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The biological effects of repeated exposure of Normal Human Bronchial Epithelial (NHBE) cells to the PM₁₀ fraction of indoor dust



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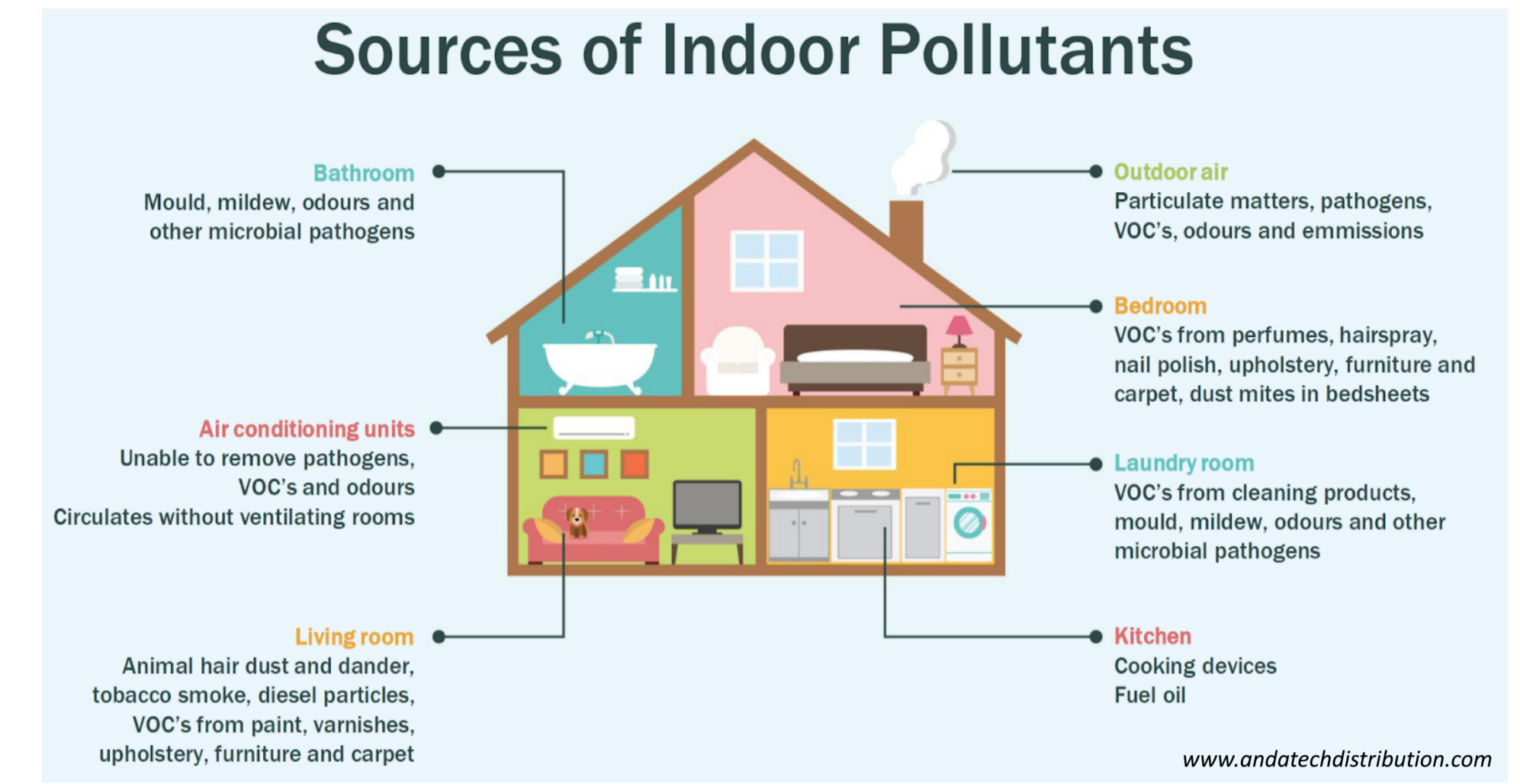
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INTRODUCTION

