Alternative models in predictive toxicology to evaluate the effects of pesticides on the establishment of fatty liver diseases

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INTRODUCTION. Fatty liver or steatosis is a global concern, and non effective therapy is available. The disease is correlated with metabolic dysfunctions, accumulating lipids in the liver in proportions higher than 5% of organ weight. The fatty liver has different etiologies, including environmental contaminants as pesticides and it is urgent the development of alternative approaches to deep investigate it. **OBJECTIVES**. In this study we aimed to establish alternative models based on the coculture of hepatic cell lines in 2D or low-cost 3D models, and using *Drosophila melano*gaster to investigate the effects of the pesticides terbacil and bromacil on pro-steatosis establishment. **MATERIAL AND METHODS.** LX-2 (Merse) and HepG2 (ATCC-Hb8075) were cocultivated at 7:3 ratio in DMEM under regular conditions or on an alginate based-scaffold on 12-wells plates. After 48h of cellular seeding, 400 µM of palmitic and oleic acids (1:1) were added to induce pro-steatotic environment. The pesticides were added to the cells at concentrations ranging from acceptable daily intake (ADI) to 1000 x more. All the chemicals were incubated for 48h. Next, triglycerides (TG), glycose, and other markers of pro-steatosis were assessed by biochemical analyses (LabTest), and qPCR evaluated the expression of genes of lipid metabolism, oxidative stress, adverse pathways and epigenetics. For *Drosophila* assays, Canton-S fly line was used, and the animals were maintained under standard diet (BDC). For assays, eggs were added to regular food containing or not 5% of coconut oil or pesticides. The pre-pupae were collected for biochemical and molecular analyses as described above. **RESULTS AND DISCUSSION**. The results demonstrated that our alternative models are responsive to the pro-steatotic environment as well as to the chemicals, increasing biochemical markers of steatosis such as TG, glucose and other investigated classical markers of the pathology, as well as by modulating gene expression in a chemical-dependent concentration manner. In our hands, our low-cost 3D cellular model is extremely sensitive to ADI pesticides doses, which are useful for toxicology investigations of the effect of pesticides and other environmental contaminants in liver health. **CONCLUSION.** Here, we present a promising alternative approach to investigate hepatic disease and chemical toxicants under 3R principles.

Key words: 2D and low-cost 3D cell culture; *Drosophila melanogaster*; environmental contaminants; fatty liver; predictive toxicology.

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