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TECNOLOGIA DA SAÚDE
DE LISBOA
INSTITUTO POLITÉCNICO DE LISBOA



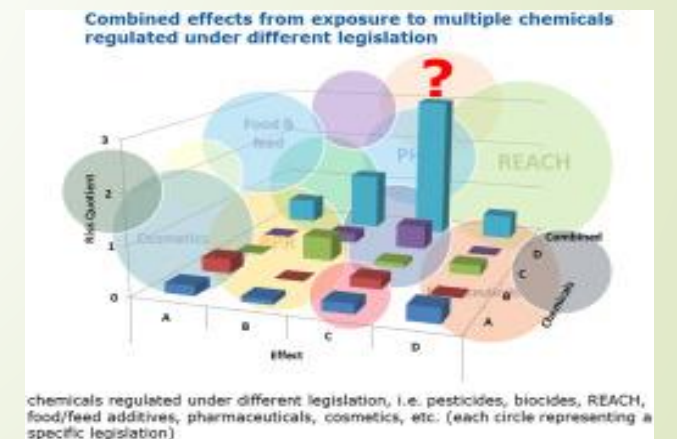
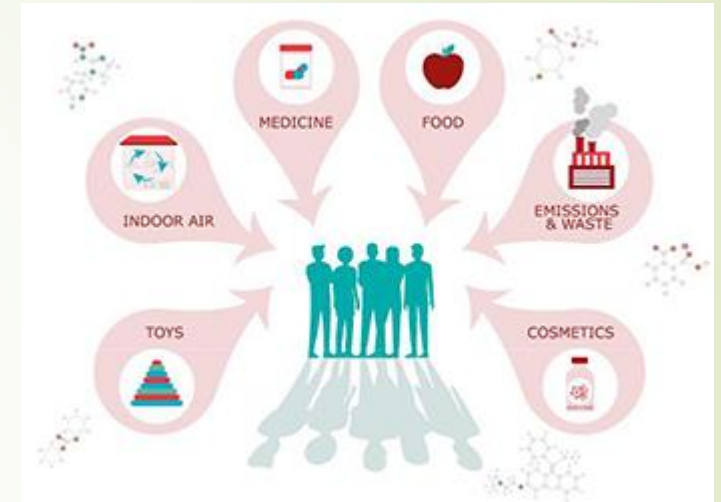
From Prediction to The Endpoints
10th INTERNATIONAL CONGRESS OF THE
TURKISH SOCIETY OF TOXICOLOGY
October 16-19, 2019, Lykia World Hotel, Belek, Antalya-TURKEY

Studies of chemical mixtures from real exposure to *in vitro*

Carina Ladeira

The combination effects of chemicals

- ▶ Humans and wildlife are exposed to an intractably number of different combinations of chemicals via food, water, air, consumer products, materials and goods.
- ▶ **Chemical Mixtures:**
 - ▶ **Combined exposures** – exposure to multiple chemicals via one or several sources and routes.
 - ▶ **Aggregate exposure** – sum of exposures to one chemical via several sources and routes.
 - ▶ **Intentional mixtures** – manufactured products with well known composition.
 - ▶ **Unintentional mixtures** – coincidentally formed and variable mixtures originating from one or several sources.



The combination effects of chemicals

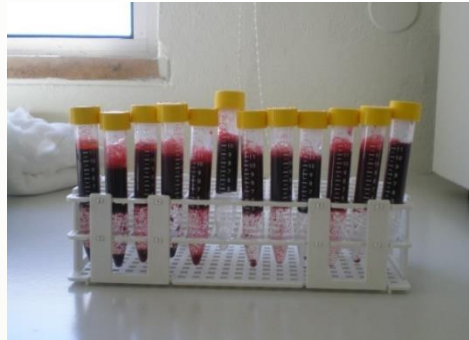


- The risk assessment of chemicals for regulatory purposes does only in rare cases take into account the “real life” exposure to multiple chemicals.
- Biomonitoring chemical concentrations in wildlife or humans is a mean to identify realistic co-exposure.
- Prediction of mixture effects based on individual chemicals is often limited by lack of knowledge of their toxicity and toxic MoA.
- **Aim:** evaluate combined cyto/genotoxicity of selected mixtures, using the concentrations measured in occupational setting real scenarios, and expose *in vitro* human lymphocytes, in a translational Toxicology approach.

