

Deciphering the toxicity of polycyclic aromatic hydrocarbons in HepG2 cell line

Patrícia I. Morgado^{1,*} and Luisa Jordao¹

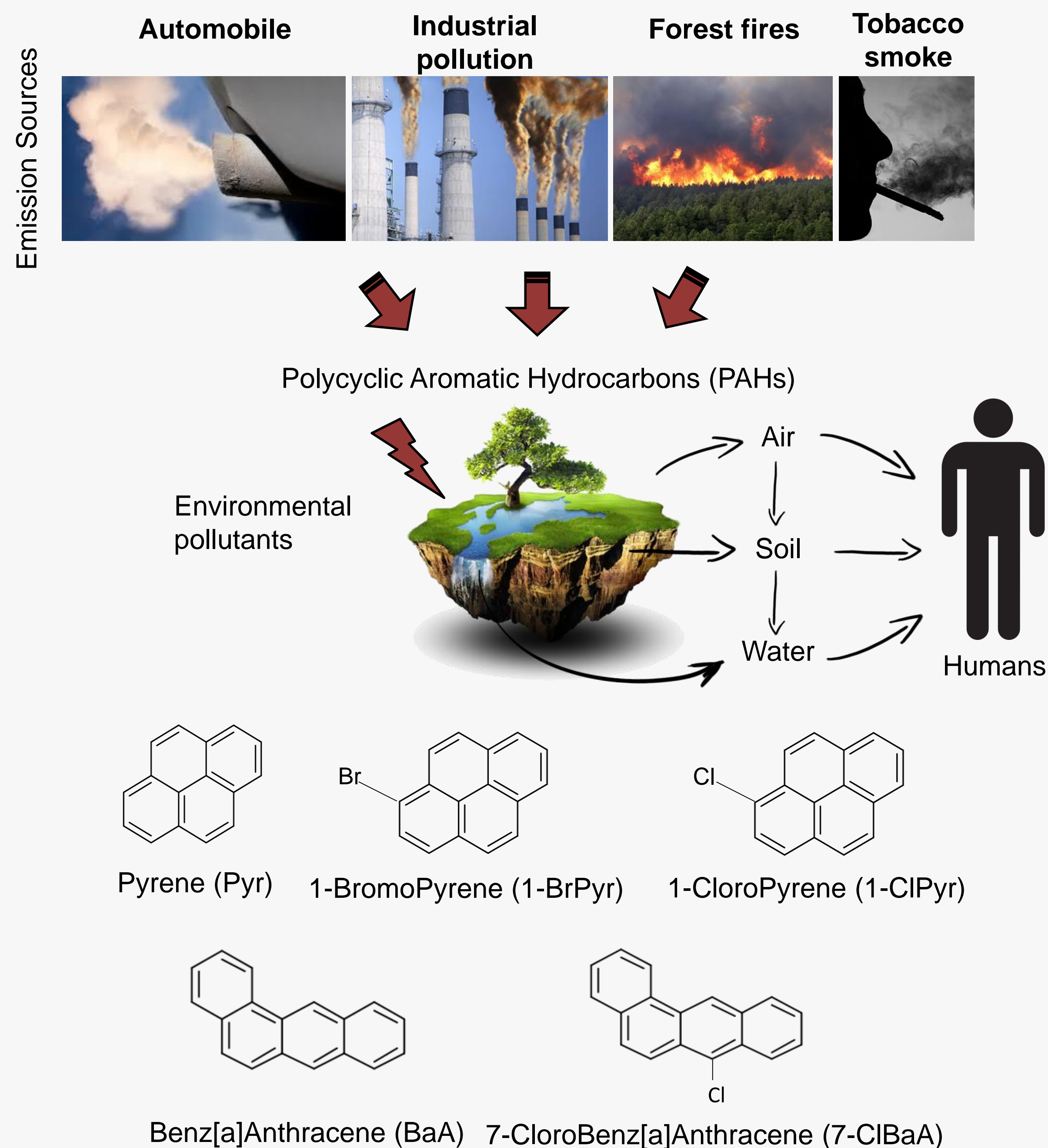


¹ Instituto Nacional de Saúde Doutor Ricardo Jorge, Departamento de Saúde Ambiental. Avenida Padre Cruz, 1649-016 Lisboa, Portugal

* Present address: Centro de Estudos de Doenças Crónicas (CEDOC), NOVA Medical School/Faculdade de Ciências Médicas, Universidade NOVA de Lisboa, 1169-056 Lisboa, Portugal. patriciaicmorgado@gmail.com; maria.jordao@insa.min-saude.pt

