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FLOW CYTOMETRY AS A TOOL TO ASSESS CYTOTOXIC EFFECTS OF THE NON-IONIC SURFACTANT TRITON X-100 IN *TETRAHYMENA PYRIFORMIS*

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Flow cytometry (FCM) is a powerful technique that has spread in the last decade through all areas of biological sciences. Although FCM has been widely used as a routine method in the evaluation of proliferation and cell viability in toxicological studies, very few works have been achieved with protozoa. Among protozoa, *Tetrahymena pyriformis* is one of the most commonly used ciliated protozoa for laboratory research and it was used in this work as a model organism to assess the cytotoxic effects of the non-ionic surfactant Triton X-100.

Structural and functional changes in *T. pyriformis* populations induced by Triton X-100 were assessed by FCM associated to the use of the Live/Dead ® Cytotoxicity and Viability kit from Molecular Probes. Kinetic changes in esterase activity and membrane integrity were evaluated by monitoring calcein-AM (CAM) hydrolysis and its retention, and ethidium homodimer (EthD-1) exclusion, respectively. In control suspensions of live (untreated) cells most cells took up and processed CAM, and accumulated calcein in the vacuoles, but almost none incorporated EthD-1. In contrast, the majority of dead cells (treated with a solution of methanol and acetone 50%, v/v) stained with EthD-1 but not with CAM. Furthermore, in *T. pyriformis* cells injured with Triton X-100, a progressive decrease in the green fluorescence mean intensity was associated to a progressive increase in the red fluorescence mean intensity suggesting that i) the decrease in esterase activity preceded the loss in membrane integrity and ii) an increase in the number of functional/structural condemned cells. Moreover, a decrease in the number of green cells (CAM^+, $EthD\text{-}1^-$) and an increase of the red ones (CAM^-, $EthD\text{-}1^+$) was observed with increasing Triton X-100 concentrations, evidencing a dose-dependent response.

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