



## Image-based biomarker detection for hepatotoxicity screening using the HepaRG cell model

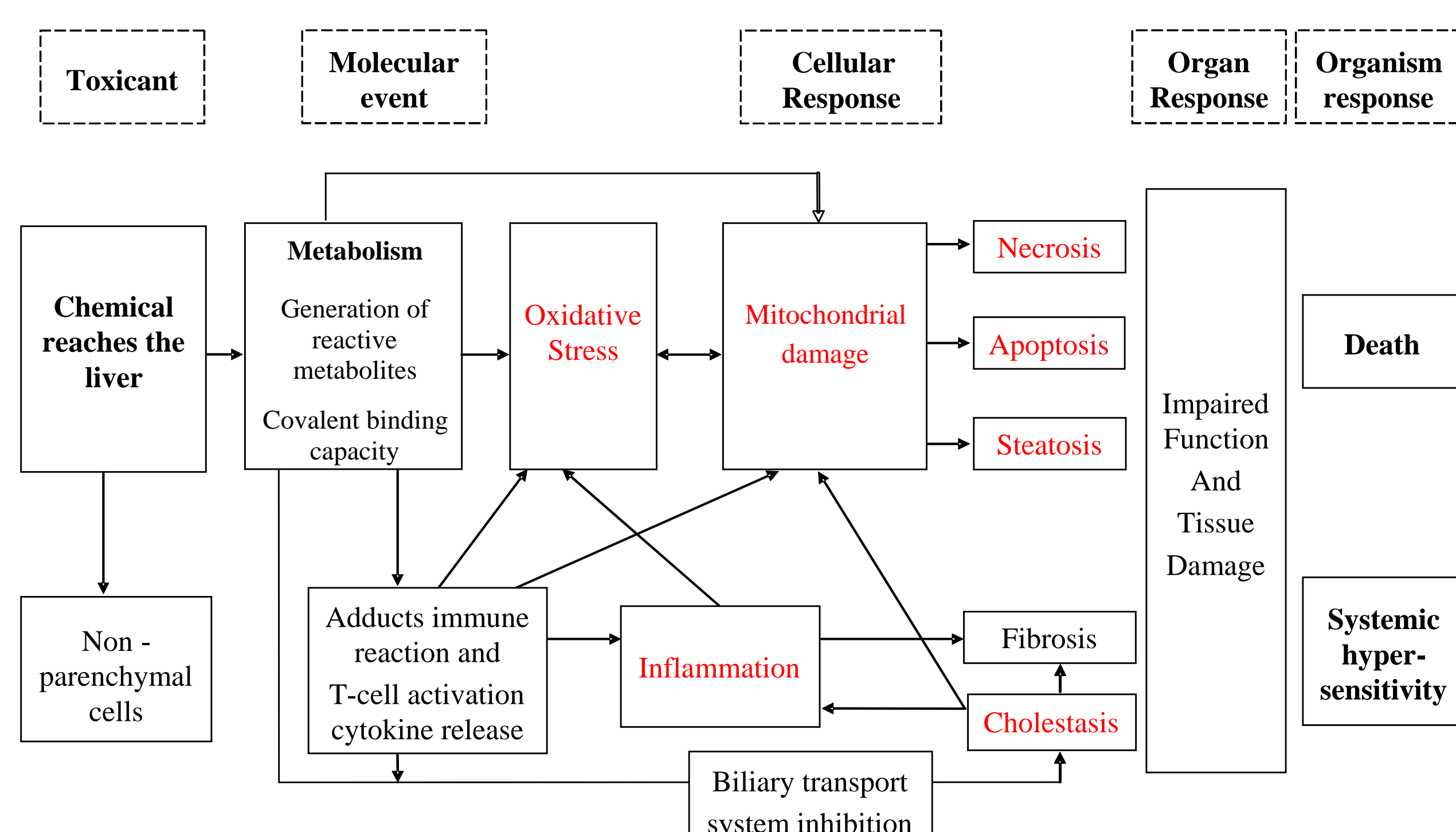
Milena Mennecozi, Brigitte Landesmann, José M. Zaldívar, Georgina Harris, Maurice Whelan

