

## DOCTORAL THESIS

# Hydrophobic organic compounds (HOCs) and algal derived organic materials (AOM) in drinking reservoirs around the Pearl River Delta Region: effects of chlorination and protecting effects of dietary antioxidants against genotoxic disinfection byproducts (DBPs)

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**Hydrophobic Organic Compounds (HOCs) and Algal  
Derived Organic Materials (AOM) in Drinking Reservoirs  
Around the Pearl River Delta Region: Effects of  
Chlorination and Protecting Effects of Dietary Antioxidants  
Against Genotoxic Disinfection Byproducts (DBPs)**

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## **Abstract**

Urinary bladder cancer has been causally linked with chlorination disinfection byproducts (DBPs). Humic substances and algal organic matter are well-known DBP precursors, but organic pollutants such as PAHs can also be oxidized and halogenated into DBPs (e.g. Cl-PAHs), which could be more genotoxic. This research focused on hydrophobic organic pollutants (HOCs), usually deposited and accumulated in particulate matter such as sediments, and their association with genotoxic DBPs following chlorination. Our concern is that with increase in extreme weathers due to global climate change, such as heavy rainfalls and intense typhoons (e.g. in Hong Kong and the Pearl River Delta region), would likely trigger a rise in sediment flux. With the widespread use of nano-sized material/particles (<100 nm) in commercial products, fine particles in the aquatic environment will pose a challenge to the water facilities. Therefore, high levels of HOCs adsorbed/absorbed on particulate matter could get into water treatment plants, penetrate filters and exposed to disinfection, which may be of human health concern.

In this study, sediment samples were collected from six reservoirs along the Dongjiang River in the Pearl River Delta region from upstream to downstream: Xinfengjiang Reservoir (XFJR), Xili Reservoir (XLR), Shenzhen Reservoir (SZR), Shiyan Reservoir (SYR), Plover Cove Reservoir (PCR) and High Island Reservoir (HIR). HOCs were extracted with an organic solvent mixture (Dichloromethane,

Hexane and Acetone in the ratio of 1: 1: 1). Level of human activity increased from upstream to downstream, based on our previous investigation. After characterizing basic sediment contaminants, HOCs were extracted and exposed to chlorination. Genotoxicity of HOCs before/after chlorination was analyzed, while effect of vitamin C (VC) and Epigallocatechin gallate (EGCG) against genotoxicity was examined. Our overall objectives were: 1) to investigate effects of chlorination on genotoxicity of HOCs in sediments of drinking water reservoirs; 2) to examine link between the toxicity and contamination level; and 3) to identify effects of VC and EGCG on protecting cells against genotoxicity upon exposure to chlorinated DBPs.

In the first study, following chlorination of the HOCs, chlorinated whole solutions (Cl-WS) and chlorinated hydrophobic organic compounds (Cl-HOCs) were examined of: 1) genotoxicities by Comet assay (DNA damage in human epithelial colorectal adenocarcinoma cell line Caco-2) and SOS chromo test; and 2) effects upon adding antioxidants. The results showed that chlorination increased genotoxicity of HOCs. Genotoxicity in the chlorinated solutions (i.e. Cl-WS and Cl-HOCs) positively linked with contamination gradient. PAHs served as a precise predictor of genotoxicity in the Cl-HOCs, indicating that other persistent organic contaminants (e.g. dioxins), having similar contamination route and partitions in the aquatic environments as those of PAHs but changed little during chlorination, caused the genotoxicity. This study demonstrated for the first time that dietary antioxidants (i.e. VC and EGCG) protected human cells *in vitro* against DNA damage from exposure to chlorinated solutions or DBPs.

