Halogenated polycyclic aromatic hydrocarbons associated with drinking water disinfection.

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INTRODUCTION

Disinfection by-products (DBPs) have been identified in chlorinated water. This fact justifies the growing concern about the potential health effects of emerging unregulated DBPs, some of which appear to be more genotoxic than the regulated DBPs[1]. Polycyclic aromatic hydrocarbons (PAHs) are among the most persistent contaminants detected in environmental samples such as river sediments and tap water. A few studies have already proven that water disinfection can lead to the formation of halogenated derivatives of PAHs, such as chlorinated and brominated PAHs[2]. The available toxicological studies have shown that these compounds possess, in general, greater mutagenicity than the corresponding parent PAHs.
Our research group has also shown that exposure of HepG2 cells to a dose-range of 6-Cl-benzo[a]pyrene (6-ClBaP) and BaP resulted in cytotoxicity above 50 µM and that, at the equimolar doses of 100 and 125 µM, 6-ClBaP was able to induce a significantly higher level of DNA damage than BaP[3]. The present study had two main objectives: 1) identification of the major chlorinated and brominated derivatives of benzo[a]anthracene (BaA) and pyrene (Pyr) formed as disinfection by-products and 2) evaluation of their potential hazard to humans, through the characterization of their potential cytotoxic and genotoxic effects in a human cell line.

METHODS

1 Synthesis and Characterization of: 1-ClPyr, 1-BrPyr and 7-ClBaA

- 1-ClPyr and 7-ClBaA were prepared by adaptation of the method described by Mitchell et al.[4], using *N*-chlorosuccinimide in DMF. 1-BrPyr was purchased from Sigma-Aldrich.
- Characterization by ^{1}H-NMR (Nuclear Magnetic Ressonance) and ^{13}C-NMR and bidimensional NMR (COSY, HSQC, HMBC).

2 Optimization of SPE-GC-MS* Methodology

- **GC-MS conditions:** Electronic impact (EI), 1ml/min He, T_{trap} = 240 ºC, $T_{transferline}$ = 230 ºC; $T_{manifold}$ = 80 ºC; $T_{injector}$ = 270ºC.
- **SPE conditions:** 250 mL water samples, C18 cartridges; extration with acetone.
- **Calibration curves:** Concentration from 5 to 35 µgL^{-1}, standards injected directly in the GC-MS system.

***** *Solid phase extraction-Gas cromatography-Mass spectrometry*

3 Evaluation of Citotoxic and Genetoxic effects

- **Celular line:** HepG2
- **Citotoxicity:** Neutral red test[5]
- **Genotoxicity:** Comet assay[6]
- **Tested concentrations:** 0, 10, 50, 100, 150, 200 µM for all compounds and 0, 0.5, 1, 2.5, 5, 10, 15 µM for BaA and 7-ClBaA.

4 Formation of Cl-PAHs under water treatment plants (WTPs) disinfection conditions

- 300 mL water samples with C = 1 and 10 µgL^{-1} for both PAHs (2 distinct experiments).
- Subjected to chlorination with NaOCl under acidic conditions for 30 min.

RESULTS

1

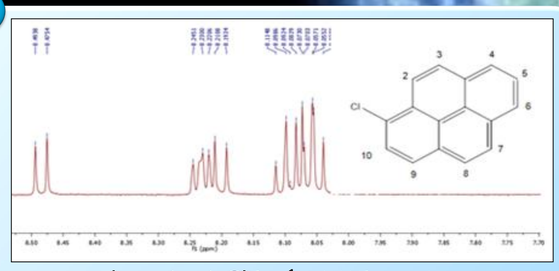

Figure 1 – 1-ClPyr ^{1}H-NMR spectra.

- 1-ClPyr: 7% yield; 7-ClBaP: 5% yield.
- ^{1}H-NMR spectra of 1-ClPyr (Fig.1) discloses the disappearence of H1 in comparison with Pyr spectra.
- MS spectra reveals the presence of chlorine: m/z (%): 238 (32) $[M+2]^{+}$, 236 (100) $[M]^{+}$ (Fig. 2).
- Similar interpretation was made for 7-ClBaA.

