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Mixtures of genotoxicants: does the principle of additivity applies?

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Until recently, chemical risk assessment was strongly focused on single compounds. However, in practice, humans are exposed to chemical mixtures instead of just a single compound. Consequently, there is a need to evaluate the combined effects of co-occurring chemicals on human health, including genotoxicity. Different types of combined effects have been described, whereby for non-genotoxic endpoints, the principle of additivity is assumed to generally apply.

The aim of this study was to investigate whether the principle of additivity is also justifiable for genotoxic mixtures. To this extent, two types of binary mixtures were evaluated for their potential to induce chromosome damage *in vitro*. The first consisted of two well-known reference genotoxicants with a similar mode of action, i.e. ethyl methanesulfonate (EMS) and methyl methanesulfonate (MMS). The second mixture contained two genotoxic mycotoxins, i.e. deoxynivalenol (DON) and zearalenone (ZEN), known to co-occur in food and feed.

First, *in vitro* micronucleus (MN) data in TK6 cells for the individual compounds were collected in absence of S9 metabolic fraction. Next, benchmark concentrations of the two compounds were compared by applying a specific module in the PROAST software to establish a relative potency factor (RPF), thereby taking into account the optimal parallel fit of the curves. The RPF was then used to express the concentration of one compound as a function of the other and to select a range of binary mixtures expected to induce responses covering different parts of the concentration-response curve. More specifically, binary mixtures with varying concentration ratios of the two compounds of interest (i.e. 1:1, 1:3 and 3:1) were identified and tested in the *in vitro* MN assay. The collected experimental data of the mixtures were then compared to the responses predicted based on the data of the two individual compounds using the PROAST dose-addition model of the BMD approach in R. A first set of experiments was done with EMS-MMS mixtures. The experience gained in these tests was then used for the mixture testing with DON and ZEN. The experimental results of the EMS-MMS mixtures were close to the fitted curve based on the data of the single compounds, indicating that the principle of additivity is applicable to mixtures of EMS and MMS. Analysis of the mixture results with DON and ZEN is currently being finalized.

Keywords:
DNA damage, combined effects, *in vitro* MN assay, BMD approach, dose-addition.