- World Health Organization reported 8 million deaths every year globally related to air pollution. Among these, 4.3 million were attributed to air pollution from house-hold sources and 3.7 million die because of ambient air pollution (1)
- Related health effects have been studied mainly from outdoor pollution but as we spend 90% of our time indoor, the awareness about the impact of indoor air quality has emerged more recently (2)
- The most severe human health effects of air pollution have been attributed to particulate matter (PM) (3)
- The chemical composition of indoor particles is very complex because it is a mixture of different sources and components (4)
- Exposure to indoor PM is associated with adverse health effects related essentially to respiratory and cardiovascular diseases (5)



AIMS OF THE STUDY

Study the biological markers of cellular and molecular alterations of NHBE differentiated human cells cultured at the Air-Liquid Interface (ALI) after repeated exposure to aerosolized indoor PM₁₀ issued from standard reference material "Organic Contaminants in Dust House" (SRM® 2585, NIST®) in order to mimic realistic exposure conditions *in vitro*

RESULTS

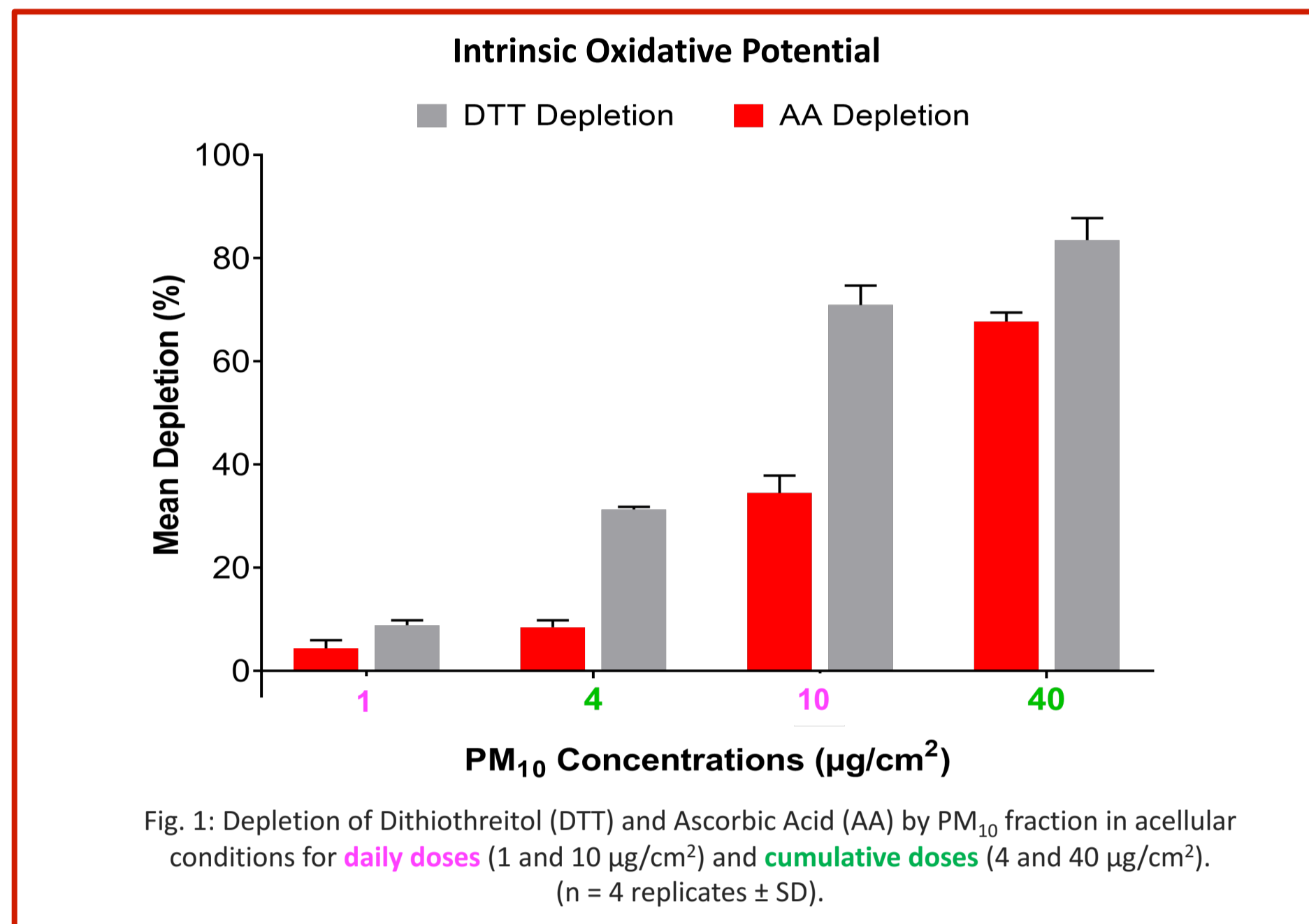


Fig. 1: Depletion of antioxidant substrates increased in a dose-dependent manner for both daily and cumulative doses, suggesting an intrinsic oxidative potential

Intrinsic Inflammatory Potential
 PM₁₀ endotoxin level : 2660 ng/g of PM₁₀

- Endotoxins are issued from gram – bacteria and could contribute to pro-inflammatory process. Endotoxins are present in SRM® 2585 PM₁₀ whereas no viable fungal nor bacterial flora was detected

