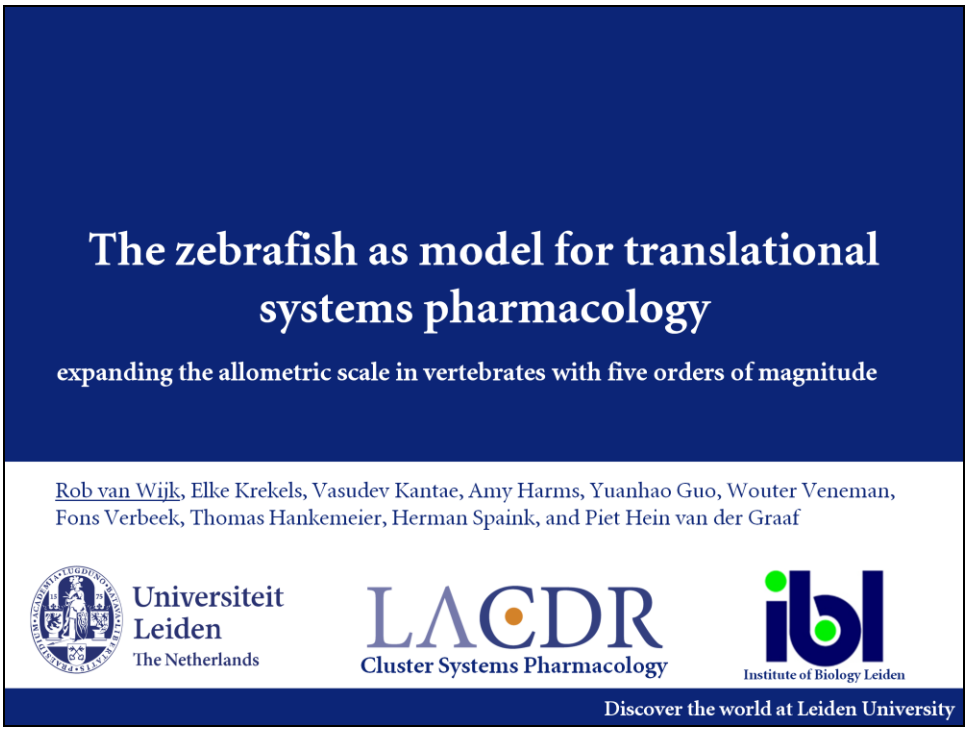
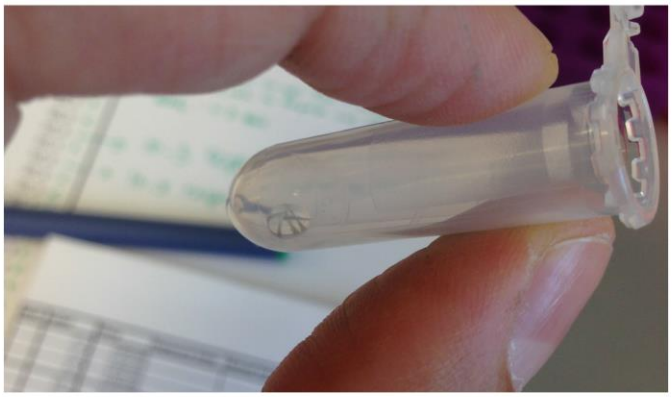
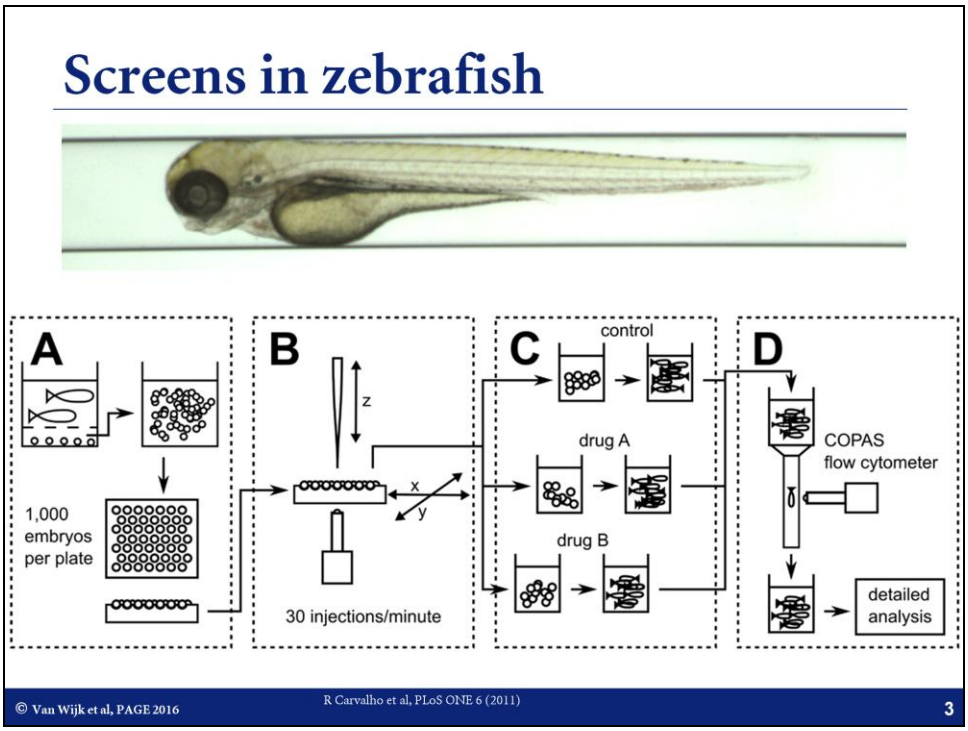


