ECO 4

Comet Assay to Detect the DNA Breakages in the Tissue of the Purple Clam (*Saxidomus purpuratus*) and the Blood of the Olive Flounder (*Paralichthys olivaceus*) Exposed to 5 PAHs

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Comet assay is a potential monitoring tool because DNA strand breakage may be produced by a wide range of agents. The comet assay, also called the single-cell gell electrophoresis (SCGE) assay, is rapid and sensitive method for the detection of DNA damage in cells. This study was performed for the identification of DNA damage in the cells from flounders and clams exposed to PAHs. As a control experiments, flounder and clam cells were exposed to H_2O_2. The cells exposed to H_2O_2 were displayed a typical nuclei movement. DNA damage of cells were significantly increased when the isolated cells from the blood of flounders and the tissue of clams were *in vitro* exposed to the different concentrations (5, 10, 50, 100 ppb) of five kinds of PAHs (benzo[a]pyrene, pyrene, fluoranthene, anthrancene, and phenanthrene). For the *in vivo* test, flounders and clams were exposed to the different concentrations of BaP for 4 days. The results showed that DNA strand breakage was effected by the concentration of BaP and the duration of exposure. In high concentration of BaP, the mean tail lengths of nuclei was longer than it in low concentration, while the mean size of head DNA decreased.

In this research, both *in vitro* and *in vivo* genotoxicity of PAHs could be biomonitored by the comet assay. Especially, clams and flounders seem to be useful as materials for monitoring genotoxic damage by comet assay.