3 Genotoxicity

For small molecules, mutagenicity is a very severe development hurdle, since gene mutagens are also regarded as potential carcinogens and may cause germ line mutation, a hazard, which is acceptable only under rare circumstances (e.g., late-stage cancer treatment). Therefore, a hazard for mutagenicity is generally not accepted for drugs. As a consequence, evaluation of the mutagenic potential is often done very early in the candidate screening process. Fortunately, for this endpoint, various in silico tools are available which allow a relative reliable prediction, especially when combining two complementary QSAR systems, one a rule-based expert system (e.g., Derek Nexus, ToxTree) and the other a statistical-based system (e.g., MultiCase, Sarah Nexus, TopKat, Leadscope Model Applier) (Serafimova et al. 2010). The sensitivity and negative predictivity of these systems has reached a level that they are now accepted by regulatory agency for the prediction of the absence of a mutagenic potential in case of impurities (Valerio and Cross 2012; Sutter et al. 2013.). Such methods are also suitable for screening in the early research phase, where high throughput is needed.

Since, for regulatory assessment, a *Salmonella typhimurium* reverse mutation assay with five bacterial strains ("Ames test") is mandatory before FiM, in vitro screening of the different candidates in downsized variants of the regulatory assay is frequently performed. There are several screening formats of the Ames test offering higher throughput and less substance need than the regulatory test performed according to OECD guideline (e.g., Mini Ames, Micro Ames, Ames II, and Ames MPF[6] assay) (Escobar et al. 2013). Positive results in the gene mutation assay are likely to be considered to represent a no-go for further development of the drug candidate, as evaluation of the biologic significance of the finding would require extensive in vivo assessment of the mutagenic and carcinogenic potential prior to start of clinical studies. However, as outlined in ICH S2 (R1) (2011), purity of the test substance should be considered in order to exclude impurities as cause of the mutagenicity.

In addition to the bacterial gene mutation, chromosomal damage or recombination may occur resulting in clastogenic and/or aneugenic effects. In regulatory testing, this endpoint has to be evaluated in a mammalian cell genotoxicity assay. Therefore, screening for such effects is also common in the drug discovery process. From the multitude of available in vitro tests, the micronucleus test is the most frequently applied screening assay due to the potential of automation and thus relatively high throughput (Kirsch-Volders et al. 2003). The other well-established mammalian tests – the mouse lymphoma *Tk* gene mutation assay and the metaphase chromosome aberration test – are much more labor intensive and may still be used as pivotal regulatory assays.

Different from a positive result in the gene mutation assay, a positive result in the in vitro mammalian cell assay does not automatically result in the termination of

[6] Microplate format