Real time in vitro evaluation of the carcinogenic potential of contaminants

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The Genotrace project, combining targeted research and technology transfer, aims at delivering an innovative test to improve the safety of chemical products, drugs, human and animal food and the environment. The project consortium is led by INRA (French National Institute for Agricultural Research) and includes CNRS (French National Center for Scientific Research), the University Toulouse III – Paul Sabatier and the company Biopredic International. The Genotrace project has just received the support of the French National Research Agency (ANR) for three years.

Among the current short-term assays developed to assess DNA damage induced by a physical or chemical agent, the micronucleus assay (MN) represents a reliable and precise method that is already validated as a regulatory test in a battery of predictive tests for carcinogenesis. The Genotrace project aims at bringing major technological innovations to develop a new MN assay, that will allow to monitor both dynamically and in real time the production of chromosome damages and the signal of a genotoxic reporter on HepaRG cells (HepaRG cells are human hepatic cells that can metabolize chemical compounds, this metabolization step is required for many carcinogenic compounds).

What is the micronucleus assay?

As the name suggests, the MN test is based on the presence of DNA breaks visible as small pieces after coloring. Two mechanisms contribute

to the formation of micronuclei: a break of chromosome or a defect in the chromosome distribution, at the end of the cell division stage. This test is performed either on cells, generally lymphocytes from an animal or a human exposed to genotoxic products or on cells cultured in vitro. Whilst the assessment of disruptions in vivo cannot be foreseen, the MN test, in its in vitro version, does not enable a real time evaluation of the disruptions that will lead to the formation of micronuclei, and thus prevents the identification of the mechanism causing the chromosome fragments.

A new test for real time monitoring

The new test developed within the Genotrace project will rely on fluorescent biotracers recently generated by the academic partners (INRA, CNRS, University Toulouse III - Paul Sabatier). The first biotracer will allow visualizing the chromatin (the DNA filament associated to proteins, forming the chromosomes), without cellular toxic effect, thus allowing the dynamic monitoring of the cellular chromatin in real time. Second, the expression of a specific gene will allow the evaluation of any associated activation of the DNA damage pathway. Therefore the test will provide information on the micronuclei origin, whether induced by mechanisms of DNA breaks (clastogenic) or produced through the abnormal chromosomes distribution during mitosis (aneugenic). To take into account the metabolism of many chemical compounds, these biotracers will be stably expressed in the HepaRG® cells, human liver cells that are metabolically active, optimized for the MN test by the industrial partner (BIOPREDIC International).

The developed in vitro MN assay will be adapted to a medium- to high throughput straightforward readable assay, thanks to the implementation of high content screening imaging protocols and the development of an image analysis and classification-based pipeline. The assay will bring new capacities to the classic MN assay and may lead to breakthroughs in

the prevention and/or the diagnosis of exposure to genotoxicants present in the environment, food or future drug candidates.

The innovative genotoxicity test developed by the Genotrace stakeholders will be able to answer today's recommendations, with significant improvements in the scientific, technical and economical fields. Overall, the Genotrace project aims to a better safety of all chemicals, drugs, and foods exposure of human, farm animals/pets and environment.

Provided by INRA