Key message: How can pharmacological modeling improve research in zebrafish, and how might zebrafish research improve translational pharmacology

Screens in zebrafish

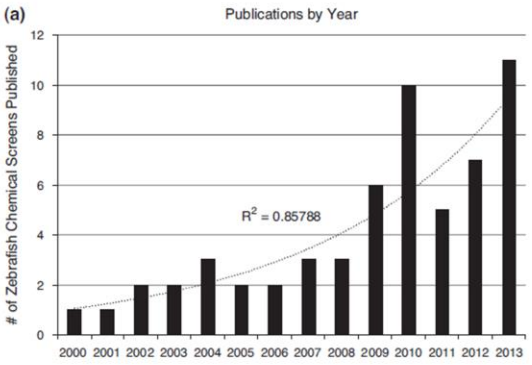


Key message: The zebrafish embryo/larva is a relatively new model organism. Because of its small size, transparency and external fertilization, it is very suitable for high throughput research



Key message: An example of a high throughput screen, where fertilized eggs are infected by robotic injection, exposed to different drugs, and analysed by flow cytometry.

Screens in zebrafish



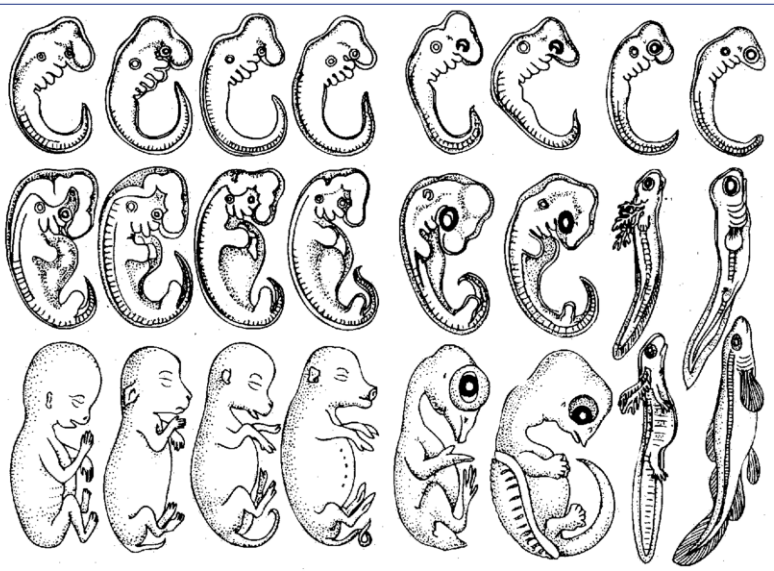
Abbott gsk AstraZeneca Lilly MERCK Bristol-Myers Squibb NOVARTIS Johnson & Johnson Pfizer

AJ Rennekamp et al, Curr Opin Chem Biol 24 (2015)
A Fleming et al, Drug Discov Today Dis Models 10 (2013)

4

Key message: These high throughput screens are increasingly performed in this model organism, not only in academia but also in industry (see p.21 for source table for companies)

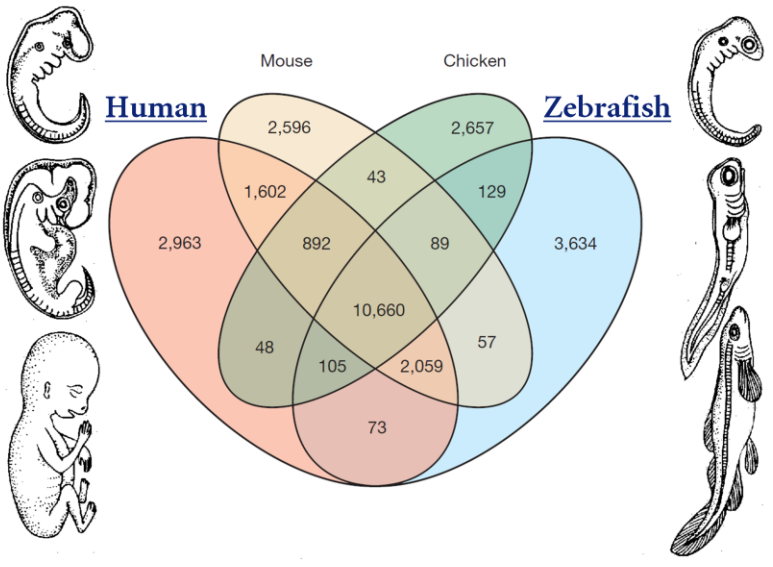
Strong homology with higher vertebrates



KV Kardong, Vertebrates p191 (1998)

Key message: Zebrafish is an attractive model organism because high throughput is possible, but it remains comparable with higher vertebrates, as this old textbook image visualizes.

