The novel ToxTracker reporter system provides mechanistic insight into the genotoxic properties of compounds and materials.

Giel Hendriks[1], Remco Derr[1], Branislav Misovic[2], Bruno Morolli[2], Fabienne Calléja[2] and Harry Vrieling[2].

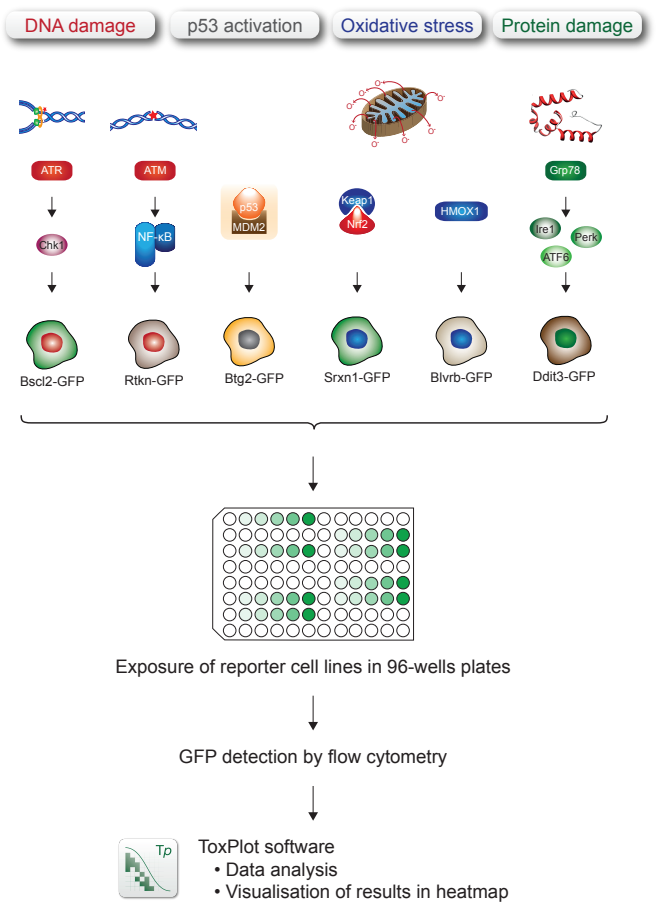
[1] Toxys B.V., Albinusdreef 2, 2333 ZA, Leiden, the Netherlands. g.hendriks@toxys.com
[2] Department of Human Genetics, Leiden University Medical Center, Leiden, the Netherlands

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.

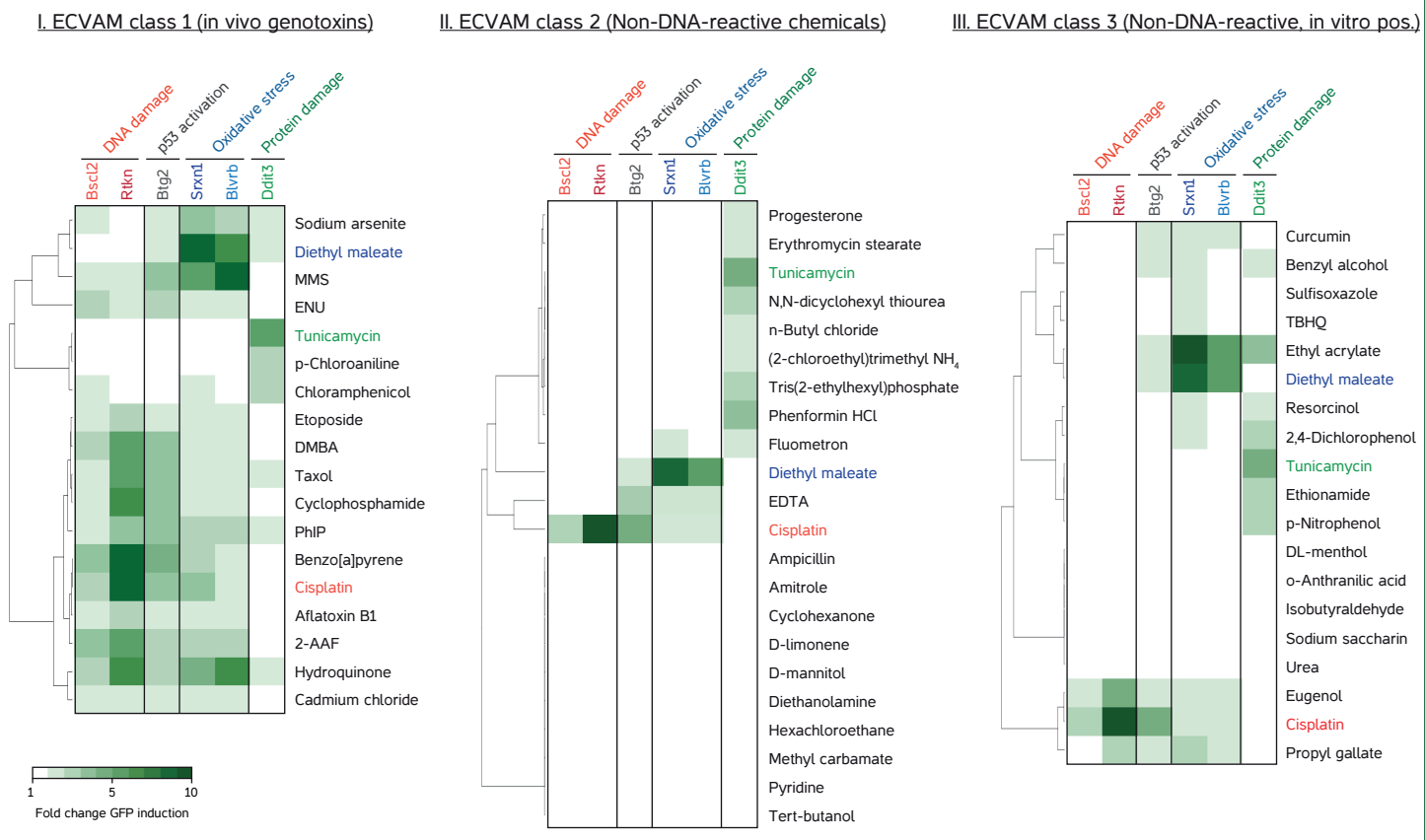
Results
The ToxTracker assay is a mammalian stem cell-based assay that detects activation of specific cellular stress signalling pathways as the result ofexposure to compounds. ToxTracker can discriminate between induction of DNA damage, oxidative stress and protein damage by quantitative assessment 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.