Polycyclic Aromatic Hydrocarbons (PAHs) are persistent pollutants present in the environment with known mutagenic and carcinogenic properties. In the present study the effect of exposure to single or multiple doses of benzo[a]anthracene (BaA), pyrene (Pyr) and three halogenated PAHs (1-ClPyr; 1-BrPyr and 7-ClBaA) were evaluated in a liver-derived human cell line (HepG2). Cytotoxicity as accessed by the classic MTT and neutral red showed a mild toxic effect in response to single or multiple dose exposure for up to 72h; except for multiple dose exposure to BaA and 7-ClBaA (cumulative concentration of 4 μ M) and single exposure to 10 μ M BaA. Furthermore, a selective mitochondrial and lysosomal toxicity was observed for Pyr and BaA series, respectively. In order to understand the underlying molecular mechanisms responsible for this effect, ROS production, mitochondrial membrane depolarization, lysosomal pH, DNA fragmentation and apoptosis mediators were evaluated after exposure to single PAHs doses. All compounds were able to trigger oxidative stress after 24h as measured by catalase activity and a good correlation was found between mitochondrial membrane depolarization, lysosomal pH increase and MTT and neutral red assays, respectively. The evaluation of cell death mediators showed that caspase-3/7 but not annexin-V pathways were involved in toxicity triggered by the studied compounds. In conclusion, the studied PAHs, especially 1-BrPyr and BaA, exhibit cytotoxic effects when accumulated, and may have adverse effects to humans after long periods of exposure.