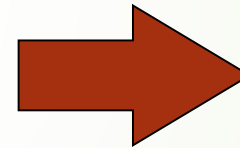
Methodology

- Blood collection by venepuncture.
 - Workers, controls and donors.
- Isolation of peripheral blood lymphocytes.

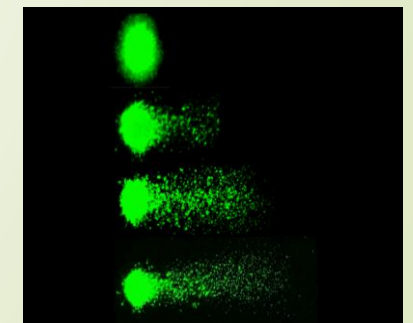
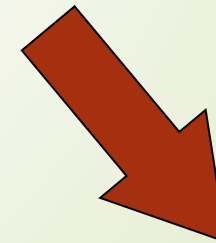
- Comet assay:
 - Alkaline comet assay for DNA damage.
 - Modification with FPG for oxidative DNA damage.
- CBMN assay:
 - MN, NPB, NBUDs.



Peripheral whole blood

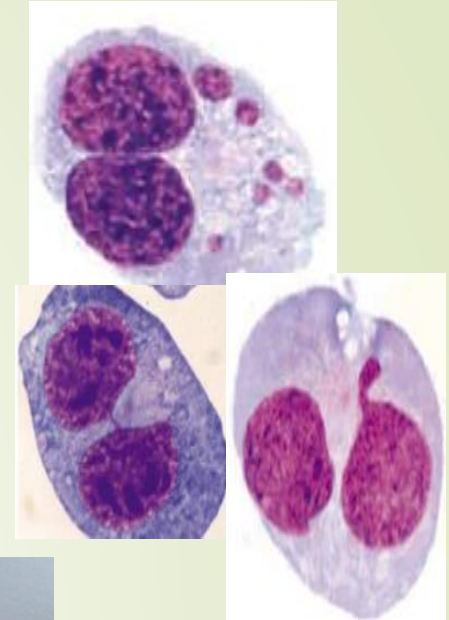
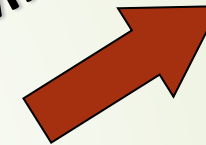


Lymphocytes



Comet assay

CBMN assay



Cytostatics exposure – human biomonitoring

- Selection of most consumed anticancer drugs and based on their different MoA: 5-fluorouracil, cyclophosphamide and paclitaxel.
- Exposure assessment: surfaces and air monitoring.

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ASSESSMENT OF GENOTOXIC EFFECTS IN NURSES HANDLING CYTOSTATIC DRUGS

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TABLE 2. 5-Fluorouracil in Both Hospital Samples

	Hospital A (n = 28)	Hospital B (n = 105)
Contaminated (> LOQ)	14 (50%)	9 (8.57%)
Mean (ng/cm ²) ± SD	16.42 ± 3.41	19.94 ± 3.39
Range	10.95–60.51	10.66–41.25
p Value	0.208	

Note. Limit of detection (LOD) = 3 ng/cm². Limit of quantification (LOQ) = 10 ng/cm².

