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Supported by the Bo Holmstedt Memorial Foundation for describing a feasible method for the solution of a toxicological problem under maximum respect of the 3R-principle (Reduce, Refine, Replace animal testing)

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Utilising chemiluminescent methods for animal-free toxicology tests

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The use of molecular endpoints to identify and classify potentially toxic compounds provides significant potential for highthroughput in vitro screening. We have developed a highly reproducible and robust method of direct quantification of gene expression based on chemiluminescence. Seven target genes were chosen for their ability to inform on toxicity and mechanism and assays for their expression were developed and optimised. To assess genotoxicity, p53, RAD51C and CSTA were selected after a study by Westerink et al (2010) showed that these targets provided good predictive indices of genotoxic potential in the group of compounds screened. Additionally, we have developed gene-based tests for a group of enzymes involved in steroidogenesis (CYP21, 19, HSD17B2). For the purpose of standardisation, an assay was also developed for beta-actin to be used in parallel with the other assays. Our assay method involves the direct quantitation of RNA transcripts by the use of chemiluminescent -labelled complementary probes and is capable of analytical sensitivity of 10-50 attomol of target with a routine coefficient of variance of less than 15%. In addition to their high sensitivity, these assays have the ability to distinguish between targets differing by a single base pair providing extraordinary specificity for the intended target. These tests are being applied in combination with cell culture technologies to evaluate potentially toxic compounds in a high throughput format. The exploitation of genetic endpoints will provide a basis for a rapid screening technology allowing accurate assessment of the mechanistic action of new drugs / chemicals.