The ToxTracker reporter assay

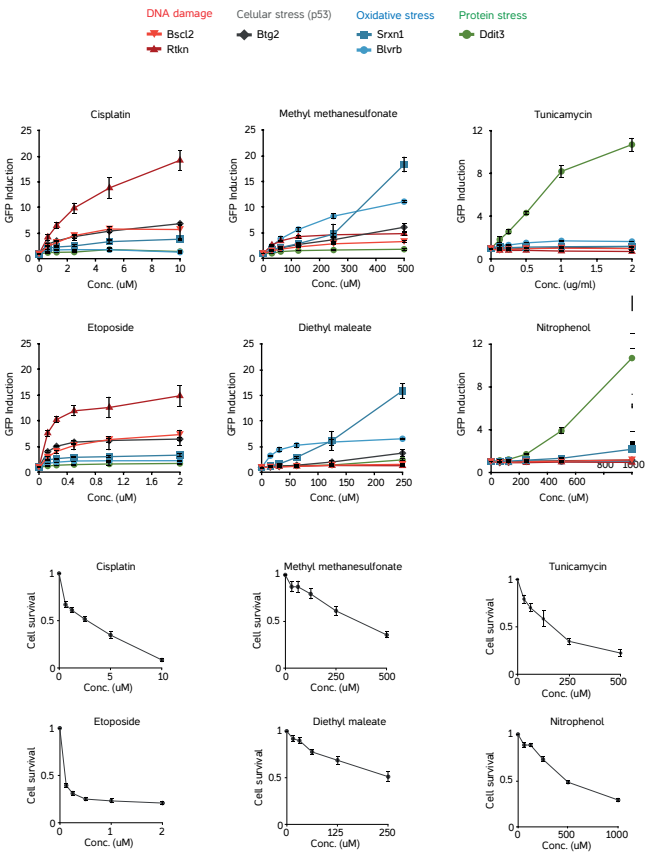


Validation of the ToxTracker assay



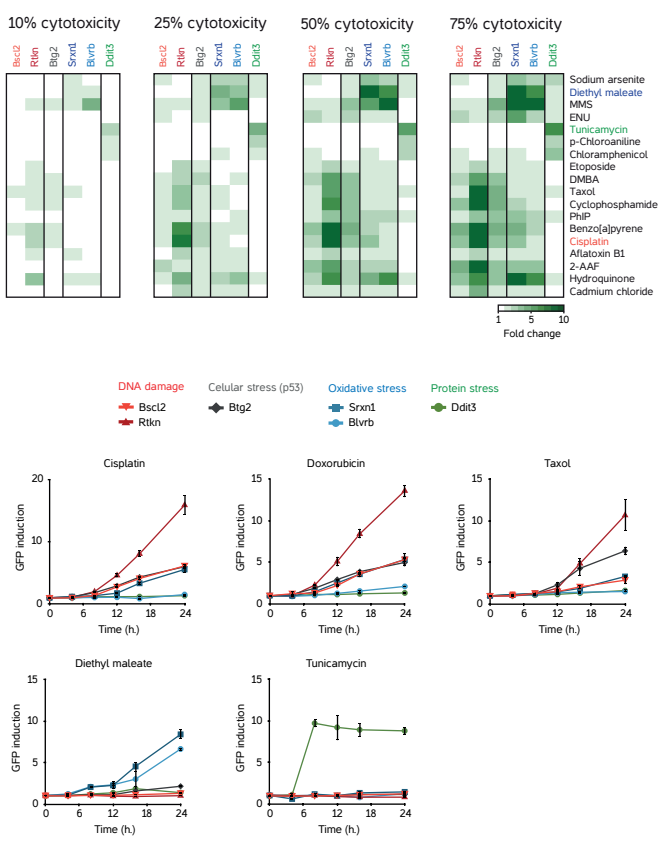
Preferential activation of ToxTracker reporter cell lines. Induction levels of the different GFP reporters were calculated for all compounds at an equitoxic concentration that induced 50% cytotoxicity. GFP levels were determined by linear regression of the GFP induction data points of the two doses encompassing 50% cytotoxicity. Induction of the GFP reporter upon exposure to a selection of ECVAM-recommended carcinogens that (I) should be positive in an *in vitro* genotoxicity assay, (II) a selection of non-carcinogens that should be negative in an *in vitro* genotoxicity assay and (III) a collection of compounds that are non-carcinogens or non-genotoxic carcinogens but that occasionally scored positive in a conventional *in vitro*/*in vivo* genotoxicity test.

