:MODI.000006562.93049.36)).

No adequate calculation methods are currently available for calculating blood:plasma ratios. A “Custom input” can be provided. As initial default approach (when no in vitro data are available), the blood:plasma ratio can be set to 1.

To include an in vitro measured Fup, select “Custom input”.

Two approaches can be selected:
- Berezhkovskiy ([https://doi.org/10.1002/jps.20073](https://doi.org/10.1002/jps.20073))
- Rodgers and Rowland ([https://doi.org/10.1002/jps.20502](https://doi.org/10.1002/jps.20502))

Note that when using the method of Rodgers and Rowland the human and rat partition coefficients are the same, as the underlying physiological data are assumed to be the same.
2. Metabolism tab

Scaling factor is automatically adjusted based on the selected enzyme source. The value of the scaling factor can also be manually changed.

The calculation method for estimating the free concentration in the in vitro incubation is automatically adjusted based on the selected enzyme source.

Add the in vitro measured intrinsic clearance according to the provided units. Select the enzyme source that was used (hepatocytes, microsomes or S9).

Provide the number of cells/ml or mg S9 or microsomes/ml that was present in the in vitro incubation to calculate the free fraction in the in vitro incubation.
3. Intestinal uptake tab

| QIVIVE tools |   |
|--------------|---|
| **Intestinal uptake human** | **Intestinal uptake rat** |
| \( k_a \) (/h) | \( k_a \) (/h) |
| 1 | 1 |
| \( F_a \) | \( F_a \) |
| 1 | 1 |

Include the intestinal uptake rate \( (k_a) \) and the fraction absorbed \( (F_a) \). By default, the fraction absorbed is assumed to be 1. \( k_a \) can be calculated from a Caco-2 Papp value.
4. PBK result

PBK model results for all organs can be downloaded as .CSV file. The simulation can take some time. This "Making plot" bar indicates that the simulation is ongoing.

Select which species needs to be displayed in the graph and select the exposure route.

Click "Run model" to initiate the calculation.

Perform the simulation at different dose levels to find the dose level that leads to plasma or tissue concentrations that are equivalent to the in vitro biological effect concentration of interest.

Rerun the model each time a change has been made in the "Scenario" field or when changes are made in the different input tabs.

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