Mixture Risk Assessment of Atrazine, Chlorpyrifos and Endosulfan

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Exposure to pesticides can occur via a number of pathways and routes. The pesticides featured in this study belong to three different classes, namely triazines (atrazine), organophosphorous (chlorpyrifos) and organochlorine (endosulfan), each exhibiting different modes of action. Newer methods are emerging in response to the complexities of chemical mixture exposures and effects. We describe here the use of the xCELLigence system for label-free and real-time monitoring of cell - viability. The xCELLigence system uses specially designed microtiter plates containing interdigitated gold microelectrodes to non-invasively monitor the viability of cultured cells using electrical impedance as the readout. The continuous monitoring of cell viability by the xCELLigence system makes it possible to distinguish between different perturbations of cell viability, such as senescence, cell toxicity (cell death), and reduced proliferation (cell cycle arrest). The ability of pesticides to activate the Pregnane X Receptor (PXR) was tested by monitoring luciferase activities in a stably transfected hPXR/HepG2 cell line, which was exposed to the above mentioned pesticides individually and in combinations. MTT test was also performed with the same concentrations as used for xCelligence study.

Experimental conditions optimization for Cryopreserved hepatocytes

Liver tissue

Collagenase perfusion

Hepatocytes

Cryopreserved 24 h
Specific Protocol

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Adhesion optimization
Collagen and Medium
2 wash every 1h30

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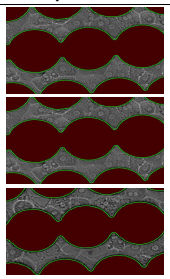
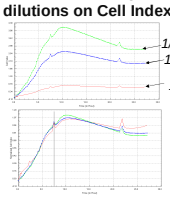
Exposure (20h)
1% FCS Medium

Optimal adhesion & viability



1/10
1/5
1

Effect of Collagen dilutions on Cell Index



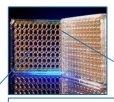
No difference on normalized cell Index

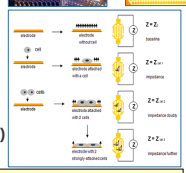
Objective of the study:

The purposes of this project is to evaluate in an *in vitro* study of the toxicological interactions (synergistic, additivity, potentiality) resulting from the exposure to relevant pesticides mixture of French consumers via diet. Three pesticides was chosen :
ATRAZINE, CHLORPYRIFOS and ENDOSULFAN.

Xcelligence system for label free and Real-time monitoring of cell viability

RTCA, xCELLigence (Roche)



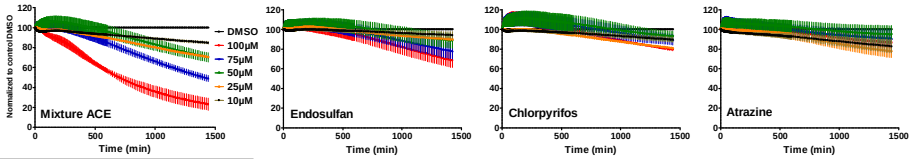


MIXTURE PESTICIDE CYTOTOXICITY

- Freshly Isolated Human hepatocytes were exposed to pesticides for 24h at five concentration after around 20h-22h of seeding in E-plates
- The data was normalized with DMSO: CI

For each compound at each concentration we calculate :

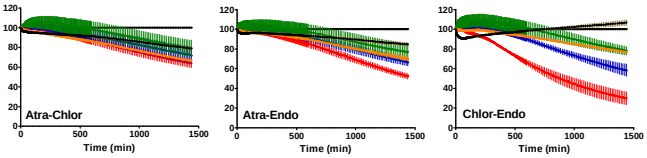
$$DMSO_{Ti} - CIPEST_{Ti}^{Ci} = \%effect.$$



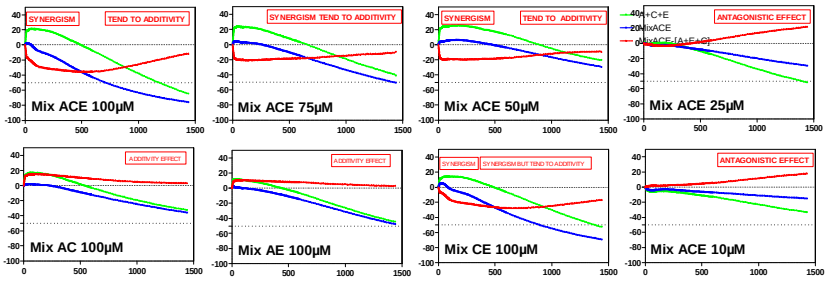
Additivity Test of the interactions we traced with given formula

$$\%effect.MIX_{ti}^{ci} - \left[\%effect.ATRA_{ti}^{ci} + \%effect.ENDO_{ti}^{ci} + \%effect.CHLOR_{ti}^{ci}\right] =$$ **RED CURVE**

Blue CURVE **GREEN CURVE**

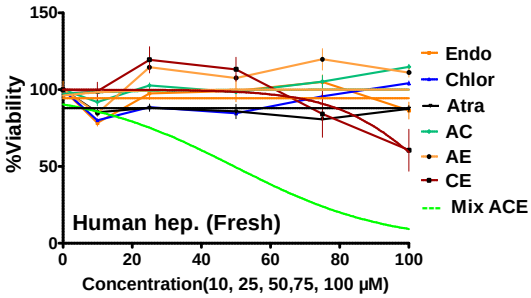


THE SLOPE OF THE RED CURVE, INDICATES THE INTERACTION

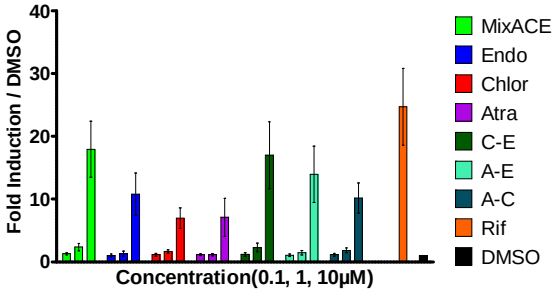


- Interaction leading to additivity synergisn or antagonism depends of the concentration and the time of exposure of the cocktail and also for its combination. Cocktail ACE have the dose dependent synergistic to antagonistic effect from higher to lowest concentration. For combinations only CE shows synergistic effect which tends to additivity while the other two combinations (AC,AE) show the additivity effects even at highest concentration i.e., 100µM

MTT test with Fresh Human Hepatocytes



PXR Transactivation by Pesticdes



The pesticides ranking according to their ability at 10µM as follows: (mixACE ≈ Endo-Chlor > Endo-Atra > Endo ≈ Chlor-Atra > Chlor ≈ Atra)
There is no significant difference b/t Mix ACE and CE and C & E seems to be act synergistically.

CONCLUSION and Perspectives:

- **Exposure to Mulitiple environmental chemicals increases the necessity to develop models to insight the toxicity of chemical Mixtures. For Chemical mixtures toxicity assessment, real time Cellular Index Measurement System improve the analysis of interaction.**
- **CI measurements give usefull kinetics data to improve analysis of the interaction of chemicals both as individual and in combinations.**
- **Real time Impedance is more sensitve as compare to MTT test.**
- **xCelligence and PXR (pregnane X Receptor) data modelisation of dose response with more sophisticated algorithm will be very helpful for understanding of chemical behaviour and their interactions both at lower and higher concentrations.**