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Combined genotoxicity of chlorinated products from tyrosine and benzophenone-4

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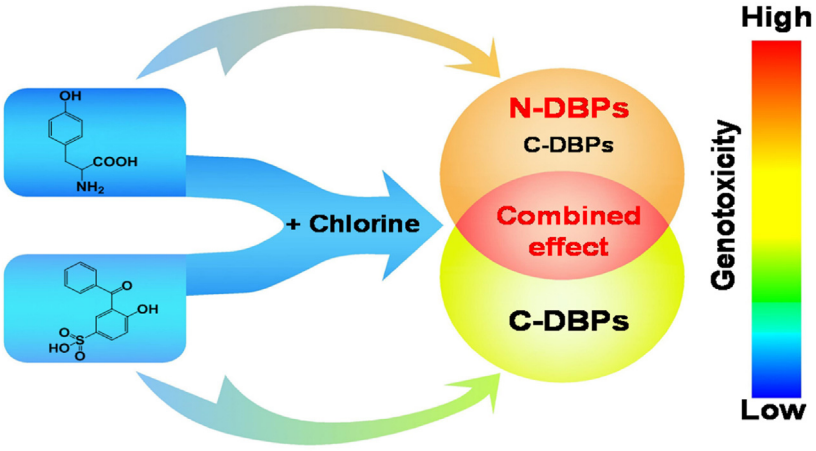
HIGHLIGHTS

- Genotoxicity of chlorinated mixture (GCM) is not predicted by that of single (GCI).
- pH is an important factor affecting difference between GCM and GCI (G_Δ = GCM-GCI).
- $G_\Delta > 0$ occurred at pH 5.0–6.1 and $G_\Delta < 0$ occurred at pH 6.3–8.0.
- G_Δ is determined by N-DBPs decrease in mixture and combined effects between DBPs.
- TON ratio can be used to estimate the G_Δ value.

GRAPHICAL ABSTRACT



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ABSTRACT

The toxicity of disinfection by-products (DBPs) from a single precursor was studied intensively. Here we examined the genotoxicity when two precursors (tyrosine (Tyr) and benzophenone-4 (BP-4)) were chlorinated together and separately. We sought to examine whether the genotoxicity of the mixture (GCM) could be estimated from the sum of the genotoxicities of the individual precursors (GCI), which were chlorinated separately. We determined the genotoxicity using the SOS/umu test. The results revealed that GCM was not identical to GCI. The difference in genotoxicity between GCM and GCI (G_Δ) was observed to decrease with increasing pH. GCM was higher than GCI ($G_\Delta > 0$) at pH 5.0–6.1, and lower than GCI ($G_\Delta < 0$) at pH 6.3–8.0. We found that nitrogen-containing DBPs played a dominant role in determining GCM and GCI. We propose that the total organic nitrogen (TON) ratio, $TON_{(chlorinated\ mixture)}/TON_{(the\ sum\ of\ chlorinated\ individuals)}$, is useful to estimate G_Δ.

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1. Introduction

Chlorine is one of the most commonly used disinfectants in drinking water. However, disinfection by-products (DBPs, Acronyms used in this paper is listed in Box 1) with genotoxic, mutagenic and carcinogenic activities are produced during chlorination. In fact, chlorine reacts with natural organic matter (e.g., humic/fulvic acids, proteins and amino acids) [1–3], anthropogenic chemicals (e.g., pesticides and personal care products) [4], and salts (e.g., bromide and iodide) [5], and produces a wide variety of DBPs [6]. Although most DBPs' concentrations are very low (~1–100 μg/L) [7], they may pose a human health hazard as a result of persistent exposure and/or their synergistic effects [8–10]. Many previous studies have focused on the toxicity of a single DBP [2], a single chlorinated compound [11–13] or chlorinated water samples [14–16]. However, when multiple precursors are chlorinated, little is known about the factors that cause the toxicity of the mixture and whether the mixture's toxicity can be estimated from the sum of the toxicities of the individual chlorinated precursors. Accordingly, we need to determine how and why the toxicity varies during chlorination of multiple precursors.

