In silico and *in vitro* characterization of mycotoxins of genotoxic concern.

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Mycotoxins (more than 300 compounds) are food contaminants produced as secondary metabolites by filamentous fungi. The enhanced incidence of emerging mycotoxins and modified forms and the characterization of mixtures are bringing new challenges. As the number of possible combinations is very high, it is important to develop an efficient tiered strategy to prioritize the mycotoxins (and combinations) that should be evaluated from a toxicological point of view. The genotoxic potential of individual mycotoxins was characterized by two levels of evidence, i*n silico* and *in vitro,* in three phases. The *in silico* approach was shaped with two predictive tools: DEREK, a knowledge-based expert system for qualitative toxicity prediction (phase 1) and Vega, a qualitative structure-activity relationship (QSAR) model platform (phase 2). Levels of evidence “certain, probable, plausible or equivocal” were considered positive with DEREK and “inactive or nothing to report”, negative. VEGA label “mutagenic” was positive, “no mutagenic” negative and “suspect mutagenic” equivocal. *In vitro* testing was conducted with the SOS/umu test (phase 3) with three assay conditions. As usual, the assay was carried out without and with the commonly used liver metabolic activation (liver S9). For the first time, kidney metabolic activation (kidney S9) was used to assess potential renal metabolites of the mycotoxins. Indeed, in this case, it was observed that kidney S9 was able to activate the positive control 4-nitroquinoline which is not activated by liver S9.

The concordance among the *in silico* and *in vitro* tools for the well-characterized mycotoxins, aflatoxin B1 (positive) and ochratoxin A (negative), validated the strategy. Sterigmatocystin was considered genotoxic in all phases, except when kidney S9 fraction was used. Regarding *Fusarium* mycotoxins, deoxynivalenol and fusarenone-X were negative except with DEREK. Some trichothecenes type B (nivalenol and deoxynivalenol acetylated forms) showed inconclusive results: positive (DEREK), equivocal (kidney S9) and negative (VEGA and liver S9). Zearalenone was negative in all phases. The kidney S9 fraction, revealed a higher genotoxic response for some mycotoxins: i) T-2 toxin was positive except when VEGA (negative) and the liver S9 fraction (equivocal) were used, ii) HT-2 was positive in all phases except after liver bioactivation, and iii) fumonisin B1 was predicted as negative in all phases except for the kidney S9 fraction (equivocal).