Abstract

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Title of diploma thesis: Comparison of *in vitro* methods for the study of cytotoxicity

People are exposed to a growing number of toxic substances from the environment. Endocrine disrupting chemicals (EDCs) are a broad category of molecules that are thought to cause adverse effects on the endocrine system by interfering with the synthesis, transport, degradation or action of endogenous ligands.

One of the aims of this thesis was to determine the *in vitro* toxicity of 17 selected endocrine disruptors on the human hepatocellular carcinoma HepG2 cell line. Cell viability was determined using the CellTiter96® AQueous One Solution Cell Proliferation Assay colorimetric method, the principle of which is the reduction of MTS to the colored product formazan by mitochondria in viable cells. The cytotoxic potential of the compounds was expressed by using the toxicological parameter IC_{50}, which was measured in three time intervals (6, 12 and 24 hours).

For 14 substances: Atrazine, DHEP, Bisphenol A, Carbofuran, 3-hydroxycarbofuran, Cypermethrin, DDE, DES, MEHP, PCB 118, PCB 153, PFOA, PFOS, Propiconazole, IC50 > 100 μM (respectively > 250 μM) was determined, indicating that these substances are non-toxic to the HepG2 line. Cytotoxic substances include TBT, TPP/TPHP, TDCPP and 3-MC, which was used as a standard. The highest toxicity was shown by TBT, whose IC_{50} was 0.1063 μM at an incubation time of 24 hours. Toxicity decreases in in the order TBT > 3-MC > TPP/TPHP > TDCPP.

Cellular luciferase reporter assays are key tools for analyzing AhR interaction. In the second experimental part, we analyzed the cytotoxicity and AhR activation of selected endocrine disruptors (Bisphenol A and the substance 3-methylcholanthrene) using a new secreted Metridia luciferase (pMCS-DRE) responding to the AhR on the HepG2 cell line. The method used was the Dual-Luciferase® Reporter Assay System.

The possibility of measuring luminescence from the medium using cells transfected with the pMCS-DRE construct was experimentally confirmed, which for future research means that the experiment can be continued without cell lysis.