Institute for Health and Consumer Protection, European Commission-Joint Research Centre, Ispra (Italy)

### Abstract

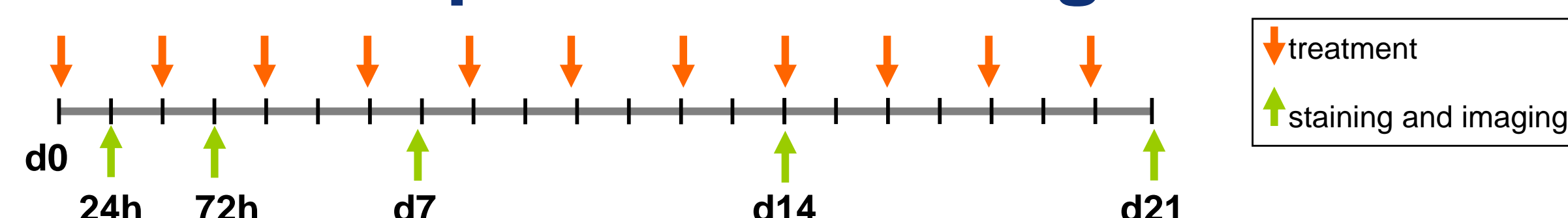
In this study we used a mode-of-action (MoA) inspired experimental design to analyse multiple cellular phenotypic changes related to hepatotoxicity. The aim is to identify biological key events associated with toxicity pathways that can be used as biomarkers for *in vitro* toxicity testing. HepaRG cells were employed as the cellular model. This cell line is derived from a human hepatocellular carcinoma and when seeded at low density, differentiates into bi-potent hepatic progenitors and divides before acquiring morphological and functional characteristics of adult human hepatocytes. Differentiated HepaRG cells express the major liver functions, including P450s involved in xenobiotic metabolism, phase II enzymes, transporters and nuclear receptors at levels comparable to those found in primary hepatocytes. Based on our experience in acute toxicity testing, we exposed the HepaRG cells to Acetaminophen (APAP) for 21 days (repeated dose toxicity). Quantification of immuno-fluorescently stained biomarkers expressed by treated HepaRG cells, including cell loss, nuclear size, nuclear morphology, DNA content, mitochondrial membrane potential expressed by treated HepaRG cells was then performed using an epifluorescent automatic microscope and high content imaging. Concentration response data were acquired and analyzed to characterize both the technical performance of the system and its ability to predict repeated dose hepatotoxicity. In addition, a computer-based prediction modelling was developed, using the generated *in vitro* experimental data.

### Hepatotoxicity MoA Hypothesis



Mode of action is a biologically plausible sequence of key events leading to an observable adverse health outcome. MoA for hepatotoxicity is not yet really defined. Extensive literature research has led to the proposed hypothesis that mitochondrial damage plays a crucial role in various hepatotoxicity pathways. Mitochondrial injury can therefore be used as a marker for chemically induced hepatotoxicity.

