In vitro genotoxicity assessment of an antiseptic formulation containing silver nanoparticles

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The increase of antimicrobial resistance is one of the most concerning public health problems worldwide. A material composed of kaolin containing silver nanoparticles on its surface (AgNPs-kaolin), is being evaluated to be applied in animal feed as an alternative to antibiotics. AgNPs-kaolin safety is being assessed following the EFSA "Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain: Part 1, human and animal health".

An *in vitro* genotoxicity evaluation has been carried out following the strategy recommended in the EFSA guidance, under good laboratory practices (GLPs). Given the nature of the AgNPs-kaolin, the assessment has included three *in vitro* assays: i) the mouse lymphoma assay (MLA) to evaluate the induction of gene mutations, ii) the micronucleus (MN) test to evaluate the induction of structural and numerical chromosomal aberrations, and iii) the standard and formamidopyrimidine glycosylase (Fpg)-modified comet assay, as a complementary assay to evaluate the induction of pre-mutagenic lesions, in particular strand breaks and oxidized bases.

The MLA was performed following the OECD TG 490. Both AgNPs-kaolin and Ag/AgNPs released from the antiseptic formulation after agitation in cell culture media, were tested on L5178Y $Tk^{(+/-)}$ cells. Five concentrations in the range of 0.37-10 mg/mL, determined by previous cytotoxicity assays, were tested. The MN test was performed following the OECD TG 487. Both AgNPs-kaolin and released Ag/AgNPs were tested on TK6 cells, at four concentrations in the range of 0.0185-0.5 mg/mL, determined by previous cytotoxicity assays. For the comet assay, both AgNPs-kaolin and released Ag/AgNPs were tested on TK6 cells, at five concentrations in the range of 0.0067-0.5 mg/mL, determined by previous proliferation assays. Given the nature of the testing material, metabolic activation was not used in any of the assays. Negative results were obtained from every *in vitro* assay in every condition. Due to the complexity of the formulation, an *in vivo* evaluation is being carried out, following EFSA recommendations.

Funding: Project EFA 1983/16 OUTBIOTICS co-financed by "Fondo Europeo de Desarrollo Regional" (FEDER) through the European Program of territorial cooperation POCTEFA 2014-20 (INTERREG POCTEFA). Thanks to the Government of Navarra for the pre-doctoral grant.