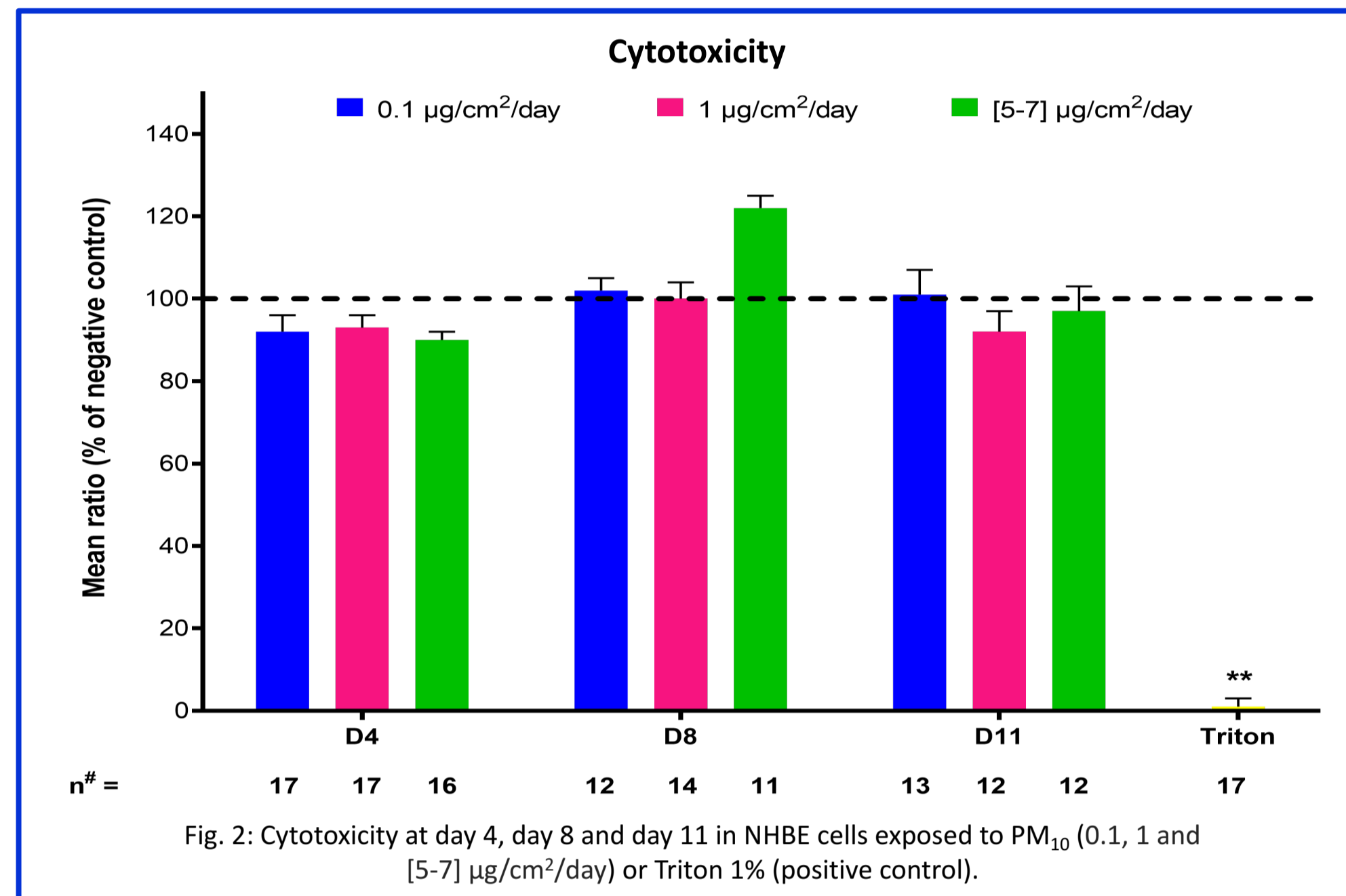


Fig. 2: No significant cytotoxicity was observed whatever the conditions tested in accordance with homogenous TEER measurements (data not shown)