### Experimental Design

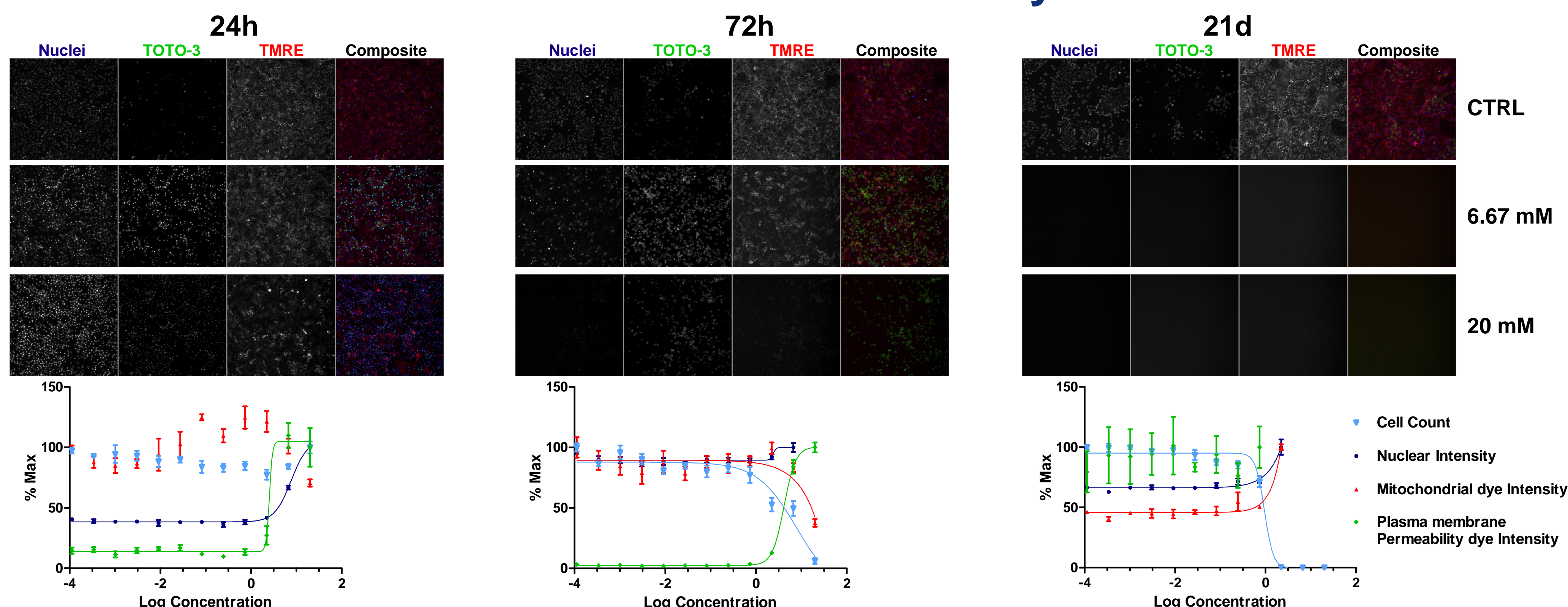


Eleven concentrations of APAP, ranging from 20 mM to 0.3  $\mu$ M, were tested in triplicate. 1:3 plate-to-plate dilutions were performed. Treatment was repeated every second day. Treated HepaRG cells were stained with Hoechst, Toto-3 and TMRE. High content images were acquired using Cellomics ArrayScan VTi platform (Thermo Scientific) and analysed through Cytotoxicity Bioapplication Software Module.

