AEROSOLIZED FLAME RETARDANT EXPOSURE INDUCES MOLECULAR AND STRUCTURAL DAMAGE IN LUNG SLICES

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INTRODUCTION: Flame retardants (FRs) are widely used to reduce flammability in various commercial products, including electronics, construction materials, furniture, textiles, and plastics. Their extensive use has led to environmental dispersion, resulting in their presence in the air, water, soil, and even the food chain. Inhalation of FRs may cause adverse effects on human health, raising the need for New Approach Methodologies (NAMs) that better replicate the respiratory system function and xenobiotics exposure. Porcine Precision-cut Lung Slices (pPCLS), due to their anatomical and functional similarity to human lungs, have emerged as a promising alternative for toxicological assessment that preserves both the cell composition and the extracellular matrix structure of the lung tissue. Moreover, pPCLS are compatible with cultivation at air-liquid interface (ALI) and aerosol exposure systems, enhancing their relevance for realistic exposure modeling. OBJECTIVE: Evaluate the effects of the FR tributyl phosphate using an *ex vivo* pPCLS to evaluate mechanisms of pulmonary toxicity. MATERIALS AND METHODS: The pPCLS were obtained using the Tissue Slicer DTK-3000W apparatus. Single and repeated exposures were conducted on pPCLS, which were exposed to aerosolized flame retardant tributyl phosphate using the Vitrocell® Cloud 12 chamber. Tissue viability was assessed, and morphological analysis was performed. After processing, histological examination was conducted using hematoxylin-eosin and Mallory staining. Markers of reactive oxygen species (ROS), mitochondrial activity and active Caspase-3, were evaluated by indirect immunofluorescence. Additionally, molecular mechanisms involving K-RAS, TNF-α, p53, MT-MMP, and Cleaved Caspase-3 were analyzed by western blot. RESULTS AND CONCLUSION: The findings demonstrated that exposure to tributyl phosphate aerosols in the pPCLS model revealed a concentration-dependent tissue damage. This was evidenced by increased levels of ROS, decreased mitochondrial activity, and activation of caspase-3. Additionally, the expression of molecular markers such as K-RAS, TNF-α, p53, MT-MMP, and cleaved caspase-3 showed modulation of inflammatory and apoptotic pathways, supporting the applicability of the model for evaluating and elucidating mechanisms of pulmonary toxicity.

Keywords: Pulmonary toxicity assessments; New Approach Methodologies. Precision Cut Lung Slices.