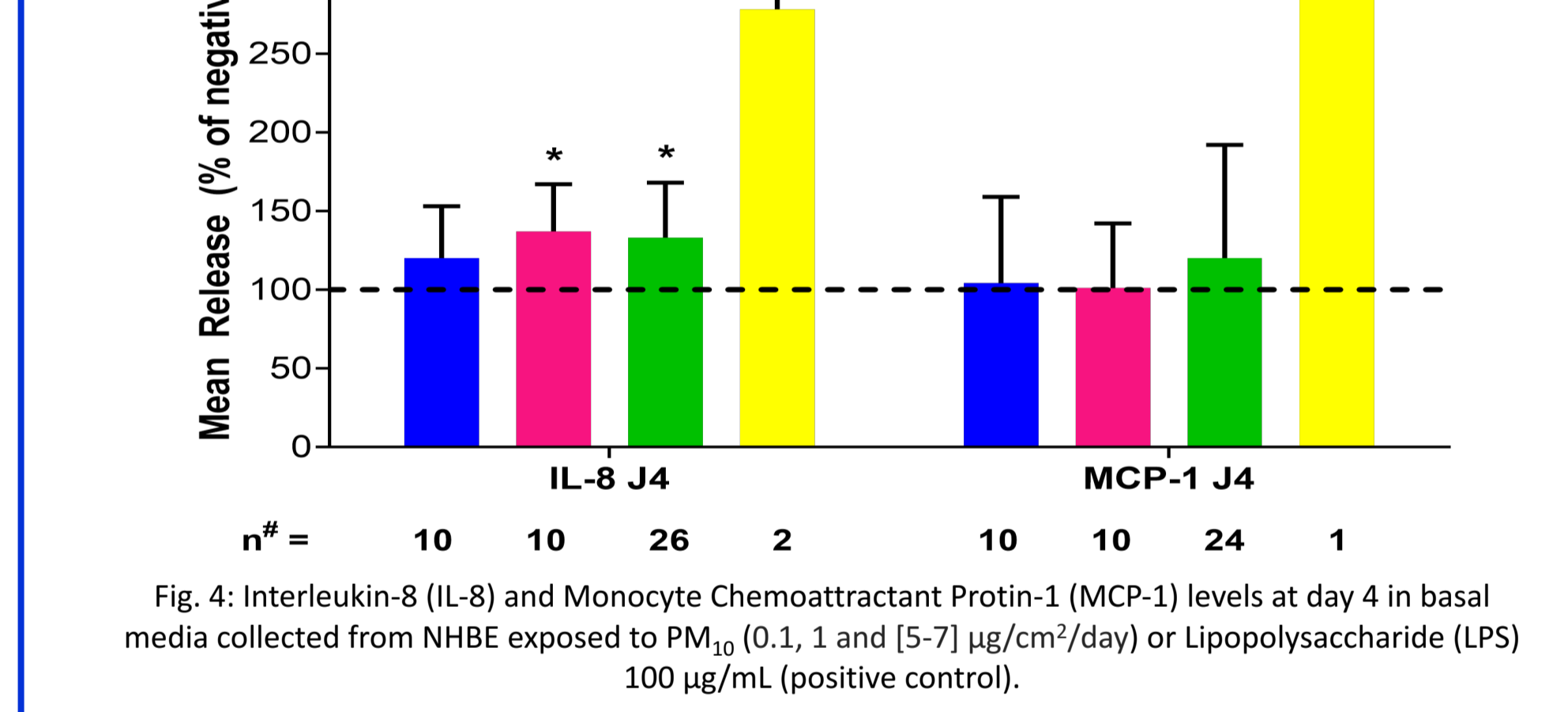


Fig. 4: A weak but significant increase in IL-8 production was observed at day 4 only for the two highest doses

