FORMATION OF EMERGING DISINFECTION BYPRODUCTS IN WATER AND EVALUATION OF POTENTIAL GENOTOXIC EFFECTS: THE CASE OF CHLORINATED POLYCYCLIC AROMATIC HYDROCARBONS

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Disinfection byproducts (DBPs) are formed when disinfectants used in water treatment plants (WTPs) react with natural (or anthropogenic) organic matter present in the source water. Many studies have addressed health risks posed by a life-time exposure to DBPs through chlorinated drinking water or through dermal or inhalation exposure routes. Experimental studies have revealed genotoxic and carcinogenic effects of some DBPs and epidemiological studies evidenced potential associations between chlorinated drinking water and bladder or colorectal cancer. In addition, a possible link between chlorinated drinking water and reproductive/developmental effects has been hypothesized.

Many DBPs have been identified in treated water, which justifies the growing concern about the potential health effects of emerging unregulated DBPs, some of which appear to be more genotoxic, in some assays, than the regulated DBPs. Polycyclic aromatic hydrocarbons (PAHs) are among the most persistent contaminants detected in environmental samples such as river sediments and tap water. Water chlorination can lead to the formation of chlorinated derivatives of PAHs (Cl-PAHs) and the few available toxicological studies have shown that Cl-PAHs possess greater mutagenicity than the corresponding parent PAHs.

The present study had two main objectives: 1) identification of the major chlorinated derivatives of benzo[a]pyrene (BaP) and fluoranthene (Fluo) formed as chlorination by-products and 2) evaluation of their potential hazard to humans, through the characterization of their potential genotoxic effects in a human cell line. To synthesize chlorinated standards of PAHs, a newly two phase (water/*n*-hexane) method was developed for BaP and Fluo. 6-Cl-BaP was obtained as the major chlorination

product of BaP, and 3-Cl-Fluo and 1,3-Cl_2-Fluo of Fluo. The formation of these BaP and Fluo chlorinated derivatives was also observed under WTPs chlorination conditions after at 0.5 until 24 h of exposure. The effects of equimolar concentrations of 6-Cl-BaP *vs.* BaP and of 3-Cl-Fluo/1,3-Cl_2-Fluo *vs.* Fluo on cell viability and DNA integrity were assessed by the neutral red uptake (NR) and the comet assay, respectively. Exposure of HepG2 cells to a dose-range of 6-Cl-BaP and BaP showed that both compounds are cytotoxic above 50 µM and that, at the equimolar doses of 100 and 125 µM, 6-Cl-BaP is able to induce a significantly higher level of DNA damage than BaP. On the other hand, no changes of cell viability were observed after exposure to several concentrations of Fluo and its derivatives. Likewise, none of the compounds was able to significantly induce DNA damage.

In conclusion, the present data confirmed that chlorinated derivatives of BaP and Fluo are formed during WTPs chlorination procedures and allowed the identification of their major chlorinated derivatives that should be further analysed in drinking water. On the other hand, the results from the comet assay evidenced a higher DNA damaging effect of Cl-BaP comparatively to its parent compound, suggestive of a more potent genotoxic effect. In spite of the negative results found for Fluo and its chorinated products, further genotoxicity studies are still needed to allow a definite conclusion. Although health risks of DBPs are small compared to health risks of waterborne diseases, the identification of hazardous Cl-PAHs in water emphasizes the need of development of new and safer water disinfection methods.