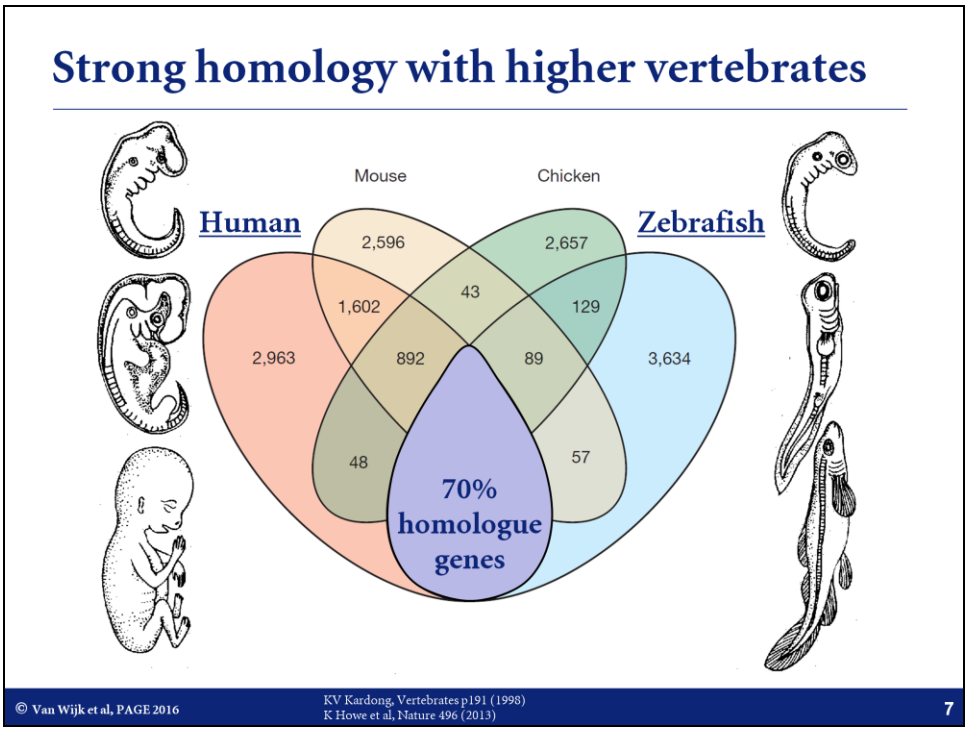
Strong homology with higher vertebrates



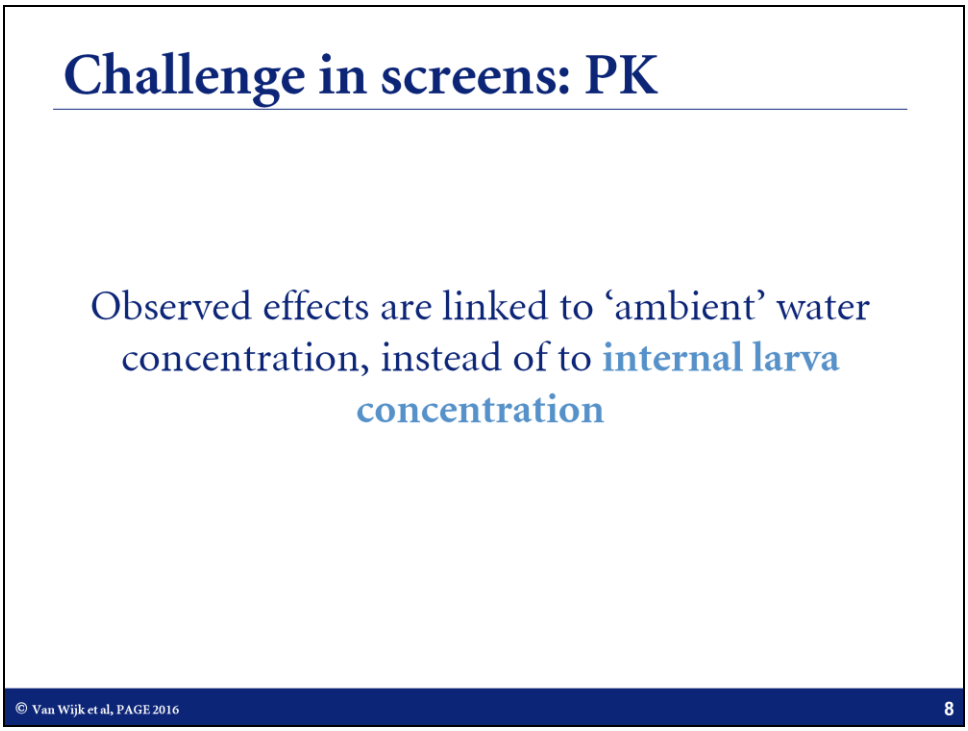
KV Kardong, Vertebrates p191 (1998)
K Howe et al, Nature 496 (2013)