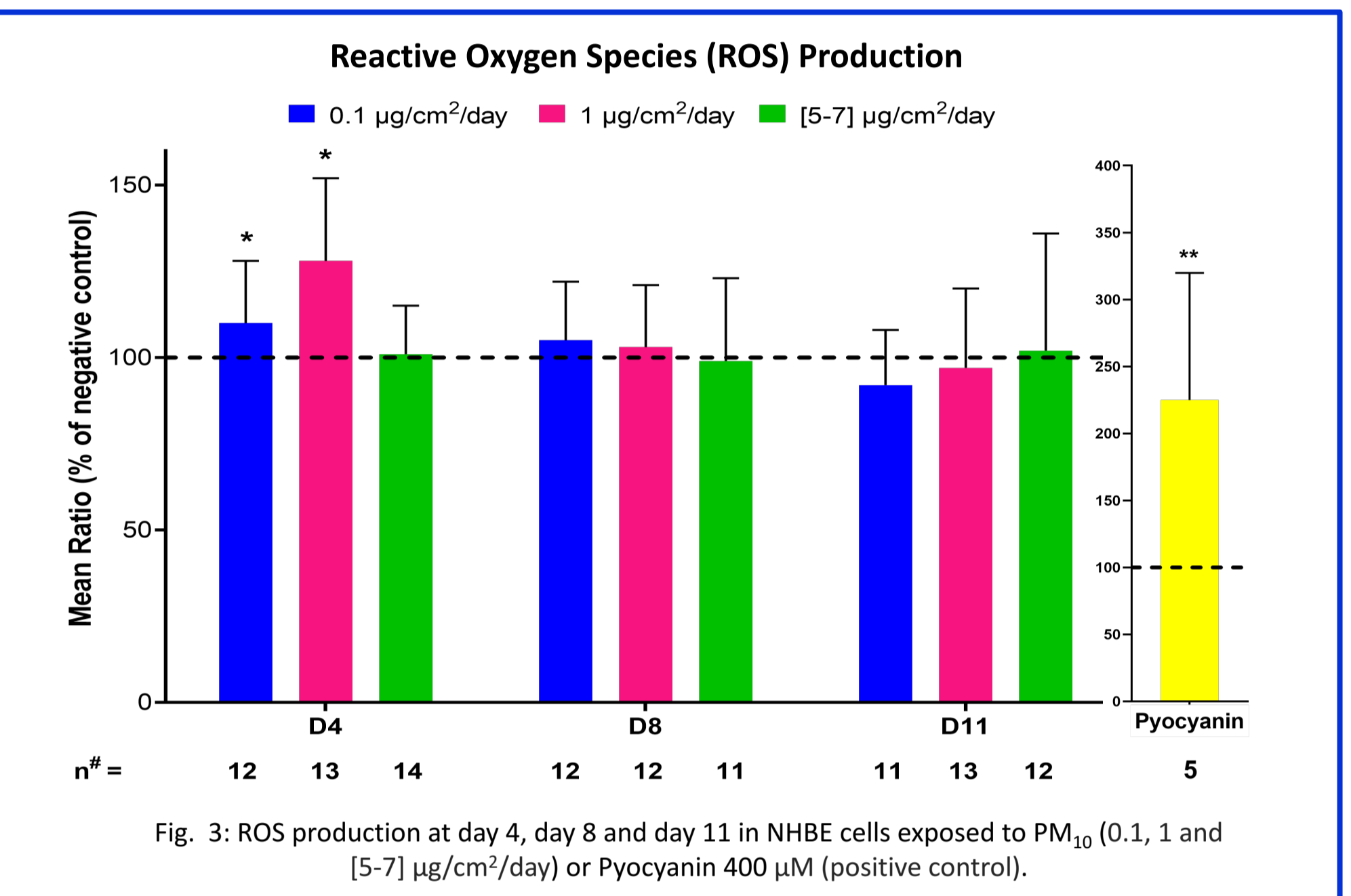


Fig. 3: A transient and significant increase of ROS production was observed only at day 4 for the two lowest doses

CONCLUSIONS & PERSPECTIVES

- Limited biological responses were detected despite the chemical content, the intrinsic capacity of these PM₁₀ to generate ROS and the presence of endotoxin. The differentiated NHBE cell model appears thus resistant to the toxic effects of PM₁₀ fraction isolated from SRM® 2585 in our realistic exposure conditions
 - To complete and explain this behaviour, expression of a panel of gene involved in oxidative stress and genotoxicity is currently being analyzed
 - Moreover, since the mucus produced by bronchial cells has antioxidant properties that could potentially limit the ROS effects, bronchial mucins will be further quantified

Perspective

- The NHBE cells will be co-exposed to SRM® 2585 PM₁₀ and supernatants collected from human macrophages exposed to SRM® 2585 PM_{2.5} (Poster n° 1185, "Impact of repeated exposure to indoor dust on human macrophage polarization profile"). The possible effect on inflammatory response will be thus studied in NHBE cells

MATERIALS & METHODS

Chemical Family	Total number of substances
Polycyclic Aromatic Hydrocarbons (PAHs)	66
Polychlorinated biphenyl (PCB) congeners	42
Chlorinated pesticides	14
Polybrominated diphenyl ether (PBDE) congeners	18
Phosphorous flame retardants	5
Perfluorinated alkyl acids (PFAAs)	8
Synthetic musks	5

