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The Story of BENZOTRIAZOLES

EVALUATION OF IN VITRO CYTOTOXICITY AND GENOTOXICITY OF CURRENTLY USED BENZOTRIAZOLES ON ADULT HUMAN STEM CELLS AND INTERNAL EXPOSURE OF CZECH POPULATION.

Chapter I: Introduction

Benzotriazole (consisting of three nitrogen atoms and fused benzene ring) and its derivates (BTRs) are high production volume emerging contaminants extensively used as corrosion inhibitors in detergents, ultraviolet stabilisers in plastics or antifreeze agents in aircraft de-icing fluids. Nowadays, they can be found in virtually all types of water.

Human population is exposed probably by diet (application of contaminated biosolids and water on agricultural fields), dermal contact but also by inhalation (dust particles). Despite their ubiquitous presence in both environment and human urine, and equivocal results from genotoxicity tests (with bacteria and mammalian cells), BTRs have not been tested for cytotoxic and genotoxic effects to human cells yet. Hence, the risks assessment focused on possible health outcomes and safe dose for humans has to be done.

Chapter II: Cytotoxicity, genotoxicity and BMD

Human liver stem cells (HL1-hT1) were used as biological model for assessment of potential adverse effects of 5 BTRs (1H-BTR, 1M-BTR, 4M-BTR, 5M-BTR and 4OH-BTR). Cytotoxicity was evaluated by combination of biological dyes Resazurin (AB) and Neutral Red (NR) which indicates the metabolic (mitochondrial) activity and membrane integrity of human cells, respectively.

Potential to interact and disrupt DNA was assessed by Comet Assay, which is sensitive to DNA strand breaks in cells.

In nutshell, after the exposure the cells were embedded in agarose on a microscopic glass and lysed followed by electrophoresis.

Upon electric current, undamaged DNA in a supercoiled state remains intact while damaged DNA extend to anode to form a comet-shaped structure. Single cells are then visually assessed by measuring the amount of DNA in comet "tail" which refers to the amount of damaged DNA (fig.1).

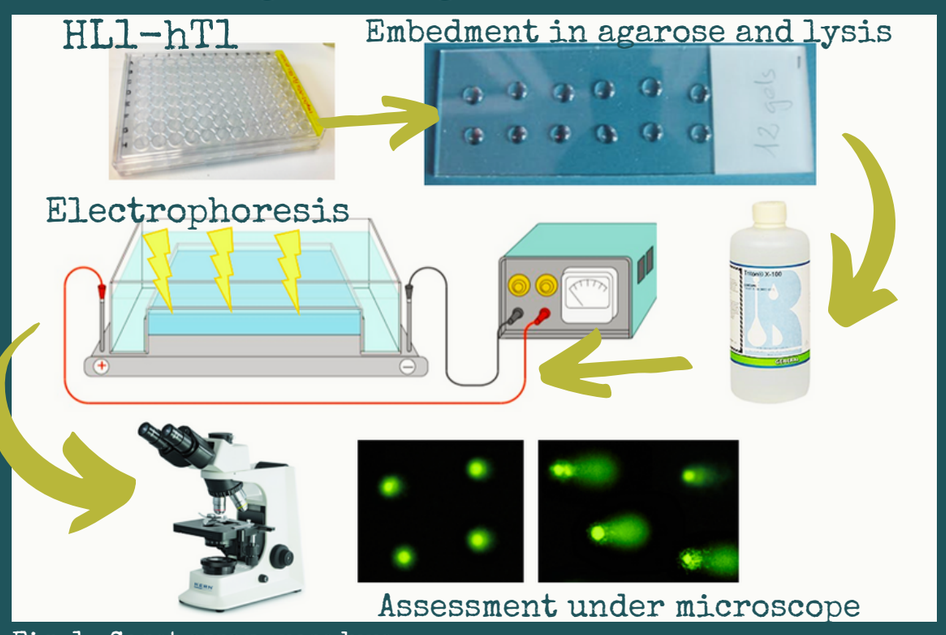

Fig. 1- Comet assay procedure

The benchmark dose (BMD, the dose that produces a predetermined change in the response rate of an adverse effect compared to control treatment) was calculated for both genotoxic and cytotoxic effects using Proast69.2 and it can serve as a reference point for subsequent health risks assessment .

Chapter III: Internal exposure to BTRs in Czech population

Liquid chromatography-electrospray ionization tandem mass spectrometry method will be developed for determination of BTR and its 6 derivates. Urine samples will be deconjugated and liquid-liquid extracted for the measurement of total concentrations. The levels of 5 BTR-based UV-stabilizers and 4 benzothiazoles will be evaluated as well.