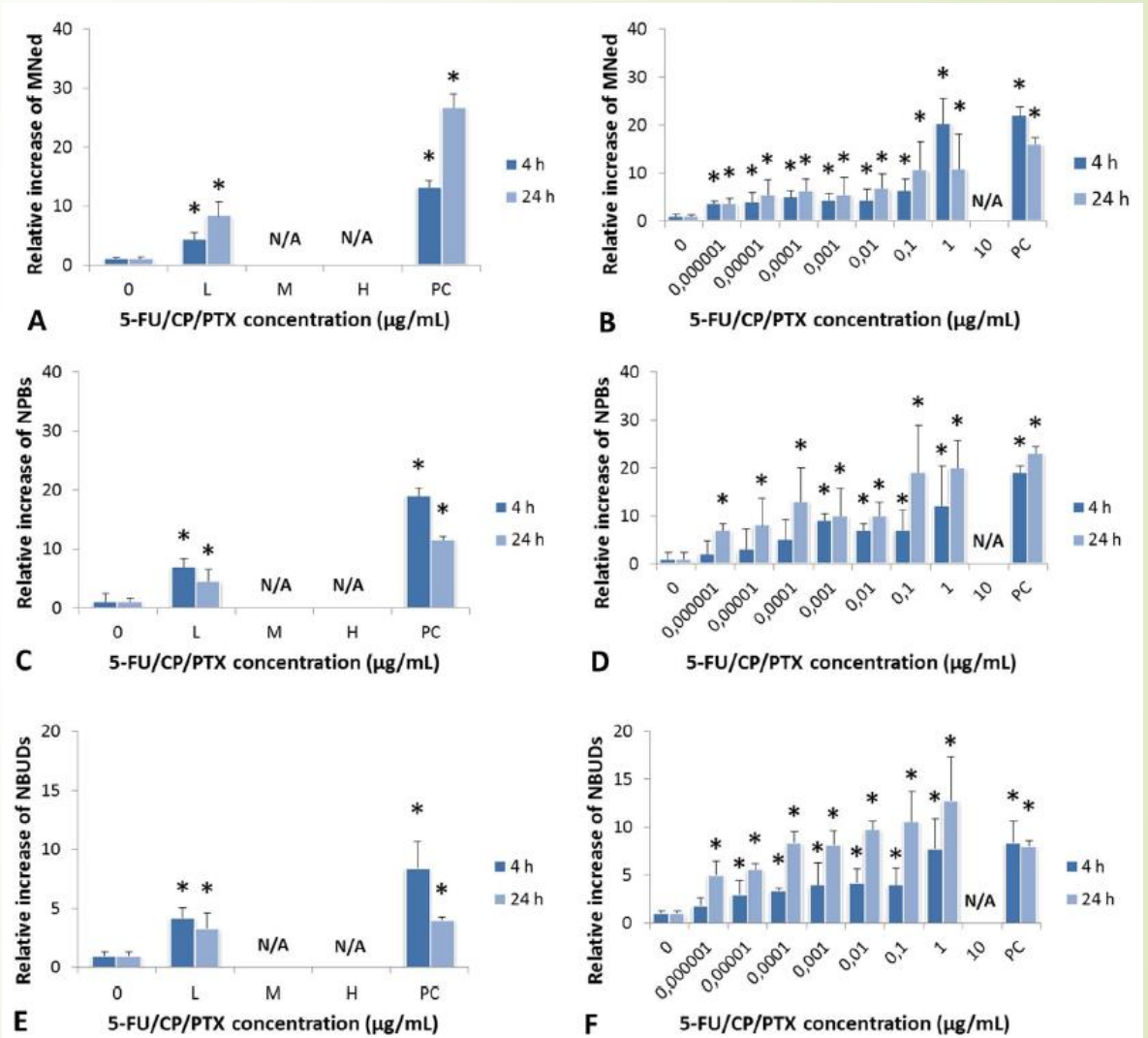
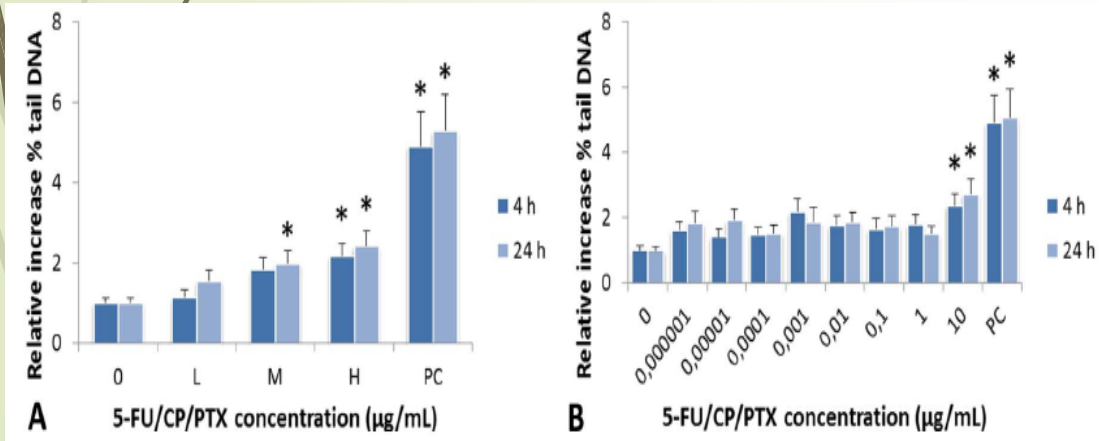
TABLE 3. MN in lymphocytes

