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# Increase of cytotoxicity during wastewater chlorination: Impact factors and surrogates

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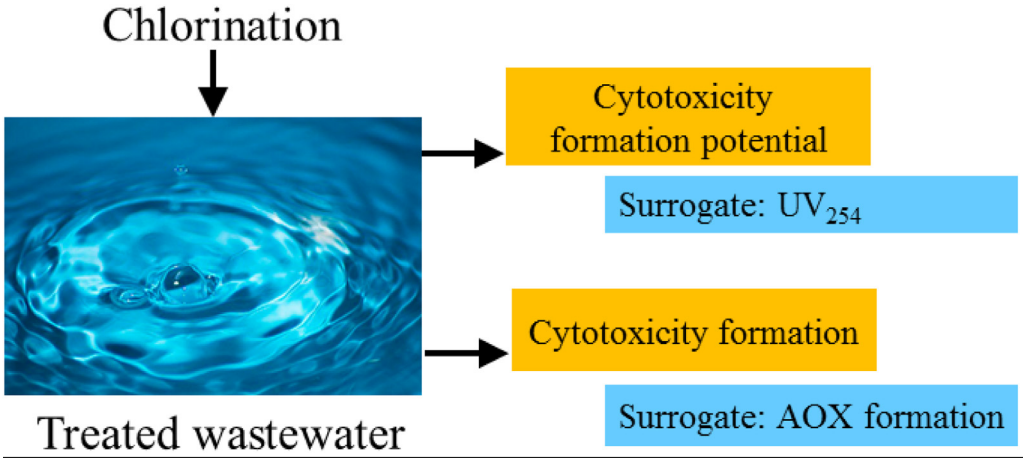
## GRAPHICAL ABSTRACT




## HIGHLIGHTS

- Factors affecting cytotoxicity formation were investigated.
- A method for evaluating cytotoxicity formation potential was developed.
- AOX formation could serve as a surrogate for cytotoxicity formation.
- $UV_{254}$ could serve as a surrogate for cytotoxicity formation potential.

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## ABSTRACT

Toxic and harmful disinfection byproducts (DBPs) were formed during wastewater chlorination. It was recently suggested that cytotoxicity to mammalian cells reflects risks posed by chlorinated wastewater. Here, ATP assays were performed to evaluate the cytotoxicity to mammalian cells. Chlorination significantly increased cytotoxicity of treated wastewater. Factors affecting cytotoxicity formation during

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wastewater chlorination were investigated. Quenching with sodium thiosulfate and ascorbic acid decreased the formed cytotoxicity, while ammonium kept the cytotoxicity stable. The chlorine dose required for the maximum cytotoxicity increase was dramatically affected by DOC and ammonia concentrations. The maximum cytotoxicity increase, defined as the cytotoxicity formation potential (CtFP), occurred when wastewater was treated for 48 h with a chlorine dose of $2{\cdot}DOC + 11{\cdot}NH_3{-}N + 10$ (mg-$Cl_2$/L). During chlorination, the amounts of AOX formation was found to be significantly correlated with cytotoxicity formation when no DBPs were destroyed. AOX formation could be used as a surrogate to estimate cytotoxicity increase during wastewater chlorination. Besides, the CtFP of 14 treated wastewater samples was assessed ranged from 5.4–20.4 mg-phenol/L. The CtFP could be estimated from $UV_{254}$ of treated wastewater because CtFP and $UV_{254}$ were strongly correlated.



## 1. Introduction

Water reclamation from wastewater is important because of the diminishing availability of freshwater resources [1]. In some cases, upstream wastewater after second treatment or tertiary treatment was directly discharged into receiving waterbody, thus leading to the *de facto* potable reuse. Chlorination is the most general process adopted for disinfection. Chlorination inactivates pathogens but produces hazardous disinfection byproducts (DBPs) [2,3]. Many DBPs are genotoxic and carcinogenic [3,4] and may cause adverse effects in humans exposed to chlorinated wastewater. Therefore, in the case of *de facto* potable reuse, it is important to assess the risks posed by chlorinated wastewater to ensure the human health.

