CHLAMYDOMONAS REINHARDTII AS A MODEL INDICATOR SYSTEM FOR GENOTOXICITY OF LOW DOSES XENOBIOTICS

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SUMMARY

Relevance of the problem: The development of highly sensitive bioindicator and biomarker systems for detection of low doses bioavailable xenobiotics in the environment is very important aspect of environmental genotoxicology. Moreover evaluation of the genotoxic/mutagenic potential of xenobiotics on photosynthetic organisms as primary producers needs to be done. This problem is closely related to plant biodiversity; genome protection and stability of plant populations growing in contaminated areas. Additionally the problem is also relevant to Directive 2010/63/EU aiming to firmly anchor the principle of the "Three Rs, to Replace, Reduce and Refine" the use of animals for experimental and scientific purpose in the EU Member States. According to Anex (47), there is an increasing need for new methods to be developed and proposed for validation in the European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM) (http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam).

***Chlamydomonas reinhardtii* as a model:** The photosynthetic unicellular green alga is a robust model for plant cell and meets the requirements of modern ecotoxicology for "good" test-systems postulated by Weber et al., (2000): quick methods, relatively inexpensive, good resolution, sensitivity, potential for extrapolation of results to higher eukaryotes.

Hypothesis: An eukaryotic bioindicator and biomarker system for detection of low doses xenobiotics with different mode of action could be developed based on strains *C. reinhardtii* that differ in their DNA repair capacity (two DNA repair deficient - UVS-10, *recombination*-repair deficient and UVS-14, *mismatch*-repair deficient, and two DNA repair proficient strains - 137C, wild type and CW15, cell-wall-less).

Main results: High susceptibility to zeocin and $CdCl_2$, defined as decreased cell survival, increased MDA and H_2O_2 quantities, and high primary induced DSBs levels is observed in DNA-repair deficient genotypes UVS-10 and UVS-14. It could be speculated that zeocin and $CdCl_2$ could induce two types of DNA damage in photosynthetic eukaryotes: DNA damage repaired via *recombination* repair and DNA damage repaired via *mismatch* repair.

We recommend 137C WT for revealing the presence of bioactive substances in samples with an unknown content. Two DNA repair deficient genotypes (UVS-10 and UVS-14) are convenient for receiving additional initial information about mechanism of action of the biologically active substances in samples. Further these DNA repair deficient strains could be successfully involved in genotoxicology for the revealing of low doses contaminants.

Contribution: In the thesis, two rapid, high sensitive and inexpensive bio-tests based on the 137C WT were developed for the genotoxicology purposes: 1) Bio-test for evaluation of the genotoxic and mutagenic potential of water, soil and air samples polluted with different types of xenobiotics – heavy metal (below and/or slightly above MAC), traces of pesticides, PM_{10}, waste gases, etc. Three endpoints were used: colony forming ability ("clonal" assay), induced "visible" mutants and biomarkers for oxidative stress - MDA, H_2O_2 and pigments. 2) Bio-test for the bioactivity analysis of multifunctional bio-mineral products (bio-transformed duck guano granulated under different temperatures and pressure conditions) for the agriculture and ecology. Spot-test, micro-colony cell survival assay, and DSB induction were applied as endpoints.

In addition these not expensive, reliable and quick *C. reinhardtii* bio-tests could be recommended for using in plant ecotoxicology because the results obtained in algal cells could be extrapolated to higher plants.