In the second study, I tested the following hypotheses: 1) sediment HOCs or the chlorinated solutions could induce formation of reactive oxygen species (ROS) in Caco-2 cells, which contribute to oxidative damage in the cells; 2) sediment HOCs could induce EROD activity in the cells; 3) VC and EGCG could protect the cells from oxidative damage; and 4) VC could reduce dioxin-like toxicity induced damage in the cells. We measured ROS generation using fluorescence probes, including dihydroethidine (DHE) and dichlorofluorescein diacetate (H<sub>2</sub>DCF-DA), to respectively detect O<sub>2</sub><sup>-</sup> and H<sub>2</sub>O<sub>2</sub>. Oxidative damage in the cells, including DNA oxidative damage (8-hydroxydeoxyguanosine, 8-OHdG) and lipid peroxidation (malondialdehyde, MDA), were also analyzed. Chlorination induced 2.8 - 5.3 folds of increase in H<sub>2</sub>O<sub>2</sub> formation and significant DNA peroxidation in the cells, while adding antioxidants significantly reduced both ROS induction and DNA peroxidation. However, both chlorination and antioxidants' addition remarkably reduced levels of bioassay-derived 2,3,7,8- tetrachlordibenzo-p-dioxin (TCDD) equivalents (TEQ<sub>bio</sub>) in HOCs. Thus, dioxin-like toxicity and DNA damage in Cl-WS were not positively closely correlated, but oxidative stress involved in the Cl-WS exposed cells was responsible for the DNA damage.

In the third study, I investigated effects of chlorination and VC on mutagenicity of HOCs, as well as that of VC in protecting cells from mutagenicity of Cl-WS. Algal cellular materials from *Chlorella* and humic acid (HA) were employed as controls of sediment HOCs. The *Salmonella* mutagenicity tests using strains TA98 and TA100 were conducted, with and without presence of metabolic activation by S9 (+S9

and -S9, respectively). Significant positive correlations were observed between *Salmonella* mutagenic potency (MP, TA98, -S9; TA100, +S9) and PAH levels ( $r^2 = 0.89$  and  $0.84$  respectively) in HOCs. MP values of Cl-WS (TA100, +S9) significantly correlated with PAHs content in HOCs and Cl-HOCs respectively. Mutagenic effects upon exposure to chlorinated algal cellular materials and HA solutions were more easily reduced by adding VC, compared with that to Cl-WS.

In the fourth study, chlorinated solutions were separated into two different polar fractions (chlorinated hydrophobic organic compounds, Cl-HOCs) and chlorinated hydrophilic organic compounds (Cl-HICs) by solid phase extraction (SPE) using C18. Both Cl-HOCs and Cl-HICs induced oxidative stress, DNA damage and dioxin-like toxicity in Caco-2 cells. Cl-HICs induced relatively higher ROS production and DNA and lipid peroxidation than Cl-HOCs, but DNA damage induced by the two fractions had no significant difference. Among HOCs from different reservoirs, EROD activity induced by Cl-HICs from XFJR, XLR and SZR were significantly higher than that of Cl-HOCs and Cl-WS from these reservoirs. Adding antioxidants reduced ROS induction and DNA damage, and EGCG was more effective in reducing DNA damage induced in Cl-HICs rather than that in Cl-HOCs. Adding VC reduced both Cl-HOCs and Cl-HICs from SZR and PCR induced dioxin-like toxicity. Toxicities of the two fractions showed antagonistic action.

In the fifth study, 9 local dominant freshwater algal species from three groups, green algae (*Chlorella sp.*, *Chlamydomonas sp.* and *Scenedesmus quadricauda*), diatom (*Naviculapelluculosa*, *Nitzschia palea grum* and *Synedra sp.*), and blue-green

algae (*Microcystis sp.*, *Chroococcus sp.* and *Gloeocapsa sp.*) were isolated. The algal cells were grown to log phase, harvested and employed for chlorination experiment. Yields of chloroform (CHCl<sub>3</sub>), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), dichloroacetonitrile (DCAN) and trichloroacetonitrile (TCAN) were examined and genotoxicity of chlorinated water under 8 chlorination intervals were determined using SOS Chromotest (0.5, 1, 2, 5, 10, 30, 60 and 120 min) and Comet assay (120 min). Yields of DBPs differed among algal species. Green algae and diatom were generally more effective than blue-green algae in DBPs formation within 120 min chlorination. DCAN and TCAN reached peak level before 120 min and had relative higher production in green algae (*Chlorella sp.* and *Scenedesmus quadricauda*). DBPs formation and toxicity differed among groups and also species in same group. Higher genotoxicity might also be related to the formation of intermediate DBPs when chlorination is carried out for a short period of time or with relatively lower chlorine content in water.

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