Polycyclic Aromatic Hydrocarbons

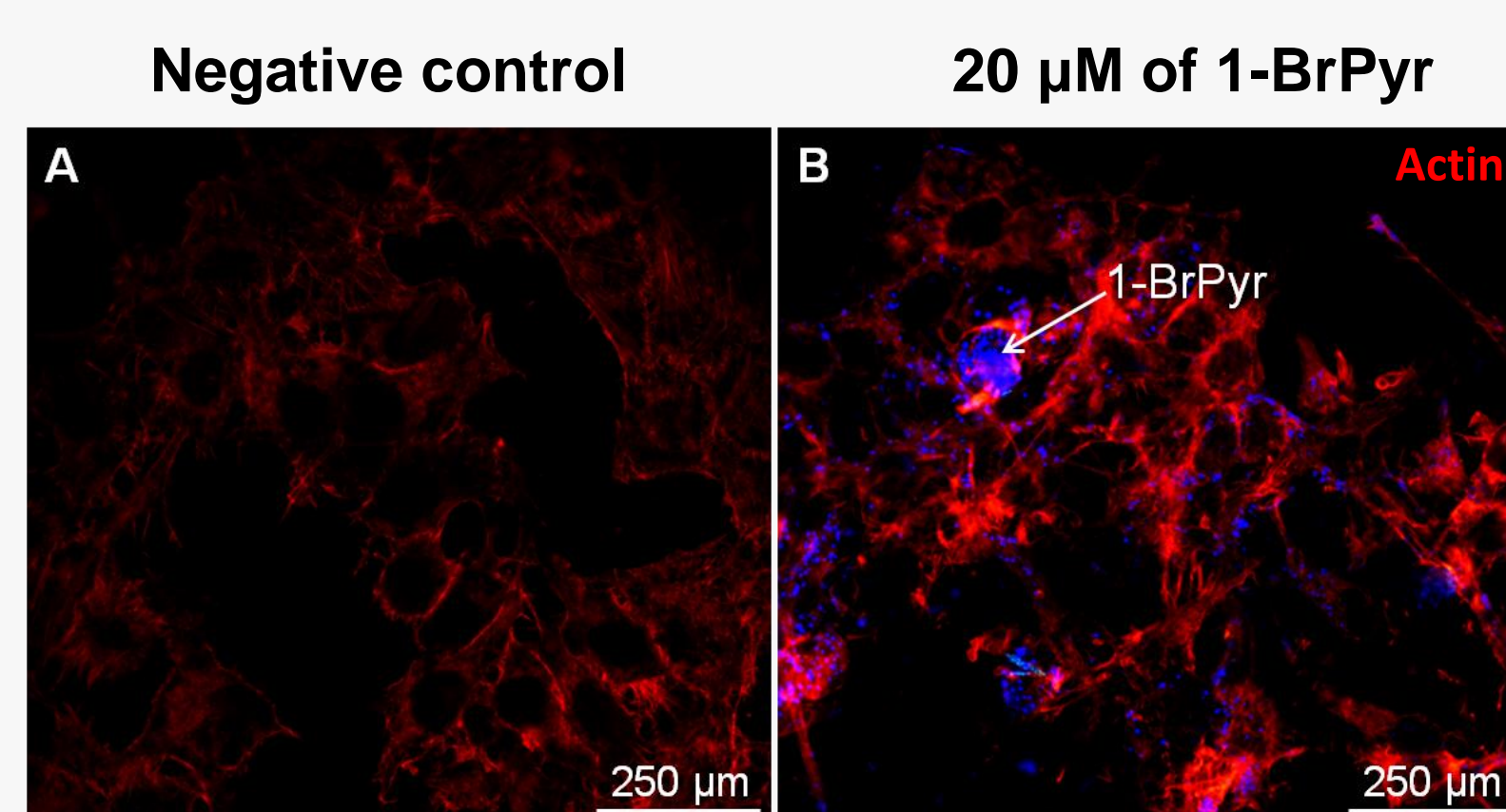


Environmental contaminants, classified as potentially toxic, mutagenic and carcinogenic

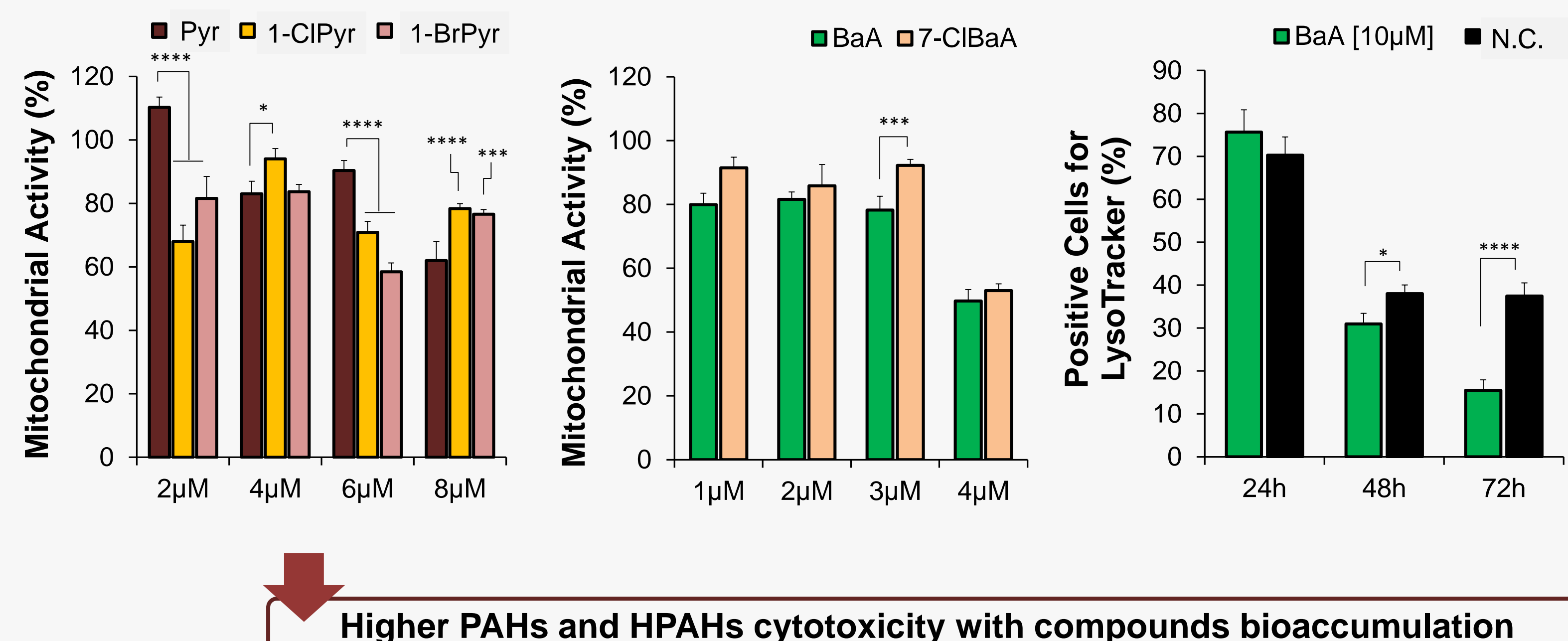
Formation of halogenated derivatives with the use of sodium hypochlorite or potassium bromide as water disinfecting agents

The halogenated derivatives (HAPHs), are generally more toxic than their parental compounds

