

ZEBRAFISH SCREENING

ADMET and Zebrafish Screening

OUR CAPABILITIES, SKILLS AND EXPERTISE

TOXICITY ASSESSMENT

- Hepatotoxicity screening predicts toxicity by assessing liver specific morphological changes
- Developmental toxicity screening detects liability for causing embryotoxicity and teratogenicity
- Acute toxicity screening assesses general toxicity and lethality caused by compounds, thereby providing a maximum tolerated concentration (MTC)
- Ototoxicity screening detects hair cell loss
- Myelotoxicity screening identifies compounds with the potential to cause anaemia

SAFETY ASSESSMENT

- Cardiac function screen assesses liability for arrhythmia (bradycardia or tachycardia). Atrio-ventricular decoupling is used as a surrogate for QT-prolongation (hERG channel blocking)
- Locomotor / seizure assay tests for neurotoxicity and seizure potential
- Bone assay tests liability for bone demineralisation or loss

- Cartilage assay tests for adverse effect on cartilage formation
- Gut motility, optomotor function and visual function assays in development

BIOANALYSIS

- Uptake into Zebrafish can vary considerably from compound to compound and logP is found to be an unreliable indicator. Therefore, Evotec has developed and validated a proprietary method for quantifying the final body burden in larvae. This has the key benefits of minimising false negatives and allowing the ranking of compounds

EFFICACY AND DISEASE MODELS

- Evotec has a number of validated efficacy and disease models including:
 - ▶ Epilepsy
 - ▶ Osteoporosis
 - ▶ Chemically-induced hearing loss
- Evotec also has models in development including: Huntington's disease (human Htt aggregation), Parkinson's disease (LRRK2 overexpression) and Alzheimer's disease (human Aβ1-42 overexpression)

The metabolism, physiology and development of Zebrafish are comparable to humans and therefore Zebrafish is highly relevant for toxicity, safety and efficacy testing. Evotec offers a suite of highly validated assays designed in collaboration with leading pharmaceutical companies to meet the needs of the industry. Benefits of Evotec's fully industrialised world leading Zebrafish platform include:

- High content *in vivo* readout using small quantities of compound
 - Reduced costs by prioritisation of better lead candidates earlier, with the identification of toxicity and safety related issues prior to traditional late stage, high cost *in vivo* models
 - Quick turnaround
 - Highly predictive model ideal for filtering compounds between *in vitro* and traditional *in vivo* models
 - Identification of superior back-up or follow-on compounds
 - Early and low cost profiling of compounds in disease models
- Evotec is constantly updating its portfolio through internal research and development.

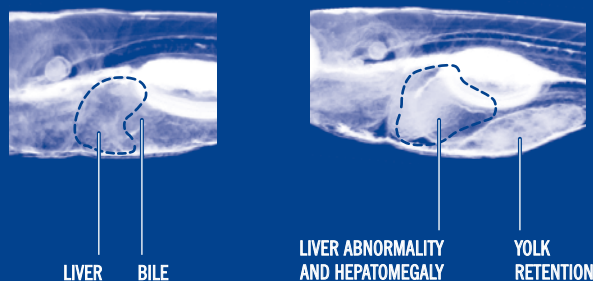
CUSTOM ZEBRAFISH ASSAYS

- Microinjection of macromolecules or small molecules with unfavourable physico-chemical properties
- Genetic manipulation for target validation studies and disease model development:
 - ▶ Transgenics antisense knock-down
 - ▶ Gene overexpression
- Phenotypic analysis of morphological and physiological changes using:
 - ▶ Expression analysis (qPCR, *in situ* hybridisation, Western blotting, immunostaining, ELISA)
 - ▶ Histology
 - ▶ Behavioural tests

Highly validated assays, designed in collaboration with leading pharmaceutical companies

HEPATOTOXICITY

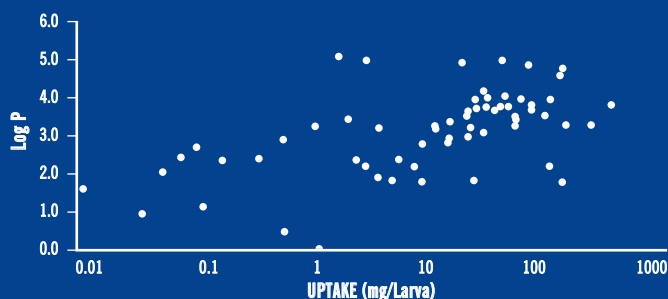
- Evotec and Johnson & Johnson presentation (SOT annual meeting in Baltimore, USA, 2009)
- Comparison between Zebrafish and HepG2 assays for the predictive identification of hepatotoxins
- Zebrafish larvae were assessed for specific liver phenotypes after exposure to 50 blinded compounds
- Overall predictivity of 91% compared to mammalian and human data



“In comparison to a targeted subset of HepG2 assays, the Zebrafish detects more hepatotoxicants and is more specific”

COMPOUND UPTAKE ANALYSIS

- Evotec and Pfizer investigated the uptake and toxicity of compounds in Zebrafish
- Five physico-chemical properties were compared against the uptake achieved in Zebrafish (ng/larvae; treated with the lowest observed effect concentration) for >70 drugs
- For further information on this project, please read: *Doshna et al, 2009 Toxicological Sciences 108 (S-1), 78*



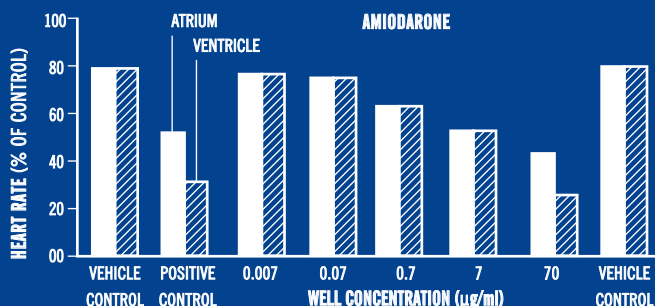
“No one physical-chemical property can adequately predict compound uptake in Zebrafish”

“Bioanalysis is necessary to confirm and measure compound uptake in Zebrafish”

Highly predictive toxicity, safety and efficacy assays ideally positioned between in vitro and traditional in vivo testing

CARDIOTOXICITY

- Evotec and Novartis presentation (SPS in Strasbourg, France, 2009)
- Cardiotoxicity testing in Zebrafish, in which 20 blinded compounds were screened for their potential to alter cardiac rhythm and cause QT-prolongation
- Overall predictivity of 94% compared to dog and human data



IDENTIFICATION OF ANTICONVULSANTS

- Zebrafish offer the potential for a primary screen to identify a wide variety of potential anticonvulsants (*Bergbman et al, Epilepsy Research 75, 18—28, 2007*)
- Zebrafish larvae undergo seizures after exposure to PTZ
- Treatment with a variety of anti-epileptic drugs such as Ethosuximide (below) prevented this effect
- Overall predictivity of 93% compared to human data

