Ex vivo genotoxicity testing as an alternative approach to evaluate waterborne contaminants threat – an assay with crayfish gill cells

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Animal testing usually adopts *in vivo* and *in vitro* methods, but several disadvantages are associated to both approaches. Moreover, the implementation of the 3R's principle (reduction, refinement and replacement) has been considered a priority, reinforcing the need of finding alternative methods. Hence, the *ex vivo* method appears as a viable alternative for a fast screening of waterborne contaminants' effects, since it combines some advantages of the previously mentioned methods, while considering 3R's guidelines. This study intended (i) to evaluate the effectiveness of the *ex vivo* approach using crayfish (*Procambarus clarkii*) gill cells, while evaluating cell viability and DNA integrity and (ii) to apply this integrative approach to assess the insecticide dimethoate's genotoxic potential. Thus, crayfish gill cells viability (in PBS) was evaluated, using the MTT tetrazolium reduction test, in an *ex vivo* assay for 2, 4 and 8 h. Cell viability was above the accepted limit (>70%) for 2 and 4 h, while it was slightly compromised after 8 h (67%). Afterwards, cells were exposed to PBS (negative control; NC) and to a genotoxic model (ethyl methanesulfonate – EMS; 5 mg L^{-1}) as positive control (PC), for 2 and 4 h. Genotoxicity was evaluated using the comet assay improved with DNA lesion-specific repair enzymes, namely formamidopyrimidine DNA glycosylase (FPG) and endonuclease III (EndoIII), to assess purines and pyrimidines oxidation, respectively. Results showed that only the 2 h exposure was found to be suitable, since NC presented low levels of DNA damage, and PC demonstrated a significant increase when compared to NC, while the NC DNA integrity began to be impaired after 4 h, invalidating this and the subsequent exposure length (8 h). To accomplish the second objective, gill cells were exposed *ex vivo*, for 2 h, to two environmentally realistic concentrations of dimethoate (20 and 40 µg L^{-1}), an insecticide with unknown genotoxic potential to crayfish. The DNA integrity was once again evaluated using the comet assay. Dimethoate demonstrated to be genotoxic to crayfish gill cells, despite not inducing DNA oxidative damage. In conclusion, the *ex vivo* method, applied to crayfish gill cells, showed to be suitable for 2 h exposures, when the comet assay was used as endpoint. Moreover, this approach might be assumed as a relevant contribution towards the improvement of strategies for a rapid and effective screening of the pernicious effects of waterborne contaminants.