

The ToxTracker reporter system is a mechanistic toxys genotoxicity assay that discriminates clastogenic from aneugenic compounds.



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Introduction

With the increasing production of new chemicals for a wide range of applications in health care, food and cosmetics, there is an urgent need for rapid and reliable toxicity assessment. For reliable prediction of human health hazards, in vitro test systems should ideally not only identify adverse properties of chemicals, but also provide insight into the type of cellular damage inflicted by novel compounds.

Results

The ToxTracker assay is a mammalian stem cell-based assay that detects activation of specific cellular stress signalling pathways as the result of exposure to compounds. ToxTracker can discriminate between induction of DNA damage, oxidative stress and protein damage by quantitative assessment of DNA replication stress, NF-kB associated DNA damage signaling pathways and various anti-oxidant or unfolded protein responses. In addition, ToxTracker can discriminate clastogenic genotoxins from aneugenic compounds based on differential DNA damage reporter induction and because of the delayed kinetics by which these reporters are activated by aneugens..

Conclusion

The integrative approach of the ToxTracker assay provides a powerful tool for in vitro carcinogenic hazard identification of chemicals by unveiling activation of specific cellular signalling pathways upon exposure and deliver insight into the underlying mechanism of toxicity.











cificity of the GFP Specificity of the GPF reporter's is largely unaffected by cytotoxicity and compound exposure times. GPF reporter induction levels w calculated at compound concentrations that induce 10, 25, 50 or Xy cytotoxicity using the panel of EVCMM-selected genotoxic carcinoge Clustering of compounds was based on the similarity in reporter activation at 50% cytotoxicity. The kinetics of GPF induction was determin by flow cytometric analysis of intact cells following 4, 8, 12, 16 and 24 h. exposure to the DNA damaging agents cisplatin and doxorubicin, microtubule disrupting agent taxol, the oxidative stress-inducer DBM and tunicamycin as activator of the unfolded protein response.

ction levels of the different GFP reporters were calculate cs of Bscl2-GFP and Rtkn-GFP following exposure to the porter cell unds at an equit DNA damaging compounds cisplatin, etoposide, mitomycin C and doxorubicin and to the mitotic spindle poisons colcernid, nocodazo inoretibine and taxol. GFP induction was determined after 4, 8, 12, 15 and 24 h. exposure times that resulted in a 15-fold increase GFP signal for the Bost2-GFP and Rith-GFP reporters after exposure to various classiogenic and neurgenic compounds were calculated ure time data points of the two doses encompassing 15 fold GFP induction data points n of the ex