Murine embryonic stem cells as a model for *in vitro* developmental neurotoxicity

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Mouse embryonic stem (ES) cells are derived from the inner cell mass of growing blastocysts. These cells are considered pluripotent and capable of differentiating into a variety of endodermal, mesodermal and ectodermal cell types including neurons. This unique feature makes ES cells a favorable tool for studying developmental processes and the pathways affected upon exposure to toxic compounds.

In the present study, we have developed a new and efficient protocol for differentiating murine ES cells into neural cells. This method is based on the formation of neuronal spheres and takes only about 12 to 14 days to produce mature neurons. The differentiation process has been well characterized my means of immunofluorescence staining of selected marker proteins specific for neuronal precursor cells as well as mature neurons and glial cells. In comparison to previous protocols our method significantly shortens the differentiation time needed for the development of mature neurons from ES cells. At the same time, the number of maturing cells is sufficiently high to be applicable in developmental neurotoxicity testing.

To assess adverse effects on neural cell differentiation and proliferation predictive toxicological endpoints have been established using flow cytometry of neuron-specific as well as glia-specific marker proteins and proliferation assays. Preliminary chemical testing revealed differences in the sensitivity of stem cells, 3T3 fibroblasts, and stem cells differentiating into neurons when compared to each other. Furthermore, we could show that the mouse embryonic stem cell model provides trustful and reliable results in the detection of developmental neurotoxicants.