ENVIRONMENT AND HEALTH

EFFECT OF LOW-DOSE POLLUTANT MIXTURES ON IMMUNE CELL LINE MODEL

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The global economy and industrial production have led to widespread contaminant release into the environment, increasing the recognized risk of immune alterations and non-communicable diseases (NCDs), like inflammatory bowel disease (IBD)[1]. BDE-47[2], a flame retardant and immune-endocrine disruptor, and Estrone[3], an estrogen with harmful effects at high concentrations, are chemicals of particular concern found in Sicilian area with relevant anthropogenic activity. The aim of this research is to study the chronic exposure to low doses of mixtures of these chemicals, mimicking real-life scenarios in *in vitro* immune model. *In vitro* assays were performed in murine macrophage cell line RAW 264.7 to study the potential immunotoxic and immunomodulatory effects induced by a wide range doses of pollutant mixtures. For this aim, cell viability assay, reactive oxygen species (ROS) production, transcriptional and epigenetic analysis were performed. To identify the sublethal doses to use in functional assays, the cytotoxic effects of single pollutants and their mixtures were evaluated by MTS assays on RAW 264.7 macrophages. Specifically, murine macrophages were treated with increasing concentrations of BDE-47, estrone E1 or BDE+E1 mixtures (3, 30, 300 nM of BDE-47 and 1, 10 nM of E1). No significant alterations in cell viability were observed for each tested concentration compared to relative controls (DMSO vehicle concentrations). To assess the ability of different BDE-47+E1 mixtures in ROS induction, DCF-DA assays were performed on treated RAW 264.7 cells. All the analyzed mix concentrations did not induce significant alteration in ROS production compared to the controls. The immuno-modulatory effects of BDE-47 and E1 mixtures were investigated analyzing the IL-6, TNF-α, NOS2 and IL-10 gene expression by qPCR. The data obtained highlighted that the mixture containing 3nM BDE-47+1nM E1 reduces the basal expression of IL-6 in treated macrophages, instead, the mixture containing 300nM BDE-47+1nM E1 induces the expression of the pro-inflammatory gene TNF-α. No modulations of all analyzed gene were observed in cells treated with 30nM BDE-47+ 1nM E1 mixture. Starting from the transcriptional results, the mixtures containing 3nM BDE-47+1nM E1 and 300nM BDE-47+1nM E1, were selected to evaluate the possible epigenetic alteration induced in RAW 264.7 cells. A set of 84 miRNAs was analysed. No significant differences in miRNA expression were observed in macrophage treated with 3nM BDE+1nM E1, instead the mix 300nM BDE-47+1nM E1 significantly modulated miRNA profile, down-regulating Let-7a-5p, Let-7c-5p, miR-423-5p, and miR-128-3p. These results show that while the MTS and DCF-DA assays showed no significant changes, functional data revealed differences in immunomodulatory activities and epigenetic effects of the mixtures. These results will drive the design for the application of these mixtures *in vivo*, in a rat model of experimental colitis. These results underscore the dose-dependent effects of pollutants, where low doses may trigger immunomodulatory responses, potentially reflecting a hormetic effect.

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2-METHYL-4-ISOTHIAZOLIN-3-ONE INFLUENCE IN THE ALTERATION OF IMMUNE RESPONSES IN *MYTILUS GALLOPROVINCIALIS*

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Methylisothiazolinone (MIT), also known as 2-Methyl-4-Isothiazolin-3-one, is a preservative added in the formulation of paints, coatings, and cosmetics products. Being a biocide, its primary role is to prevent the bacterial and fungal growth. Due to its high efficacy even at low concentrations, MIT is active against a wide range of microorganisms. However, it has been found to be able to cause allergic reactions in humans, and this is the reason why its use has been subject to regulations in several countries [1]. Therefore, the aim of the present preliminary data has been to examine the effects of MIT on *Mytilus galloprovincialis*. Specimens were exposed to two different concentrations of the substance, respectively with 200 µg/L (E1) and 400 µg/L (E2) for a period of fourteen days. Haemocyte viability has been evaluated through the employing of two colourimetric methods: the Trypan blue exclusion test and Neutral red retention assay. In addition, the immune response performed by haemocytes has been assessed through the measurement in percentage of phagocytic cell, using the yeast *Saccharomyces cerevisiae* at a concentration of $1x10^7$ ml^{-1}. Data revealed that cell viability underwent a significant reduction in both analyses: concerning the Trypan blue exclusion test at the lower concentration (E1), for the Neutral red retention assay at both concentrations (E1 and E2). Moreover, treated groups exhibited a significant reduction in phagocytic capacity (particularly in cells exposed to