

Evaluation of New Approach Methodologies (NAMs) for the detection of compounds classified as non-genotoxic carcinogens (NGTxCs)

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NGTxCs represent a major challenge for chemical risk assessment because they do not induce DNA damage through genotoxic mechanisms and therefore escape detection by standard genotoxicity testing strategies. They act through diverse and often complex mechanisms and are mainly identified through long-term *in vivo* carcinogenicity studies, which are resource-intensive and raise scientific and ethical concerns. Within the European Partnership for the Assessment of Risks from Chemicals (PARC), our group contributes to the WP 5.2.1.a working team, which aims to develop a battery of *in vitro* methods for NGTxC identification.

In a first phase, eight compounds classified as non-carcinogens or NGTxCs were evaluated using the standard and enzyme-modified comet assays in 2D and 3D HepG2 models after short- and long-term exposures. Potassium bromate and methyl methanesulfonate were used as positive controls and produced the expected responses, whereas all tested compounds were negative. In a second phase, eleven additional compounds representing diverse mechanisms of action related to carcinogenesis were assessed using the Fpg-modified comet assay in a 2D HepG2 model after short exposure. All compounds yielded negative results, including the oxidizing agents, for which additional experiments are currently ongoing.

In parallel, the Cell Transformation Assay (CTA) was implemented following the OECD guidance to assess chemically induced cell transformation associated with carcinogenic activity. The Growth in Low Attachment (GILA) assay was also established to detect anchorage-independent growth. Six compounds have been analysed to date, and the results are being compared with their expected activity profiles.

Ongoing analyses focusing on oxidative stress-related endpoints and cell transformation-based assays aim to support the development of *in vitro* approaches for NGTxC identification.

Funding: European project Partnership for the Assessment of Risks from Chemicals (PARC; HORIZON-HLTH-2021-ENVHLTH-03; 101057014; <https://www.eu-parc.eu>).