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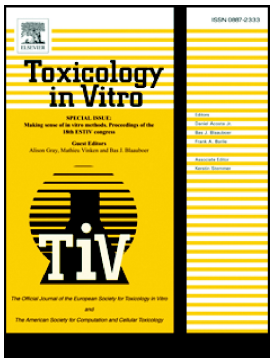

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Development of a luminescent mutagenicity test for high-throughput screening of aquatic samples

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The Salmonella reversion based Ames test is the most widely used method for mutagenicity testing. For rapid toxicity assessment of e.g. water samples and for effect-directed analysis, however, the Ames test suffers from lack of throughput and is regarded as a laborious, time consuming method. To achieve faster analysis, with increased throughput, a (downscaled) luminescent derivative of the Ames Salmonella/microsome fluctuation test has been developed through expression of the Photorhabdus luminescens luciferase in the Salmonella TA98 and TA100 strains. The applicability of this test is demonstrated by analysis of environmentally relevant compounds, a suspended particulate matter extract and an industrial effluent sample. Use of the luminescent reporter reduced the required detection time from 48 to 28 hours with a specificity of 84% for responses reported in the literature to a set of 14 mutagens as compared to 72% in the unmodified fluctuation test. Testing of the same compounds in a downscaled luminescent format resulted in an 88% similarity with the response found in the regular luminescent format. The increase in throughput, faster analysis and potential for real-time bacterial quantification that luminescence provides, allows future application in the high-throughput screening of large numbers of samples or sample fractions, as required in effect-directed analysis in order to accelerate the identification of (novel) mutagens.

Keywords: Mutagenicity testing, Ames test, Salmonella, bacterial luciferase, downscaling, bioassay

Abbreviations: EDA, Effect-directed analysis; LoD: Limit of Detection; SEM: standard error of the mean; SPM, suspended particulate matter; 4-NOPD, 4-nitro-O-phenylenediamine; NF, nitrofurantoin; 4-NQO, 4-nitroquinoline-N-oxide; 2-AA, 2-aminoanthracenel MCS, multiple cloning site; rop, repressor of primer; FAU, formazine attenuation units; BTA, benzotriazole; 1-NP, 1-nitropyrene; 4,4'-TDA, 4,4’-thiodianilline; 4-NBP, 4-nitrobiphenyl; B(h)Q, benzo(h)quinoline; GW9662, 2-chloro-5-nitro-N-phenylbenzamide; ThioTEPA, N,N’,N”-triethylenethiophosphoramide; B(a)P, benzo(a)pyrene; 4-AAB, 4-aminoazobenzene.