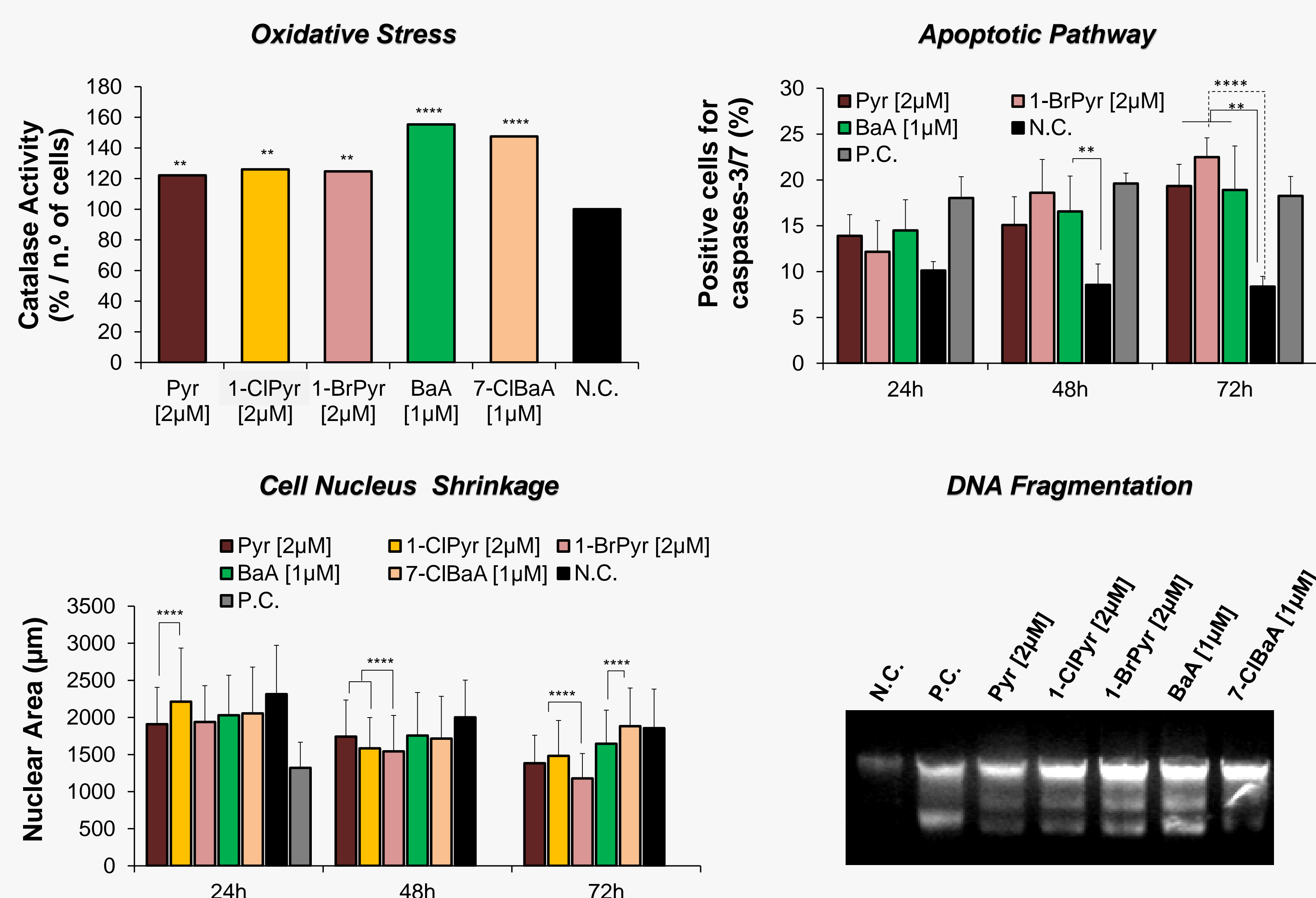
PAHs Bioaccumulation



PAHs Cytotoxicity



Programmed Cell Death Mediators triggered by PAHs



The pollutants induce ROS formation, cell nucleus shrinkage and DNA fragmentation

The pollutants induce apoptosis in HepG2 cells mediated by the activation of caspases-3/7 cascade

Acknowledgements: The authors would like to thank: Sílvia Jose (BRJ-DSA/2012) and Ana Sofia Cardoso from INSA-DSA; Riccardo Wanke and Alexandra M. M. Antunes from CQE-IST for the synthesis of 1-ClPyr and 7-ClBaA; Bettencourt group (i-Med_ULisboa) for HepG2 cell line; and FCT for financial support grant RECI/QEQ-MED/330/2012.