Specificity of the ToxTracker reporters



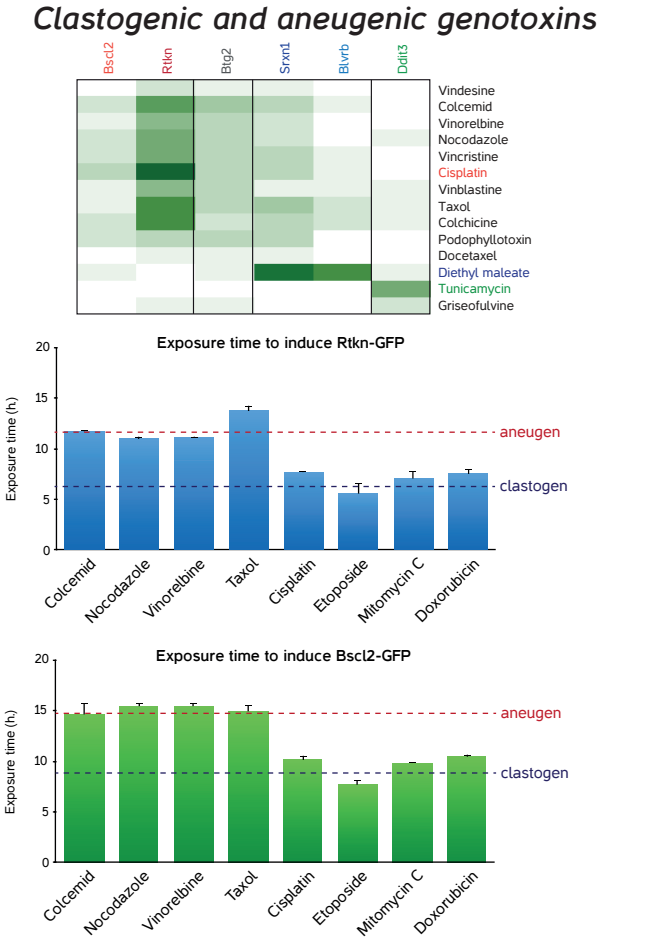
Selective activation of the ToxTracker reporter cell lines. GFP reporter cells were exposed to increasing concentrations of the DNA damaging agents CisPt and etoposide, the alkylating agent methyl methanesulphonate (MMS), the oxidative stress-inducing agent DEM and the UPR-activating compounds tunicamycin and nitrophenol. GFP induction levels in intact cells were determined by flow cytometry at 24 h. after initiation of the exposure. Cell survival was determined by flow cytometry after 24 h. exposure as the relative change in cell concentration compared to untreated controls.

Impact of cytotoxicity and exposure time



Specificity of the GFP reporters is largely unaffected by cytotoxicity and compound exposure times. GFP reporter induction levels were calculated at compound concentrations that induce 10, 25, 50 or 75% cytotoxicity using the panel of ECVAM-selected genotoxic carcinogens. Clustering of compounds was based on the similarity in reporter activation at 50% cytotoxicity. The kinetics of GFP induction was determined by flow cytometric analysis of intact cells following 4, 8, 12, 16 and 24 h. exposure to the DNA damaging agents cisplatin and doxorubicin, the microtubule disrupting agent taxol, the oxidative stress-inducer DEM and tunicamycin as activator of the unfolded protein response.

Clastogenic and aneugenic genotoxins



Differential responses of the Bscl2 and Rtkn-GFP reporters discriminate between clastogenic and aneugenic compounds. ToxTracker reporter cell lines were exposed to a selection of microtubule disrupting agents. Induction levels of the different GFP reporters were calculated for all compounds at an equitoxic concentration that induced 50% cytotoxicity. Kinetics of Bscl2-GFP and Rtkn-GFP following exposure to the DNA damaging compounds cisplatin, etoposide, mitomycin C and doxorubicin and to the mitotic spindle poisons colcemid, nocodazole, vinorelbine and taxol. GFP induction was determined after 4, 8, 12, 16 and 24 h. exposure. Exposure times that resulted in a 1.5-fold increase in GFP signal for the Bscl2-GFP and Rtkn-GFP reporters after exposure to various clastogenic and aneugenic compounds were calculated by linear regression of the exposure time data points of the two doses encompassing 1.5 fold GFP induction data points.