Poster: alternative testing methods for toxicity to reproduction

Development of a predictive *in vitro* test module for developmental neurotoxicity testing using mouse embryonic stem cells

Katrin Hayess, Dana Sittner, Birgitta Slawik, Anke Visan, Horst Spielmann, Andrea Seiler
Federal Institute for Risk Assessment (BfR) (Berlin) (DE)
e-mail: Katrin.Hayess@bfr.bund.de

The development of the nervous system is a rather complex process known to be affected by different drugs and chemicals. Therefore, regulatory test guidelines have been adopted for the prediction and assessment of developmental neurotoxicity (U.S.EPA OPPTS 870.6300 and OECD TG 426). However, current *in vivo* test methods are laborious, costly and necessitate use of considerably high numbers of laboratory animals. Validated alternative methods for developmental neurotoxicity testing are not available. Thus, standardised, predictive screens for the evaluation of developmental neurotoxicity need to be available with the ultimate goal of increased efficiency in terms of reduced animal use and higher throughput compared to whole-animal testing using the existing guidelines.

In a newly established joint project funded by the German Ministry for Research and Education our final goal is to develop standardised predictive cell-based *in vitro* assays for developmental neurotoxicity testing. Different complementary cell models which represent selected developmental stages of the developing brain *in vivo* will be investigated to predict developmental neurotoxicity *in vivo* from *in vitro* data. In the context of a complex modular test strategy, we are developing a predictive *in vitro* test module using the mouse embryonic stem cell line D3. We established a simple and fast method for differentiation of mouse embryonic stem cells into neurons, astrocytes and oligodendrocytes suitable for testing of chemicals and other compounds. The differentiation of D3 cells into neural cells was determined by analysis of neuron-specific as well as glial-specific marker protein expression using flow cytometry and western blotting, respectively. In terms of a higher throughput, the protocol was adapted to a 96-well plate format. Here, we present our achievements in establishing predictive toxicological endpoints regarding proliferation as well as neural cell differentiation. Moreover, preliminary dose response profiles of selected developmental neurotoxicants are shown.

Keywords: in vitro, developmental neurotoxicity, embryonic stem cells, neurons, differentiation, test method