6

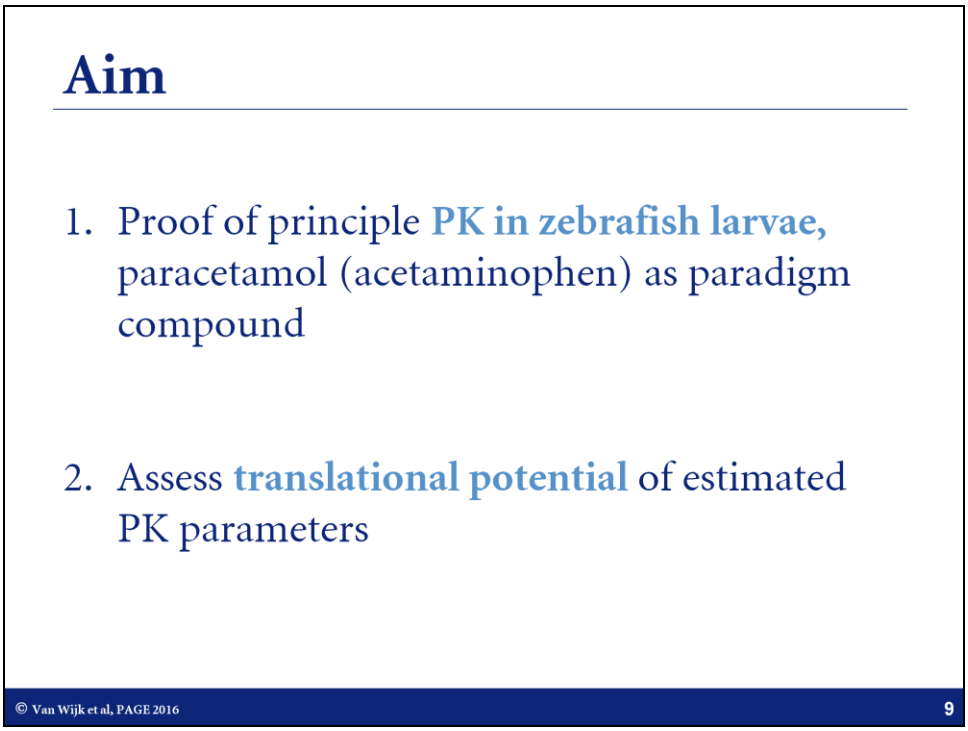
Key message: More recently, the genome of the zebrafish has been sequenced;



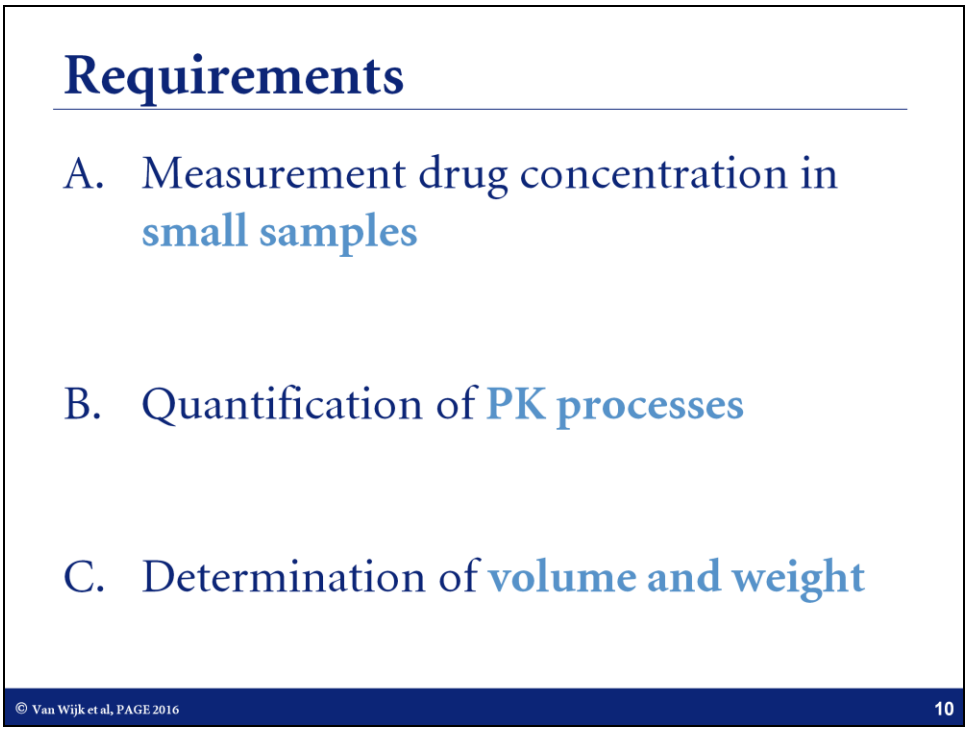
Key message: More recently, the genome of the zebrafish has been sequenced; 70% of all human genes have a homologue in zebrafish. Among those genes are phase I and II metabolizing enzymes, and numerous drug targets.



