

Lecture: alternative testing methods for toxicity to reproduction

## The three dimensional human neurosphere model identifies developmental neurotoxicants

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Current developmental neurotoxicity (DNT) testing guidelines propose investigations in rodents, which require huge amounts of animals. With regard to the 3Rs and the European Regulation of Chemicals (REACH), alternative testing strategies are needed, which refine and reduce animal experiments by allowing faster and cheaper screening.

We have established a 3D test system for DNT screening based on primary human fetal neural progenitor cells which is now embedded in the German BMBF joint project “Development of predictive *in vitro* test for developmental neurotoxicity testing”.

Within this project, different cell models are compared with regard to their DNT pre-dictability employing a set of reference compounds with different DNT potentials.

In our system first results indicate that the well known developmental neurotoxicant methylmercury effects proliferation, migration and differentiation of neurospheres in a nanomolar range, while a negative test substance, the liver toxicant paracetamol, showed interference with these processes in millimolar concentrations.

More test compounds are investigated momentarily and will give us information on the validity of the system. To allow high-throughput screening for measuring proliferation and differentiation, we have developed automated computational systems for image analyses. Macros written in the program Metamorph (Universal Imaging Corp.) allow automated analyses of gain in neurosphere size over time as one measure for proliferation, as well as pixel recognition for nuclei, neuron, astrocyte and oligodendrocyte immunocytochemical stainings as measures for differentiation.

Taken together, we have established the human neurosphere model as a system-based *in vitro* test method for elucidating the potential of chemicals to disturb human brain development. Testing more chemicals will give us an answer on the predictability of our test system.

*Keywords: neurosphere, methylmercury, proliferation, differentiation, migration*