	Mean, MN lymphocytes ± SE (range)
Controls	2.09 ± 0.312 (0–15)
Exposed	10.11 ± 2.053 (1–58)
p Value	<.001

Note. Data are given as mean in the studied population: mean ± mean standard error (range); p value is of Mann–Whitney test.

Cytostatics exposure – *in vitro* from [] real scenario

- Oncology day service workplace surfaces.
- Selected cytostatic drug mixture is potentially cyto/genotoxic and that it can induce cell and genome damage even at low concentrations.



Considerations

- ▶ Not only the mixture may pose a risk to cell and genome integrity, but also that toxicity data from a single compound might not be sufficient for the prediction of toxicity in a complex working environment.
- ▶ The presence of drugs in different amounts and with different MoA suggests the need to study the relationship between the presence of genotoxic components in the mixture and the resulting effects, taking into account the MoA of each component by itself.
- ▶ This study provides new data sets necessary for scientifically-based risk assessments of cytostatic drug mixtures in occupational as well as environmental settings.

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Genotoxicity assessment of a selected cytostatic drug mixture in human lymphocytes: A study based on concentrations relevant for occupational exposure

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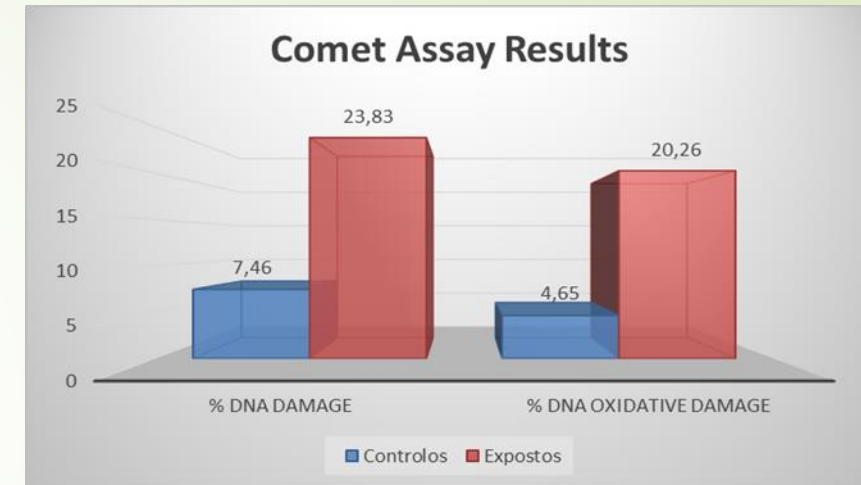
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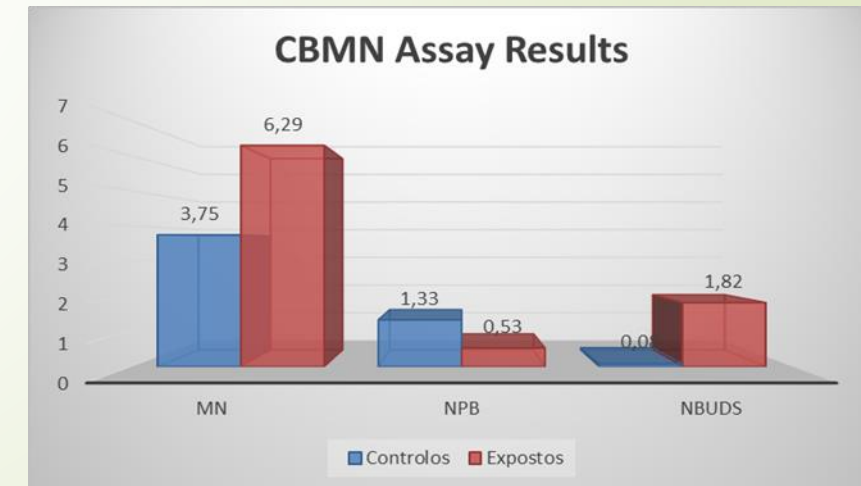
Organic solvents – Human biomonitoring