Measurements of the target compounds will be performed in summer 2021 on SPECIMEn and FireExpo subcohorts which will allow assessment of the link between the exposure and effect biomarkers (oxidative stress, DNA methylation, cortisol, haematology, thyroid biomarkers) and possible health outcomes such as cancer or autoimmune diseases.

Chapter IV: Current results

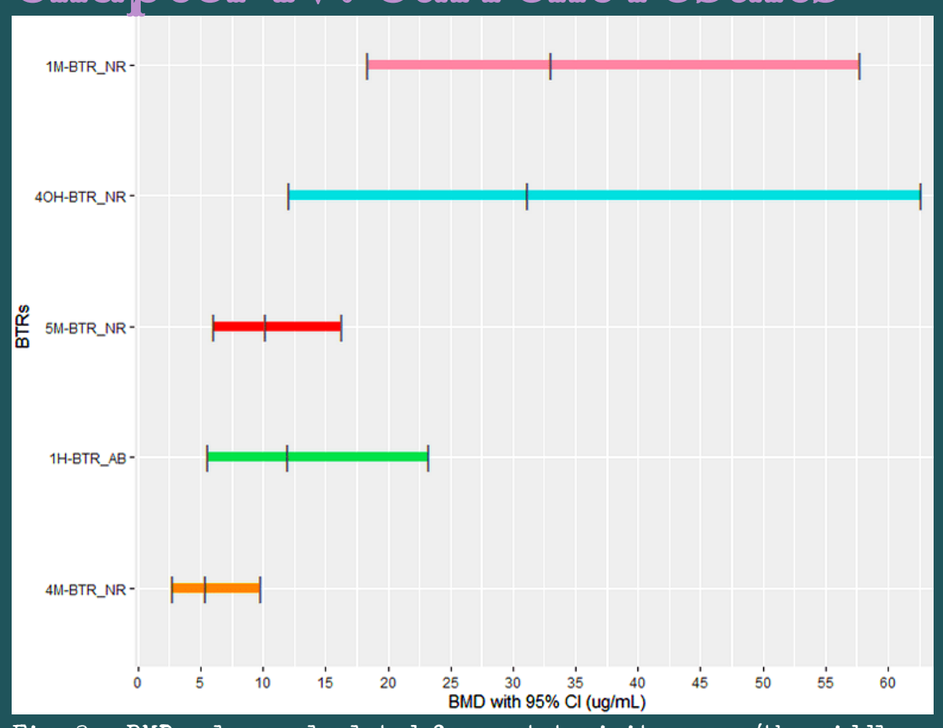

Fig. 2 - BMD values calculated from cytotoxicity assay (the middle point) accompanied by 5% upper (BMDU) and lower limit (BMDL).

Tab.1 BMD for each BTR and corresponding BMDL, assay and best model.

	1H-BTR	1M-BTR	4M-BTR	5M-BTR	4OH-BTR
Assay	AB	NR	NR	NR	NR
Best model	Exp4	Inv.Exp3	Exp4	Inv.Exp3	Inv.Exp4
BMD (BMDL)($\mu g/mL$)	11.98 (5.57)	32.99 (18.34)	5.42 (2.73)	10.21 (6.05)	31.12 (12.05)

Cytotoxicity

Pilot results suggest that integrity of cellular membrane indicated by NR seems to be more susceptible to disruption due to exposure to benzotriazoles compared to destabilization of mitochondria indicated by AB assay. Mitochondrial destabilization was more susceptible endpoint only in the case of 1H-BTR exposure (fig.2, tab.1).

Genotoxicity

The results of the comet assay on HL1-hT1 cells suggest slightly higher increase in DNA damage in lower concentrations compared to solvent control. Each BTR indicates its own pattern. However, based on raw data, the differences seem statistically insignificant. To confirm this hypothesis, ANOVA with post-hoc test will be performed on the dataset.

Epilogue

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Haber, L. T., Dourson, M. L., Allen, B. C., Hertzberg, R. C., Parker, A., Vincent, M. J., Maier, A. & Boobis, A. R. (2018). Benchmark dose (BMD) modeling: current practice, issues, and challenges. Critical Reviews in Toxicology, 48(5), 387-415.

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Hazard Identification

Hazard Characterisation

Exposure Assessment

Risks Assessment & Health Outcomes