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Establishment of an in vivo micronucleus assay using flow cytometry

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The micronucleus test is an essential part in the characterization of the genotoxic potential of a test compound. This assay is used for the detection of chromosomal damage by evaluating the formed micronuclei. Micronuclei emerge from chromosome fragments or whole chromosomes, which have failed to be incorporated correctly into one of the daughter nuclei during cell division. As genotoxic substances are known to be potentially mutagenic, the micronucleus test is an inherent part in the safety evaluation of pharmaceuticals.

In this study an in vivo micronucleus test using flow cytometry on peripheral blood was established. Experiments were performed with the alkylating compounds cyclophosphamide (CP) and ethyl methanesulfonate (EMS). Measurements were restricted to the youngest reticulocytes, displaying the transferrin receptor (CD71). This ensured that only recent DNA lesions were observed. To avoid the splenic elimination of micronucleated reticulocytes (MN-RETs) reported in rats, CD-1 mice were used through the study. Peripheral blood from the vena facialis was collected 24 hours after treatment for two consecutive days. The frequency of micronucleated reticulocytes was determined by measuring 20 000 reticulocytes using flow cytometry.

In contrast to previously published data, both EMS concentrations with 200 and 225 mg/kg bodyweight displayed only moderate effects with an overall 1.7-fold increase in MN-RETs. CP at a dosage of 20 mg/kg bodyweight resulted in a 3.8-fold inductions of MN-RETs and may serve as a validated positive control in further studies. It could be demonstrated that flow cytometry is excellent for the evaluation of the micronucleus test, as it is a reliable, time-saving method for the measurement of micronuclei in the peripheral blood of mice.

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Genetic Toxicology, Micronucleus Assay, Flow Cytometry.