- Styrene and xylene.
- Exposure assessment - none of the workers had values higher than the TWA 8h recommended for styrene (20 ppm) and xylene (50 ppm).
 - Xylene – 321 ppm (STEL = 100 ppm).
 - Styrene – 124 ppm (STEL = 40 ppm).

Genotoxic biomarkers used in the present study revealed higher mean values of MN, NBUD, DNA damage and oxidative DNA damage in workers.



DNA oxidative damage, Mann-Whitney test, $p < 0.0001$.



NBUDs, Mann-Whitney test, $p < 0.0001$.

Organic solvents – *in vitro* from [] real scenario

- ▶ The cytogenotoxic effect of styrene and xylene and their mixtures corresponding to 7 h working shift.
- ▶ []s used for *in vitro* experiments found in the occupational setting.
- ▶ Exposures:
 - ▶ styrene and xylene alone.
 - ▶ simultaneous exposure to styrene and xylene (both applied for 7 h).
 - ▶ co-exposure to xylene (2 h alone) and styrene (5 h after 2 h xylene).
 - ▶ co-exposure to styrene (2 h alone) and xylene (5 h after 2 h styrene).

Colaboration with the Institute for Medical Research and Occupational Health, Croatia.

Cr(VI), Ni and PAH exposure



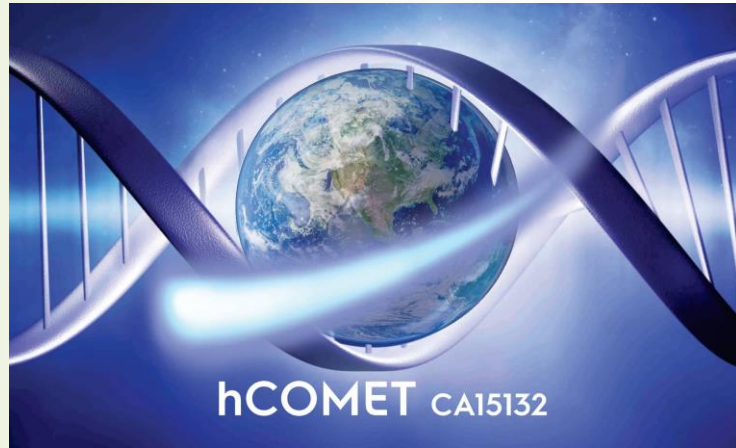
- On behalf of the HBM4EU project which intends to use human biomonitoring to assess human exposure to chemical substances, also in occupational contexts to ensure that chemicals are not to harm health or the environment.
- Exposure assessment of Cr(VI).
- Human carcinogen authorized for use in several industrial settings since is difficult to replace.
- Human biomonitoring preliminar results: Cr(VI)-exposed workers display a significant higher frequency of MN and an increased level of DNA breaks in comparison with controls.
- Real exposure data will be used to simulate exposure in *in vitro* experiments:
- Evaluation of the cytotoxicity, immunotoxicity and genotoxicity of the co-exposure to the mixture of Cr(VI), Ni and PAHs.



Final remarks

- ▶ Highlight in the information regarding mixtures of chemicals and the resulting effects of that possible combinations, taking into account the MoA of each component by itself.
- ▶ Achievement of a better understanding of risks from **combined exposure to multiple chemicals**.
- ▶ Promote the integrated assessment of **priority mixtures**, taking into account the risks of human and environmental exposure.

Acknowledgments





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