Studying the toxicities of individual DBPs does not allow the risks to be assessed satisfactorily, because DBPs may have additive, synergistic, or antagonistic effects [5] and the vast majority of DBPs have not been identified or characterized. Biotoxicity assays are, however, considered to be effective and objective to evaluate the comprehensive toxic effects [6,7] Live animals can be used in biotoxicity assays, but such assays are expensive, time-consuming, and require large water samples [8]. Using microbes, such as luminescent bacteria for acute toxicity tests and algal growth inhibition tests, is inexpensive but less relevant to human risks [9]. Testing cytotoxicity in mammalian cells has therefore become an ideal way of risk determination with convenience [10,11]. Jia et al. [12] observed the partially attenuated genotoxicity based on mammalian cells during wastewater chlorination. Yang et al. [13] found that drinking water disinfected with chlorine was less cytotoxic to mammalian cells compared with water disinfected with chloramine.

Unlike pure compounds, the complexity of treated wastewater brings about the challenge in quantitating cytotoxicity [14]. Although positive results were usually detected for treated wastewater, tests with different endpoints or methods often showed different levels of toxicity. Cytotoxicity characterized by paracellular permeability was higher than cytotoxicity detected from colorimetric assay for the same secondary sewage effluent sample [15]. The staining method 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay has been used widely for cytotoxicity test, but it is not applicable for the mixture because phenolics in secondary effluents react with the MTT and interfere with the results [16]. Therefore, few studies till now about the cytotoxicity of treated wastewater to mammalian cells have been performed, and characterization of cytotoxicity formation during wastewater chlorination has not been well investigated. Luckily, based on our previous study, the chemiluminescent method, adenosine triphosphate (ATP) assay, has been shown suitable for the cytotoxicity testing of treated wastewater [16]. The ATP assay was thusly adopted to quantify the cytotoxicity of treated wastewater and its formation during chlorination in this study.

During disinfection, the DBP formation potential (DBPFP) is used to describe the maximum increase in the amount of DBPs that could occur [17]. A relatively high disinfectant dose and long contact time are usually required during DBPFP tests [18–21]. Nevertheless, just as the DBPs themselves, risks cannot be comprehensively understood from the individual DBPFP. Formation potential of biotoxicity during chlorination is thusly investigated to evaluate the whole risk posed by chlorinated water [22,23]. Here, we proposed a new parameter, cytotoxicity formation potential (CtFP), aiming to determine the maximum risk that can occur during chlorination in a cell-based bioassay and the factors affecting the cytotoxicity formation were discussed.

Although the cytotoxicity is excellent in evaluating the risk, the inherent defect of bioassays is that they are usually costly, time-consuming and complicated. Thus, seeking out the surrogates of cytotoxicity is vital for assessing the risk with convenience. It is believed that toxicity increase during chlorination is resulted from the DBPs formation, indicating the DBPs formation is associated with the cytotoxicity formation. However, it is obviously infeasible to measure all of the individual DBPs. The adsorbable organic halogen (AOX), a collective parameter of halogenated DBPs to estimate the total organic halogen (TOX) [24], was herein studied. AOX has been investigated for decades, and much effort has been put into improving AOX measurements [24–26] and determining the mechanisms through which AOX forms during disinfection [27–29]. However, the association between AOX and cytotoxicity to mammalian cells in chlorinated wastewater, has been studied little. In particular, whether the AOX formation could serve as a surrogate for cytotoxicity formation remains to be explored.

Therefore, this study aims to investigate the formation of mammalian cell cytotoxicity during wastewater chlorination and impact factors including quenching agents, chlorine dose and contact time were discussed. Based on the characterization of cytotoxicity formation, the method for CtFP assessment was established and the CtFP of different treated wastewater was evaluated. Furthermore, the surrogates for cytotoxicity formation and CtFP were put forward, respectively.

## 2. Materials and methods

### 2.1. Water sampling and analysis

Secondary or tertiary effluent water samples were collected from three municipal wastewater treatment plants (WWTPs), labeled as WWTPs A, B, and C, in southern China. The WWTP A samples were collected at the Actiflo® outlet after a biological aerated filter. The WWTP B samples were collected at an oxidation ditch outlet. The WWTP C samples were collected at a fiber filter outlet after an anaerobic/anoxic/oxic process. The samples were kept at 2–6 °C using ice bags while being transferred to the laboratory, and all analyses and chlorination tests were performed within 24 h.