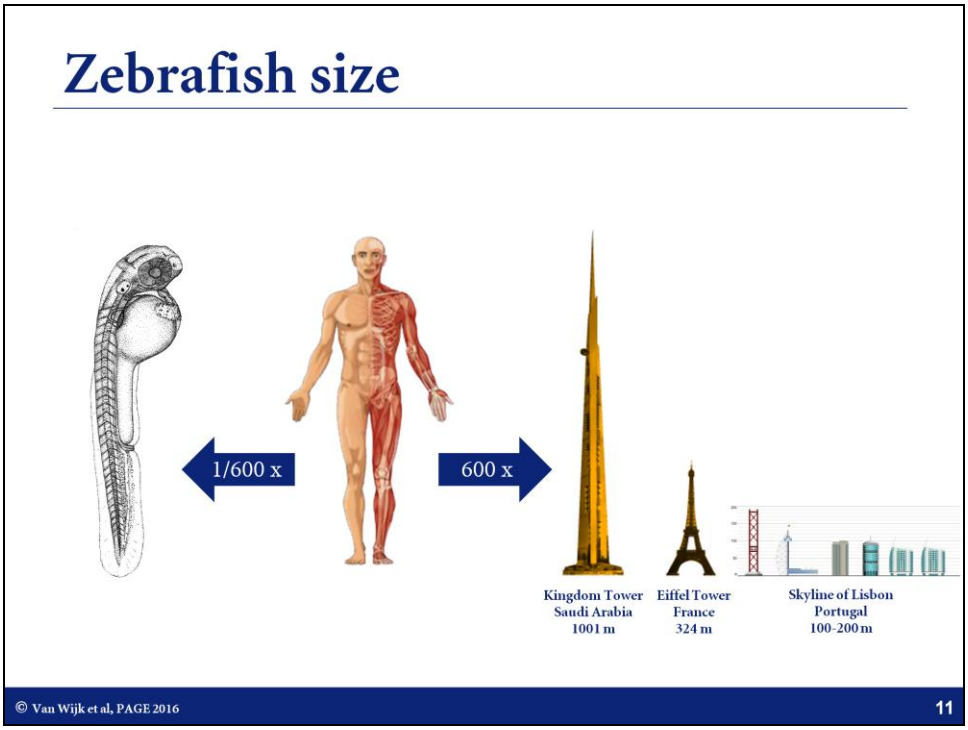
Key message: Zebrafish are comparable to higher vertebrates, but have the advantage of high throughput screens; the results of in vivo with the ease of in vitro. The challenge however is that observed effects in these screens are linked to the exposure medium (comparable to in vitro, where medium concentration around the cells is relevant), instead of to internal concentration (as would be the case in in vivo research in rodents or even humans).



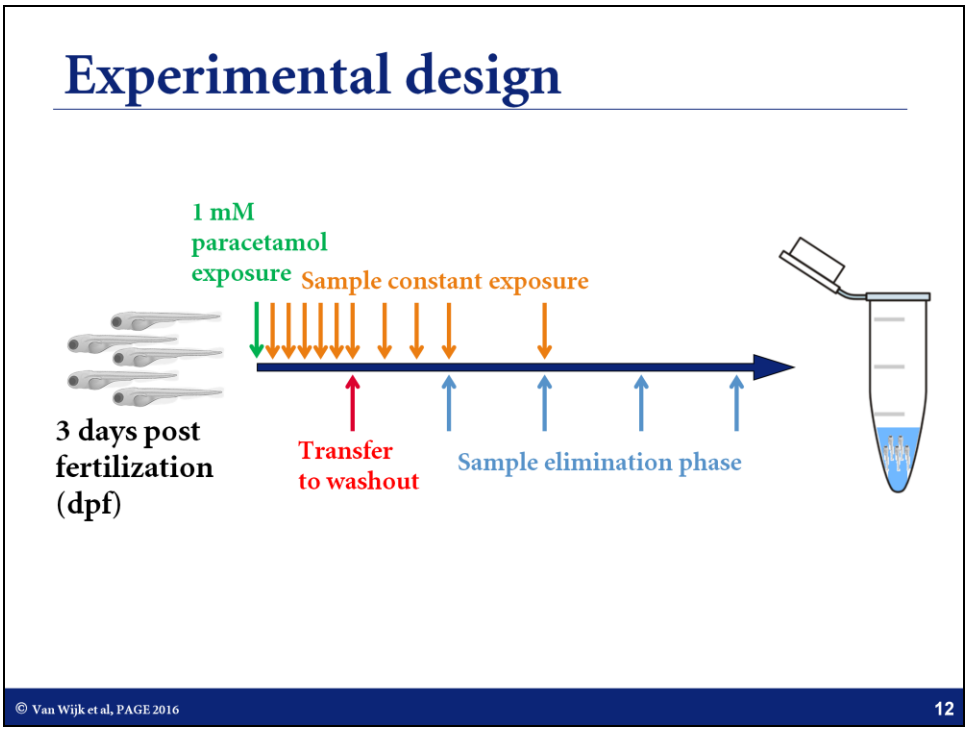
Key message: Our project aims to improve these screens by incorporating pharmacokinetics. A proof of principle experiment with paradigm compound paracetamol is performed for this purpose. Additionally, the potential for translational pharmacology is explored.



