HEPATOTOX ASSAY

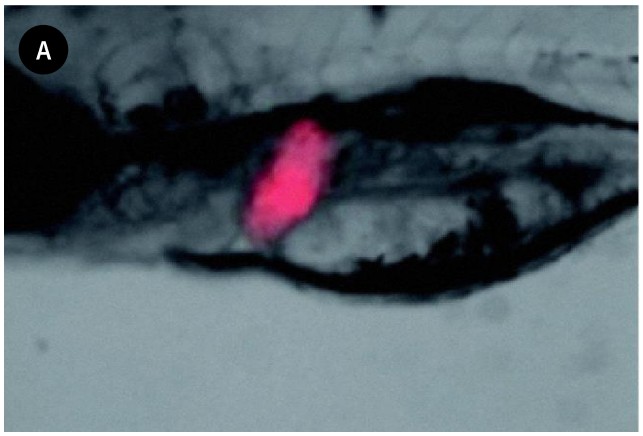
Drug-induced hepatotoxicity is one of the most important causes of drug withdrawal in the process of Drug Discovery. The low correlation of the *in vitro* cytotoxicity studies with human hepatotoxicity, especially in the early stages of drug development, makes human hepatotoxicity difficult to predict. Therefore, new methods and models to predict hepatotoxicity are needed.

Zebrafish shows a great potential to be used in early stages of discovery, thanks to its properties that include transparency, easiness to treat with compounds, high similarity with other vertebrates, cost-effectiveness and possibility to generate transgenic lines targeting specific organs and pathways. The zebrafish model has been extensively used in the field of toxicology [1] and developmental biology [2].

Mechanisms that occur during the process of development have been characterized, leading to an important knowledge about the signaling pathways that govern zebrafish liver function.

These studies have also revealed that liver precursor cells are already present at 48 hours post fertilization (hpf) (Figure 1) and are actively detoxifying at 5 days post fertilization (dpf) (Figure 2).

Because of functional similarities between zebrafish and mammalian livers, the zebrafish model is proposed for prediction of hepatotoxic agents. In Hepatotox Assays, compounds are added into 24 or 96 well plates containing larvae and subsequently, potential hepatotoxic effects can be assessed in a pluri-cellular and multi-organ context in a short period of time. Moreover, the concentration that is inducing toxicity can be measured by bioavailability assays.



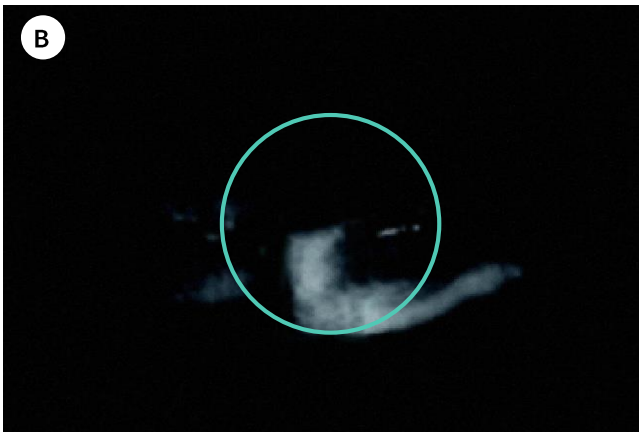


Figure 1. Liver can be directly visualized in anesthetized embryos either using a transgenic line (a) or a specific liver tissue (b).

GENE	5dpf	10dpf
MAT1A	Expressed	Expressed
MAT2A	Expressed	Expressed
GNMT	Expressed	Expressed
CBS	Expressed	Expressed

Figure 2. List of reference compounds used in the validation process.

Specifically, necrosis induction in liver (Figure 3 a,b) is a rapid assay that can help to classify potential hepatotoxic compounds. Test items are analyzed in a range of concentrations at 2 different time points using 10 embryos per condition. A reference compound and a negative control are also included (Figure 3 c).

Additionally, zebrafish potentiates assessment of other hepatotoxic endpoints described in the "Non-clinical guideline on drug-induced hepatotoxicity" such as steatosis (Figure 4), hepatomegaly and cholestasis. These endpoints can also be combined with a general toxicity analysis or hepatoprotective assays.

