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### **Stem cell based assay for in vitro developmental neurotoxicity testing.**

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A stem cell based assay for in vitro developmental neurotoxicity testing is developed. This assay makes use of ES-D3 stem cells that are grown with the intention that they differentiate into the three main cell types of the CNS, i.e. neurons, astrocytes and oligodendrocytes. The differentiation of these cell types in the in vitro system is preceded by processes like proliferation, migration and differentiation, as will be shown in pictures of the in vitro test system followed in time. The cells in the test are characterized by 3-D characteristics like size and shape, using conventional stains and light microscopy. Immunohistochemical stains for selective labelling of neurons, astrocytes and oligodendrocytes are used to identify the cell types in the culture.

Comparison of different cultures over time suggests that identical cell-types are present repeatedly. However, the total number of cells varies between the different cultures and the critical question is whether the ratio between the different cell-types per culture remains constant. This is a pre-requisite for a valid test system.

The danger of working with end-points like cell-ratios is a well known problem in the field of in vivo developmental neurotoxicity testing. A tool often used in the field of in vivo developmental neurotoxicity is objective, randomized analyses by use of a computer aided stereological toolbox (CAST) system. An explanation of applied principles of stereology, their current use in in vivo developmental neurotoxicity testing and the translation to its possible use in quantification of the in vitro system is presented.