Synchronous cultures of *Chlamydomonas reinhardtii* as a tool in investigations of anthropogenic contaminants toxicity

The natural environment undergoes progressive degradation due to increasing pollution by anthropogenic substances. Chemicals, such as heavy metals or herbicides have been introduced to the environment for many years, and mechanism of their toxicity is the subject of intensive research. In recent years, an increasing concentration of active substances of commonly used drugs, including painkillers and anti-inflammatories became an important ecotoxicological problem. Concentrations of these substances in the environment are not very high, however, due to the commonness of their application, they are continuously released into the environment in the form of effluents and municipal waste.

International organizations associated with the environment protection (OECD, ISO) recommend monitoring of the presence of anthropogenic pollutants in the environment and estimation of their toxicity. Thus they recommend toxicological tests, in which the indicator organisms, cultured under standard conditions, are treated with harmful compounds to estimate chemicals' toxicity. Unfortunately, the standard toxicological tests are based on the analysis of the growth reduction only, without taking into account neither the mechanism of the substance harmful action, nor the organism's sensitivity, related to its ontogenetic development phase.

The aim of our project is to create a precise research tool for the toxicological analysis, based on the synchronously growing population of single-celled green alga - *Chlamydomonas reinhardtii*. Due to the short life cycle, rich mutants database and the sequenced genome, *C. reinhardtii* is one of the model organisms used in biological studies of physiological, biochemical and genetic problems. In the typical culture of microalgae, each cell grows independently and in a population mature cells, the young daughter (progeny) cells and cells that are in the phase of intensive growth may be observed. The application of alternating light and dark periods leads to the cells growth "arrangement" – synchronization. In the synchronous culture all cells are in the same phase of the development (cell cycle). Working with the synchronous population of cells, it is possible to expose the cell in the defined developmental stage to the toxic substance. Such analysis may be performed on a large sample of biological material, while the results can be interpreted at the single cell level.

We have chosen three potential areas of substances harmful action that are crucial for the functioning of the plant cell: progress and regulation of the cell cycle, photosynthetic activity, induction of oxidative stress and cell's adaptation mechanisms of this stress. Therefore, for each of these areas, "reference" substance with a well-known mechanism of action was selected. Cycloheximide – a specific inhibitor of the cell cycle, mostly used in the diagnosis and therapy of cancer, in *Chlamydomonas* cells has been successfully used for characterization of cell cycle progress. Atrazine – the herbicide from the triazines group, inhibiting light phase of photosynthesis by blocking the electrons flow between the PS II and PS I photosystems. Cadmium – a heavy metal with no apparent biological function, will be used as substance inducing oxidative stress in the cells of *Chlamydomonas*, i.e. the overproduction of reactive oxygen species, which leads to damage of proteins, nucleic acids and lipids. Further, a substance of poorly recognized phytotoxicity mechanism – diclofenac, the active substance in a number of painkillers and anti-inflammatory drugs – will be tested to evaluate the correctness of test assumptions and its usefulness in chemicals' toxicity prediction. *Chlamydomonas* cells, representing various development stages, will be exposed to the "reference" toxic substances and effects of their action will be considered as the reference effect, for the ones caused by the substance having an unknown mechanism of action (diclofenac).

The final result of the proposal will be the protocol for toxicological analysis based on synchronous *Chlamydomonas* population, dedicated to verify the concept of environmental contaminants' mode of action towards algal cells. We assume, that the protocol, after a series of experiments verifying its accuracy, could be possibly incorporated into routine standard tests in many laboratories. It could be applied to CECs toxicity testing, including pharmaceuticals or personal care products. Since complete toxicological data sets for many chemicals are not available, environmental risk is hard to predict for these substances. Thus, one of the purpose of this proposal is to improve this unfavorable situation. Moreover, the deepened knowledge about toxicants' mode of action is likely to stimulate chemicals' producers and users to look for less toxic substances that could replace hazardous chemicals. Further, data developed in this proposal may offer a chance to validate techniques and methods that predict toxicity more precisely than the conventional toxicological tests, which could allow a progress in toxicological sciences.