"Organic Contaminants in Dust House" (SRM® 2585) → Size separation → PM₁₀ fraction

SRM® 2585 PROPERTIES

- Endotoxin levels → Limulus Amebocyte Lysate Assay
- Pro-oxidative intrinsic potential → Antioxidant Depletion
- Microbial content (fungal and bacterial) → Culture on MEA medium

Exposed organs: Nose and Mouth (100-10 µm), Trachea (10-2.5 µm), Bronchi (2.5-0.1 µm), Bronchioli (2.5-0.1 µm), Pulmonary alveoli (2.5-0.1 µm)

Normal Human Bronchial Epithelial Cells (NHBE) ATCC®

Differentiated NHBE culture: Mucus cells, Basal cells, Insert membrane, Cilia (X 40)

Optical microscope observation of differentiated NHBE cells after 3 weeks of culture at ALI (Hematoxylin (nucleus), Eosin (cytoplasm) and Alcian Blue (mucus) staining).

Cellular and Molecular Alterations

- EPITHELIAL BARRIER INTEGRITY → Trans-Epithelial Electrical Resistance (TEER) → Voltammeter
- CYTOTOXICITY → Cell proliferation → Microscopy
- OXIDATIVE STRESS → Metabolic activity → Fluorimetry (Resazurine)
- INFLAMMATION → Reactive Oxygen Species (ROS) production → Fluorimetry (DCFDA)
- INFLAMMATION → Cytokines levels → ELISA

Exposure of differentiated NHBE cells to SRM® 2585 PM₁₀

- SRM® 2585 PM₁₀ concentrations : 0.1, 1.0 and [5-7] µg/cm²/day
- PM₁₀ Nebulization : VITROCELL® Cloud Max system
- Repeated ALI exposures : 4 consecutive days

Timeline: Treatment (D0-D4) and Post-Treatment (D5-D11). ALI exposure on D0-D4, Day of analysis on D4 and D11.

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