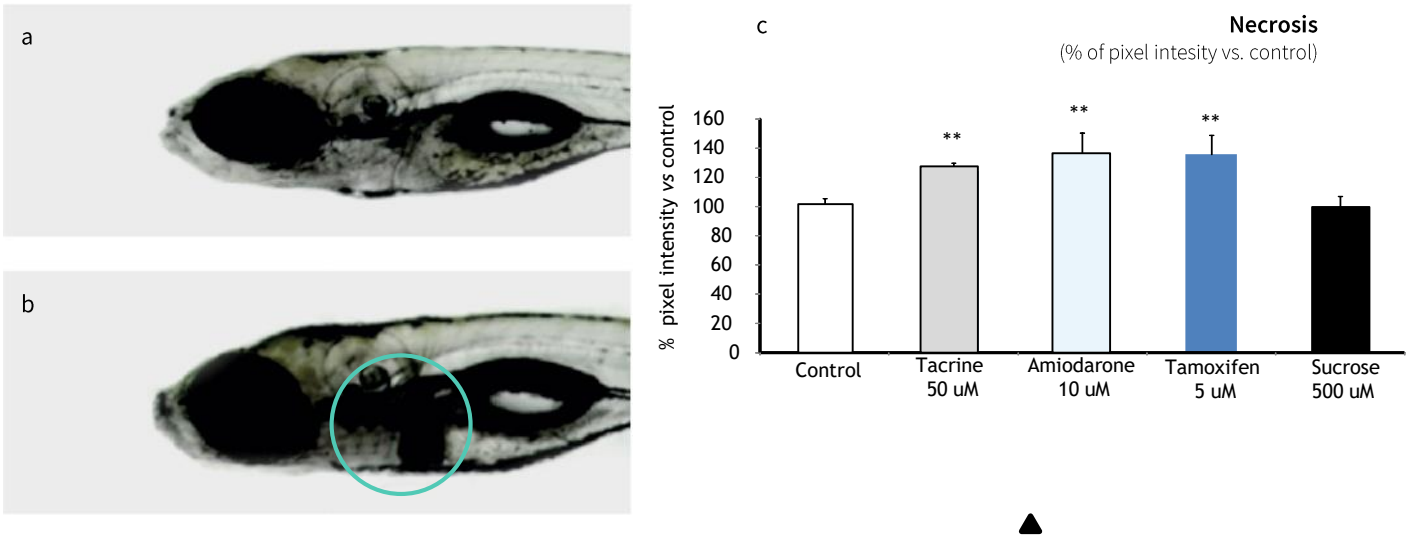


Figure 3. Liver necrosis (a) Mock-treated embryo (b) Acute liver toxicity is detected in treated embryos b by pixel intensity quantification(c) Hepatotoxic classification of compounds based on liver necrosis induction (** means p<0.01).

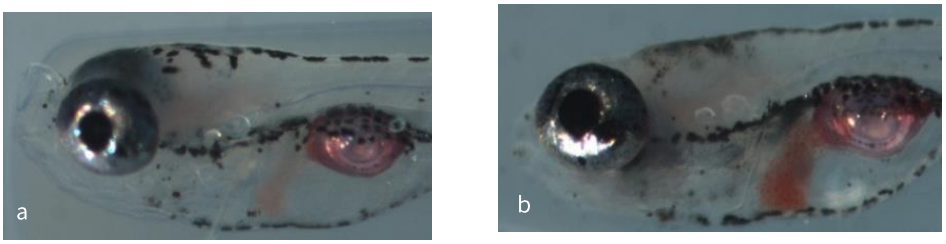


Figure 4. Liver steatosis detection. (a) Embryo with no induced steatosis. (b) Embryo showing steatosis in the liver tissue.

- The expression of different enzymes involved in liver detoxification in zebrafish indicates that this animal model can be used for prediction of hepatotoxic agents.
- Besides necrosis, zebrafish potentiates assessment of other hepatotoxic endpoints described in the "Non-clinical guideline on drug-induced hepatotoxicity" such as steatosis, hepatomegaly and cholestasis.

[1] Sadler K.C., et al. A genetic screen in zebrafish identifies the mutants vps18, nf2 and foie gras as models of liver disease. Development. 2005 Aug;132(15):3561-72. Epub 2005 Jul 6.
[2] Her GM, et al. In vivo studies of liver-type fatty acid binding protein (L-FABP) gene expression in liver of transgenic zebrafish (Danio rerio). FEBS Lett. 2003 Mar 13;538(1-3):125-33. zebrafish.Nat Genet. 2000 Oct;26(2):216-20.