2

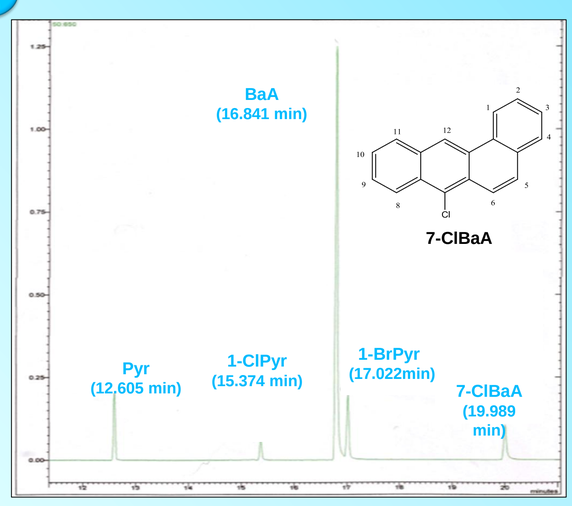

Figure 3 – Chromatogram of a mixture of BaP, Pyr and their derivatives (C = 1 mgL^{-1}).

- Calibration curves – Linearity validated by Mendel test, R^2 > 0.995; LOQ validated: RSD<10% and Relative error <25% (cromatographic separation indicated at Fig. 3).

3

Neutral red test

- 24h exposure to Pyr, 1-ClPyr and 1-BrPyr did not induce cytotoxicity in HepG2 cells (cell viability > 60%) – Figure 5.
- 24h exposure to BaA and 7-ClBaA induced cytotoxicity in HepG2 cells (cell viability < 50%).

Comet assay

- 24h exposure to Pyr, 1-ClPyr and 1-BrPyr did not cause significant increase in the level of DNA damage (DNA in tail < 5%) – Figure 6.
- 24h exposure to BaA and 7-ClBaA caused significant increase in the level of DNA damage (DNA in tail > 5%).

4

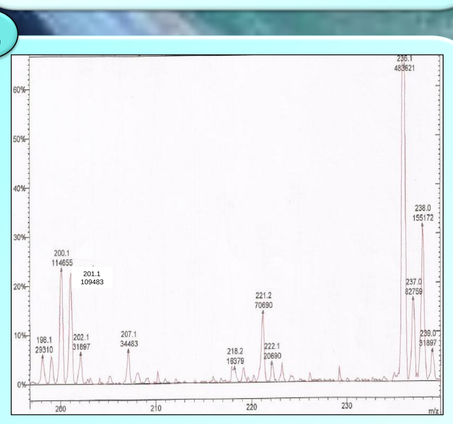

Figure 2 – 1-ClPyr mass spectra.

- Formation of 1-ClPyr (mass spectra at Fig. 2), although Pyr was still present in solution;
- Disappearance of BaA, although no chlorine derivative was identified.

DISCUSSION

Neutral red test

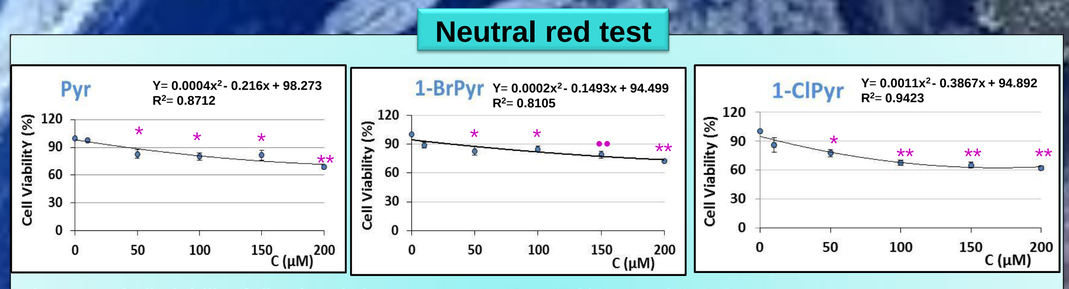

Figure 4- Cell viability following 24h exposure to Pyr, 1-ClPyr and 1-BrPyr (***p< 0.05; **p< 0.001; •p< 0.005** significantly values by Tukey's test).

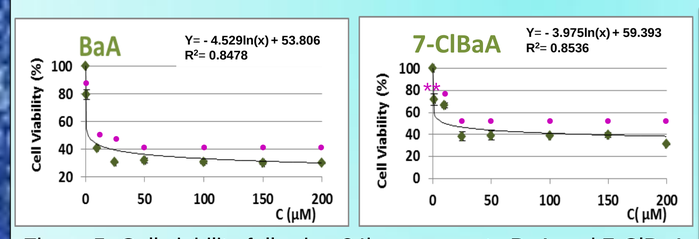

Figure 5- Cell viability following 24h exposure to BaA and 7-ClBaA (****p< 0.001; •p< 0.0001** significantly values by Tukey's test).