### Assay Principle

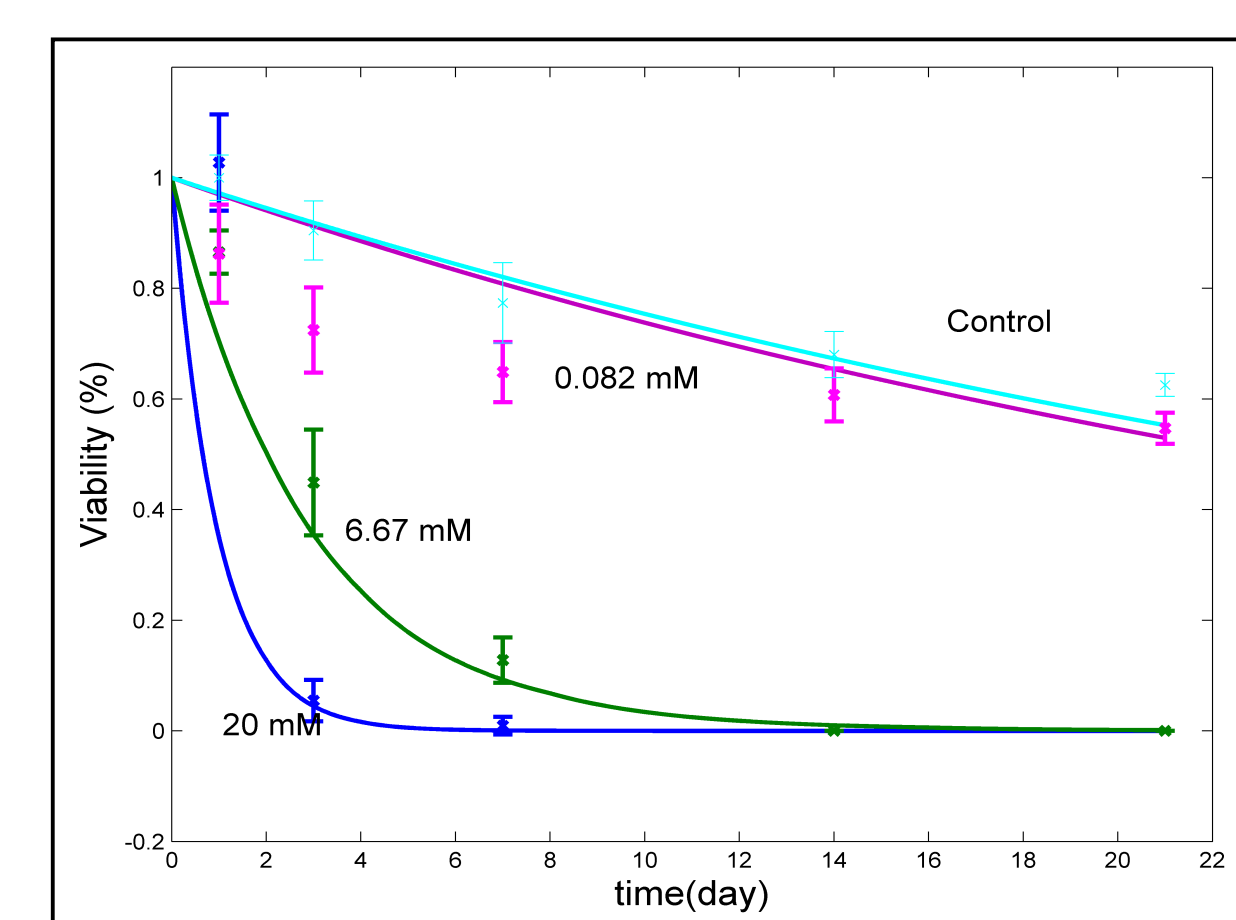
	Dye	Ex/Em	Function
	Hoechst	350/461 nm	Cell identification, nuclear area, DNA content
	Toto-3	642/660nm	Plasma membrane permeability
	TMRE	540/595 nm	Mitochondrial membrane depolarization

### In vitro APAP Toxicity



Based on our previous experiments in which we have tested acute hepatotoxicity in HepaRG cells using 92 chemicals and high content imaging, we designed a repeated dose toxicity testing study. Cells were exposed to repeated doses of APAP for 21 days. Biomarkers like cell loss, DNA content, mitochondrial membrane potential and plasma membrane permeability were measured at day 1, 3, 7, 14 and 21 and dose response curves were obtained.

### APAP Cell Viability Simulation



Based on HepaRG cells results at 72 hours a computer-based simulation model was developed. Repeated dose outcome up to 21 days was predicted using cell viability as biomarker. Predictive viability curves (lines) are plotted with the obtained cellular values (X). Error bars from 3 experiments are reported.

### Conclusions

- Plausible dose response curves proved the feasibility of long-term HepaRG cell based assays.
- The quantitative and temporal appearance of mitochondrial damage confirmed our hypothesis that mitochondria have a role in repeated dose hepatotoxicity.
- A computer-based simulation to predict cell viability up to 21 days showed dose-response curves that matched well with the obtained experimental data especially at higher doses (6,67 and 20 mM).