GT-Best short talk in the field of Toxicology

Establishment of an *in vitro* Transgenic Rodent assay for the detection of potential mutagens

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Mutagenicity is a critical endpoint in the hazard assessment of industrial chemicals, biocides and pesticides. The induction of gene mutation by test substances can be assessed *in vivo* by the transgenic rodent (TGR) assay which has been adopted by the OECD (test guideline no. 488). This assay uses the bacterial lacZ gene as a reporter gene to easily and reliably detect mutations. Multiple copies of this gene are integrated in the mouse chromosome.

There is currently no corresponding OECD method *in vitro*. We adapted an *in vitro* mutagenicity assay described by Cox *et al.* (2019) based on the protocol used for the *in vivo* TGR assay (OECD 488). In this *in vitro* version, "*in vitro* TGR assay", primary hepatocytes (PHs) isolated from transgenic mice (MutaMouse) instead of the living animal are treated with the test substance. Due to the similarity of the *in vivo* and the corresponding *in vitro* assay, this approach is aimed to predict the *in vivo* outcome better than other *in vitro* genotoxicity assays.

MutaMouse PHs were treated for six hours with N-ethyl-N-nitrosourea, Benzo[*a*]pyrene, Ethyl methanesulfonate, Mitomycin C and Azathioprine. To assess the mutagenic potential, isolated DNA from treated cultures were packaged in λ phages. Mutations of the lacZ gene were quantified by infection of *E. coli C* lacZ-galE- cultures and co-treatment with Phenyl-β-D-galactopyranoside (P-Gal). LacZ mutant frequency (MF) was evaluated by referring the number of phages containing lacZ mutations (selective conditions (with P-Gal)) to the total number of phages (non-selective conditions (w/o P-Gal)).

All tested mutagens showed an increase in lacZ MF ranging from 4- to 13-fold compared to vehicle control; the increase of the MF was concentration-dependent.

In conclusion, the *in vitro* TGR assay was able to detect the mutagenic potential of direct-acting and pro-mutagens with a performance qualitatively similar to the *in vivo* TGR assay. Further assessments using more test substances including moderate and weak mutagens are still required to corroborate the above data.

Cox et al. (2019). *Environ. Mol. Mutagen.*, *60*(4), 348. https://doi.org/10.1002/em.22277

OECD (2020). *Test Guideline No. 488,* DOI: https://doi.org/10.1787/9789264203907-en