Key message: These three requirements are essential, but difficult to achieve because of the small size of the fish.

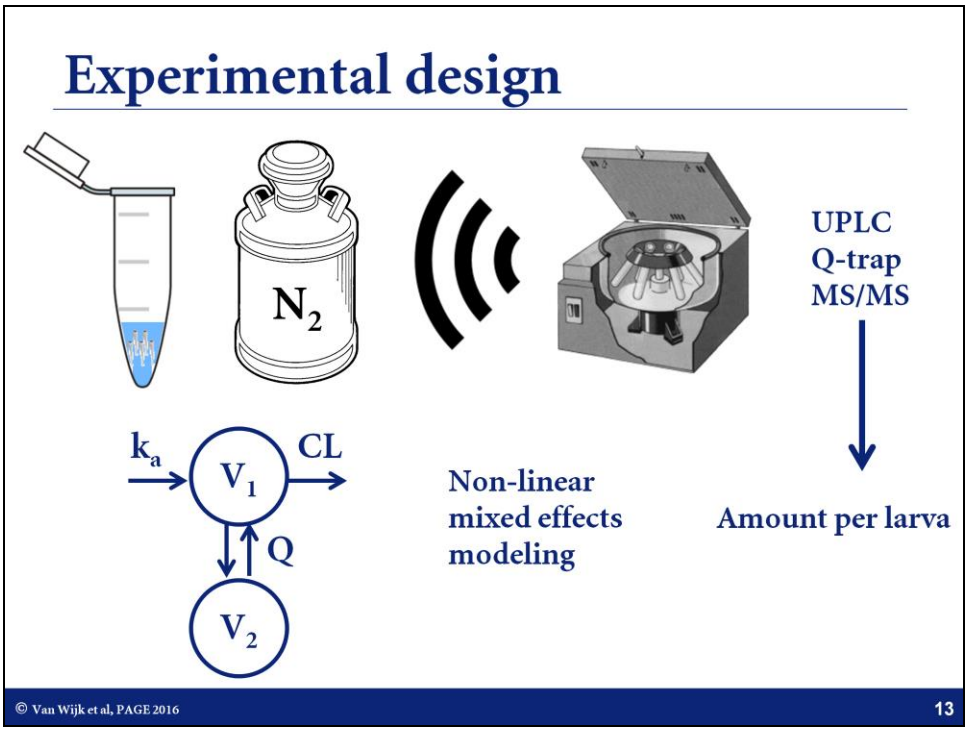


Key message: To illustrate the size of the zebrafish larva, a human would scale to the ~1 km Kingdom Tower as a zebrafish larva would to a human.



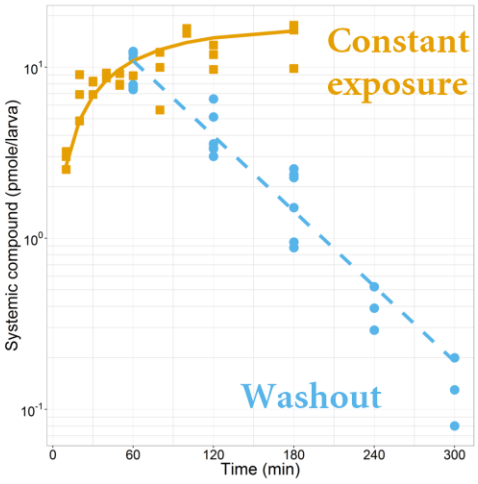
Key message: Five zebrafish larvae are therefore used as one replicate of one timepoint. Two experiments are performed; one in which the fish are constantly exposed to 1 mM paracetamol and sampled* after different periods up to 180 minutes, and the other in which fish are exposed for 60 minutes, transferred to a clean environment and sampled for up to 300 minutes.

*a sample is five zebrafish larvae in lysis buffer



Key message: The sample preparation (freezing in liquid nitrogen, sonication, centrifuging to inject supernatant in LCMS) is destructive, leading to amount per larva. Non linear mixed effects modeling is used to characterize the PK.

