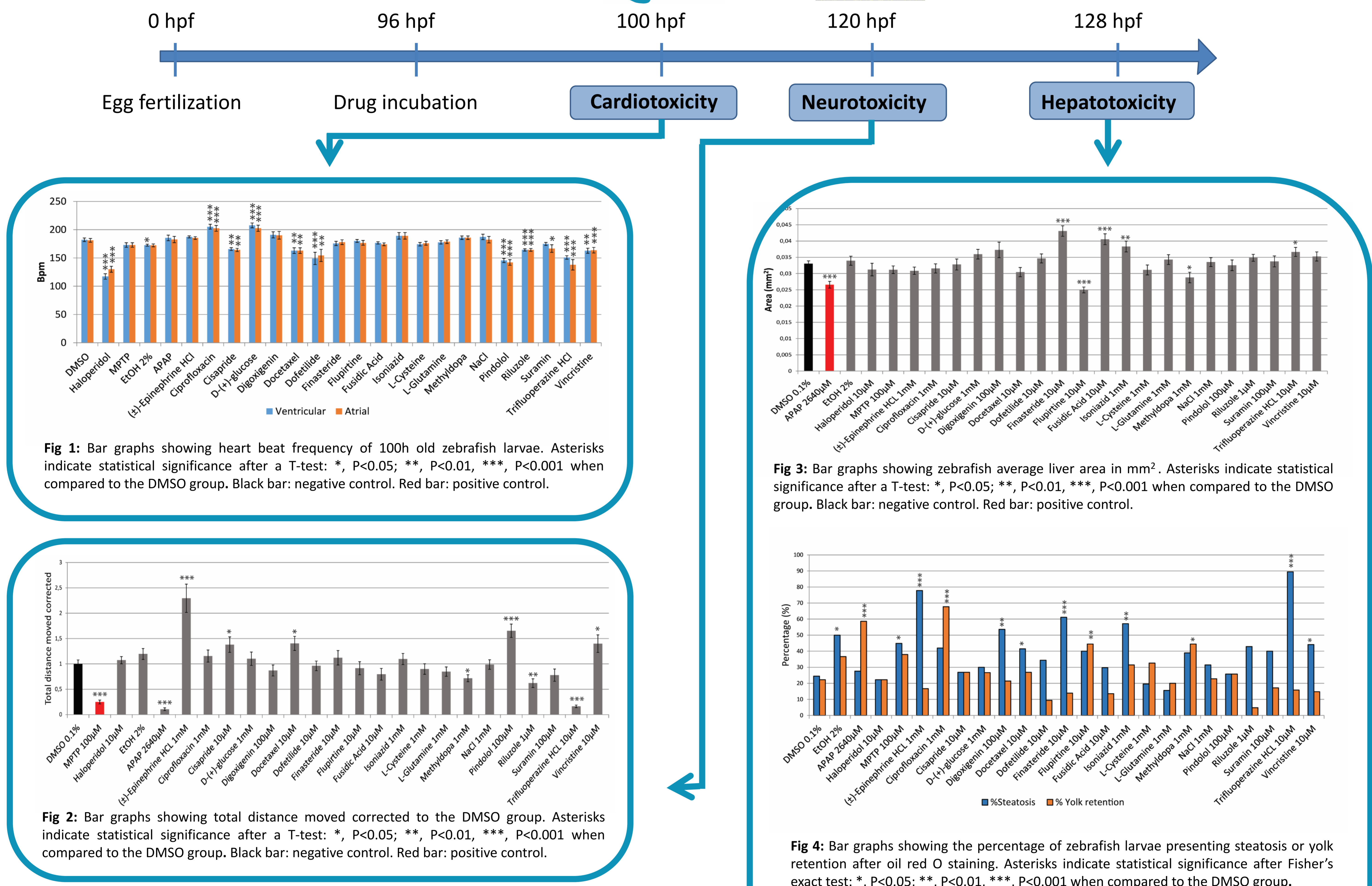# ZeGlobalTox, a new way for global toxicity assessment

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Zebrafish exploitation for high-throughput drug screening is becoming important for the pharmaceutical industry to assess toxicity and efficacy of novel drugs. Zebrafish has, from early developmental stages, fully functional organs from a physiological point of view. Thus, drug-induced toxicity is easily detected and evaluated, bridging the gap between preclinical *in vitro* and *in vivo* models in a fast and cost-effective manner.

ZeClinics has developed **ZeGlobalTox,** an innovative tool that integrates *in vivo* toxicity assessment for the brain, heart and liver in the same animal, strongly impacting the 3Rs principles. This assay _Reduces_, by up to a third, the number of animals required to assess toxicity in these organ. The new physiological parameters included in the ZeGlobalTox _Refines_ the drug toxicity assay. The high predictivity of the ZeGlobalTox (Specificity: 85% and Sensitivity: 76%) allows to _Replace_ the use of other classical species, such as rodents or other mammals.