- Cell viability > 60% for Pyr and derivatives.
- Cell viability <50% for BaA and 7-ClBaA; IC50= 3.37 µM and 12.63 µM, respectively.

Comet assay

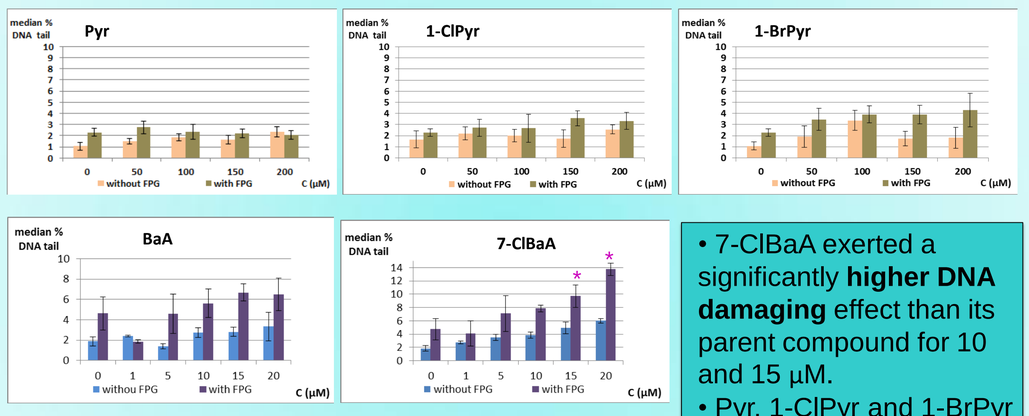

Figure 6 - DNA breakage assessed by the Comet assay following 24h exposure to different concentrations of the studied compounds (***p<0.05** significantly values by Tukey's test).

- 7-ClBaA exerted a significantly **higher DNA damaging** effect than its parent compound for 10 and 15 µM.
- Pyr, 1-ClPyr and 1-BrPyr showed no significant differences for all tested concentrations.

CONCLUSIONS

Evaluation of Citotoxic and Genotoxic effects

- BaA and 7-ClBaA showed citotoxicity and genotoxic effects.
- 7-ClBaA exerted a significantly **higher DNA damaging** effect than its parent compound for 10 and 15 µM.
- Pyr, 1-ClPyr, 1-BrPyr were neither citotoxic nor genotoxic .

Formation of Cl-PAHs under WTPs disinfection conditions

- Reaction of Pyr with chlorine – formation of 1-ClPyr (under the tested conditions).
- Reaction of BaA with chlorine - no chlorine derivatives were identified. Studies to identify reaction products are undergoing.
- New conditions are beeing tested: reaction time, chlorine concentration, etc.

[1]-Richardson, S.D., Plewa, M.J., Wagner, E.D., Schoeny, R., Demarini, D.M. (2007) *Mutat. Res.*, 636, 178-242; **[2]-**Nakamura, H., Tomonaga, Y., Miyata, K., Uchida, M., Terao, Y. (2007) *Environ Sci Technol.*, 41(7), 2190-2195; **[3]-**Rebola, M., Silva, M.J., Louro, H., Antunes, A.M., José, S.S., Silva, Z.I, Cardoso, A.S. (2010) . *European Journal of Public Health*, 40(1), 175-175; **[4]-**Mitchell, R.H., Lai, Yee-Hing, Richard, V.W. (1979) *J. Org. Chem.*, 44 (25), 4733–4735, **[5]**-Repetto, G., del Peso, A., Zurita, J.L. (2008) *Nat. Protoc.*, **3**, 1125-1131, **[6]-**Tice, R.R., Agurell, E., Anderson, D., Burlinson, B., Hartmann, A., Kobayashi, H., Miyamae, Y., Rojas, E., Ryu, J.C., Sasaki, Y.F. (2000) *Environ. Mol. Mutagen*. **35**, 206–221.

The authors wish to thank Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa for financial support by the grant BRJ-DSA/2012- Doenças Oncológicas.