Results PK model



Parameter	Unit	Parameter estimate	RSE (%)
k_a	1 / min	0.289	5.0
V_1	Larval volume	1 FIX	-
CL	Proportion of larval volume / min	0.017	5.0
RUV	%	9.73	19

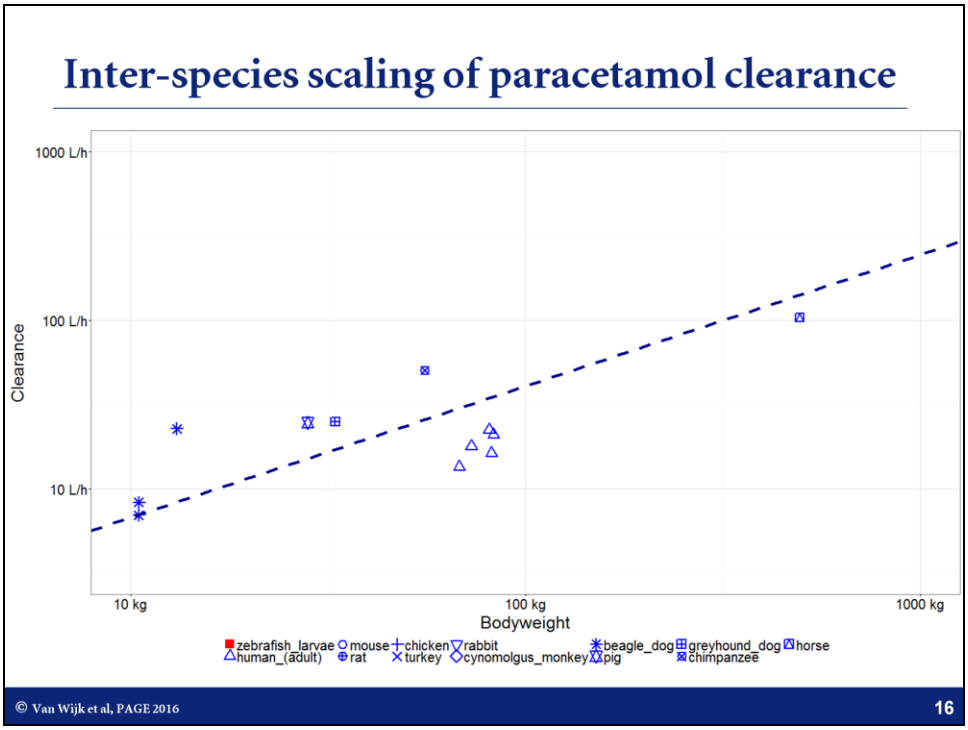
Paracetamol PK profile characterized in zebrafish larvae!

Key message: A one compartment model with zero order absorption and first order elimination fitted the data best. Preliminary data on paracetamol metabolites also show similar elimination pathways. A PK profile was successfully characterized in this small organism, meeting the first objective. Because of the destructive sample preparation, the volume of distribution is fixed to one, yielding a relative clearance.

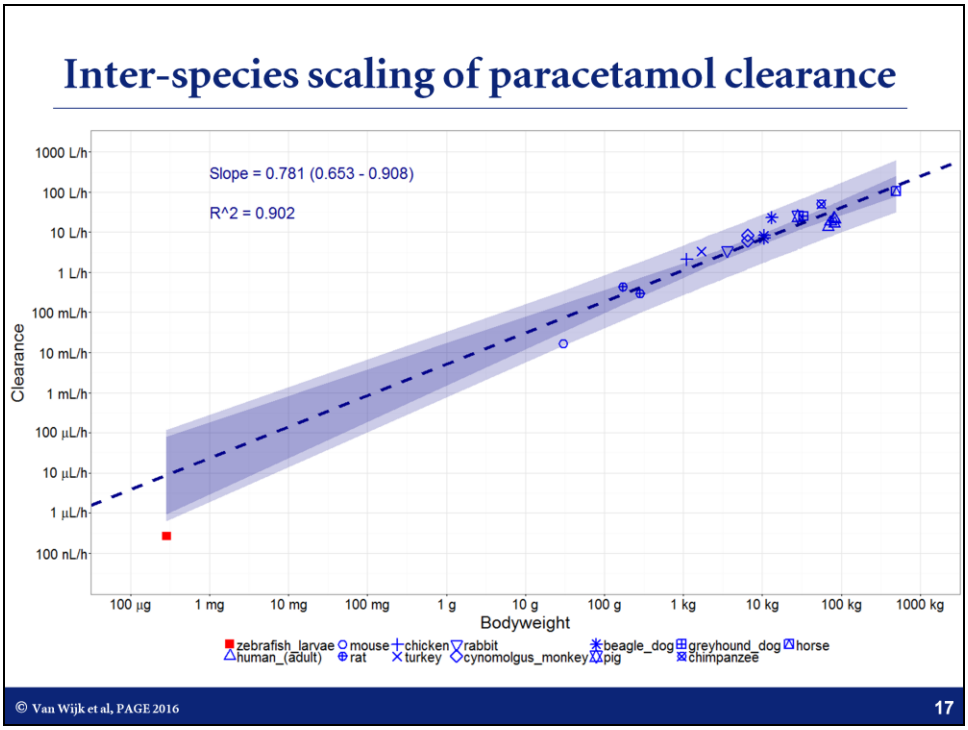
Determination of larval volume

Zebrafish larva	Relative clearance	Volume	Absolute clearance
3 dpf	1.7 % / min	260 nL	265.2 nL/h