0 hpf	96 hpf	100 hpf	120 hpf	128 hpf
Egg fertilization	Drug incubation	**Cardiotoxicity**	**Neurotoxicity**	**Hepatotoxicity**

Fig 1: Bar graphs showing heart beat frequency of 100h old zebrafish larvae. Asterisks indicate statistical significance after a T-test: *, P<0.05; **, P<0.01, ***, P<0.001 when compared to the DMSO group. Black bar: negative control. Red bar: positive control.

Fig 2: Bar graphs showing total distance moved corrected to the DMSO group. Asterisks indicate statistical significance after a T-test: *, P<0.05; **, P<0.01, ***, P<0.001 when compared to the DMSO group. Black bar: negative control. Red bar: positive control.

Fig 3: Bar graphs showing zebrafish average liver area in mm². Asterisks indicate statistical significance after a T-test: *, P<0.05; **, P<0.01, ***, P<0.001 when compared to the DMSO group. Black bar: negative control. Red bar: positive control.

Fig 4: Bar graphs showing the percentage of zebrafish larvae presenting steatosis or yolk retention after oil red O staining. Asterisks indicate statistical significance after Fisher's exact test: *, P<0.05; **, P<0.01, ***, P<0.001 when compared to the DMSO group.
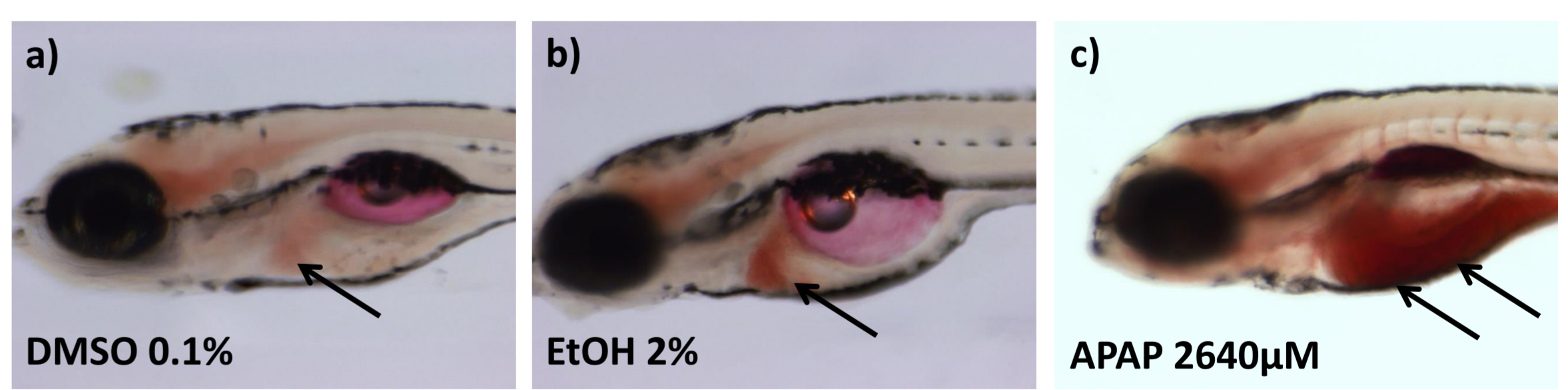

DMSO 0.1%   EtOH 2%   APAP 2640µM

Fig 5: Representative oil red O whole mount staining images of control (DMSO 0.1%), EtOH 2% and APAP 2640µM showing non-affected liver, a), liver with steatosis, b) and yolk retention, c), pointed with black arrows.

CONCLUSIONS

ZeGlobalTox is highly predictive, demonstrating its validity as an innovative tool for integrated multi-organs toxicity prediction of new drugs during preclinical drug discovery phases and, additionall, for the implementation of 3Rs recommendations.

		Cardiotoxicity				Neurotoxicity				Hepatotoxicity	
		Human				Human				Human	
		-	+			-	+			-	+
Zf	-	TN: 12	FN: 2	Zf	-	TN: 13	FN: 2	Zf	-	TN: 17	FN: 2
	+	FP: 3	TP: 8		+	FP: 2	TP: 8		+	FP: 2	TP: 4

Zf: zebrafish, **TN:** true negative, **TP:** true positive, **FN:** false negative, **FP:** false positive.

	Specificity TN/(TN+FP)	Sensitivity TP/(TP+FN)	PPV TP/(TP+FP)	NPV TN/(TN+FN)
Cardiotoxicity	80%	80%	73%	86%
Neurotoxicity	87%	80%	80%	87%
Hepatotoxicity	89%	67%	67%	89%
ZeGlobalTox	85%	76%	73%	87%

Zf: zebrafish, **PPV:** positive predictive value, **NPV:** negative predictive value, **TN:** true negative, **TP:** true positive, **FN:** false negative, **FP:** false positive.

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