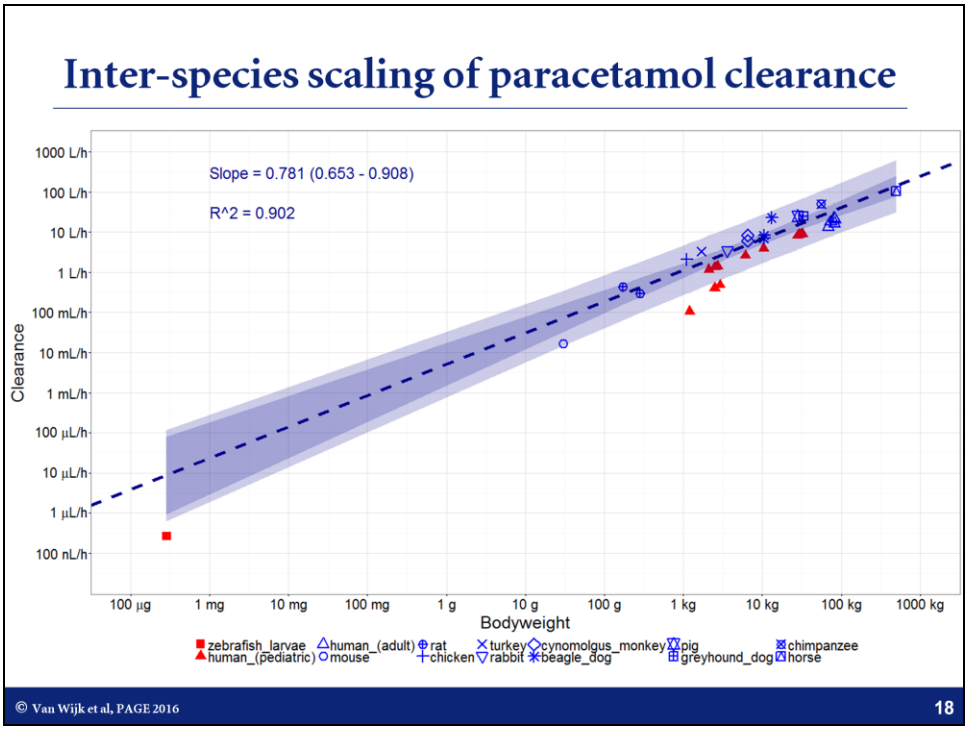
Key message: To explore the translational potential, the relative clearance needs to be transformed to an absolute clearance. The volume of a 3dpf larva was determined by 3D silhouette modeling of a series of VAST microscopic images. Assuming the volume of distribution equals the larval total volume, an absolute clearance of 265.2 nL/h was calculated.



Key message: To explore the translational potential of paracetamol clearance of zebrafish larva, a clearance-bodyweight log-log plot was constructed from literature, and linear regression was performed.



Key message: Paracetamol clearance of zebrafish larvae correlates reasonably with the linear regression.



Key message: Immature human paracetamol clearance also remains below the linear regression; this could explain the lower clearance in the immature zebrafish. This is also in line with preliminary data on paracetamol's major metabolites, which show a similar profile as neonates.

Conclusion

- A **PK profile** of paracetamol is characterized in zebrafish larvae
- Reasonable **correlation** of absolute clearance of paracetamol with higher vertebrates

Key message: In conclusion, zebrafish research can benefit from pharmacological modeling, resulting in an exposure-response relationship. The paracetamol clearance of zebrafish correlated reasonbly with that of higher vertebrates; however the translational potential of the zebrafish requires more research.

Key message: This presentation is the result of a multidisciplinary project with collaborators from four departments of three research institutes.

Big pharma screens in zebrafish

Pharmaceutical company	Assay	In-house facility/academic collaborator or zebrafish CRO
Abbott	Cardiac function	Academic collaborator
AstraZeneca	Visual function	Zebrafish CRO
AstraZeneca	Seizure liability	In-house
AstraZeneca	Tauopathy	Academic collaborator
AstraZeneca	ADME	Academic collaborator
AstraZeneca	Ototoxicity	Academic collaborator
Bristol-Myers Squibb	Embryotoxicity/teratogenicity	In-house
Eli Lilly	Bone formation	Zebrafish CRO
Eli Lilly	Primordial germ cell culture	Academic collaborator
GlaxoSmithKline	Embryotoxicity/teratogenicity	Academic collaborator
Johnson & Johnson; Pfizer	Hepatotoxicity	Zebrafish CRO
Johnson & Johnson	Embryotoxicity/teratogenicity	Zebrafish CRO
Merck KGaA	Embryotoxicity/teratogenicity	Academic collaborator
Novartis	Developmental and molecular biology	In-house
Novatis	Gastrointestinal motility	In-house and with academic collaborator
Novartis	Toxicology; determining mechanism of action	Academic collaborator
Pfizer	Safety pharmacology assays	Zebrafish CRO
Pfizer	ADME	In-house/Zebrafish CRO