The composition of source water is complicated. Thus it is difficult to study the underlying mechanism of combined genotoxicity of DBPs from multiple precursors. To simplify the study, we selected two representative precursors, tyrosine (Tyr, a natural organic matter) and benzophenone-4 (BP-4, an anthropogenic chemical). Tyr is a naturally occurring amino acid and is present in many peptides, proteins, and algae [17,18]. The largest influent concentration of the hydrolysable Tyr is 27.4 μg/L [19]. Its DBPs were identified and quantified in previous studies [20–23]. BP-4 is one of the most widely used UV sunscreens and is used in a variety of personal care products (e.g., sunscreens, lipsticks, lotions, shampoos and cosmetics) [24–26]. BP-4 has been detected in wastewater, river water, and sea water, at concentrations ranging from ng/L to high μg/L levels [27,28]. However, traditional wastewater treatment plants did not efficiently eliminate BP-4. Moreover, BP-4 could be present in reclaimed water. Consequently, it is important to determine BP-4 byproducts that are formed during chlorination [13]. Both Tyr and BP-4 are common precursors in reclaimed source waters. Furthermore, Tyr is a precursor of nitrogen disinfection by-products (N-DBPs) [20], and BP-4 reacts with disinfectants only to form carbonaceous by-products (C-DBPs) [13]. In vivo genotoxicity and cytotoxicity assays suggest that the genotoxic and cytotoxic activities of N-DBPs are higher than those of C-DBPs [29–31]. Accordingly, curiosity in the study of N-DBPs has increased rapidly.

Accordingly, we hypothesized that after chlorination, the toxicity of a Tyr and BP-4 mixture could not be equal to the sum of individual toxicities that result from chlorinating them separately. In this study, we used genotoxicity to test this hypothesis and then explore: (i) what is the main experimental condition affecting the difference between the genotoxicity of the chlorinated mixture (GCM) and the sum of genotoxicities of the individual chlorinated compounds (GCI), (ii) what is the underlying mechanism of the genotoxicity difference between GCM and GCI (G_Δ, G_Δ = GCM − GCI), (iii) develop a methodology to estimate G_Δ.

2. Materials and methods

2.1. Chemicals and solution preparation

Standards for the analysis of the following DBPs were purchased from AccuStandard (USA): (1) chloroform (CF) and (2) halogenated volatile organic chemical as a mixture of DBPs including dichloroacetonitrile (DCAN), trichloroacetonitrile (TCAN), chloral hydrate (CH), chloropicrin (CP), 1,1-dichloropropanone (DCP) and 1,1,1-trichloro-2-propanone (TCP). Two species of haloacetic acids (HAAs, including dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA)) were purchased from Bei Na Chuang Lian Ltd. (China). Dichloroacetamide (DcAcAm) and trichloroacetamide (TcAcAm) were purchased from Alfa Aesar (Germany) and J&K (China), respectively. Ethyl acetate and methyl *tert*-butyl ether (MTBE) used to extract DBPs were obtained from Fisher (USA). Tyr was obtained from Sigma-Aldrich (USA). BP-4 and phloretic acid (PA) were obtained from J&K (China). The chemical structures of Tyr, BP-4 and PA were listed in Fig. 1. The sodium hypochlorite aqueous solution was obtained from Sinopharm Chemical Reagent Co., Ltd (China). The concentration of the chlorine stock solution was determined using the *N*,*N*-diethyl-*p*-phenylenediamine ferrous titration method. All chemicals were of analytical grade and used without further purification. All reagent solutions were prepared with ultrapure water (resistivity 18.2 MΩ cm, Millipore, US). Additionally, all bottles were rinsed three times with ultrapure water and then dried in a muffle furnace at 500 °C for 4 h.

2.2. Chlorination experiments

Chlorination experiments were performed under headspace-free conditions in glass screw-cap vials that were capped with Teflon-faced septa and kept in the dark. Hydrochloric acid and sodium hydroxide were used to adjust the pH (pH = 5.0–8.0). 50 mM

Box 1: Acronyms used.

DBPs	Disinfection by-products	TCP	1,1,1-trichloro-2-propanone
Tyr	tyrosine	HAAs	haloacetic acids
BP-4	Benzophenone-4	DCAA	dichloroacetic acid
GCM	genotoxicity of chlorinated mixture	TCAA	trichloroacetic acid
GCI	the sum of genotoxicity of chlorinated individual precursor	DcAcAm	Dichloroacetamide
G_Δ	the difference between genotoxicity of chlorinated mixture and the sum of genotoxicity from chlorinated individual precursor	TcAcAm	trichloroacetamide
TON	total organic nitrogen	MTBE	methyl *tert*-butyl ether
N-DBPs	nitrogen-containing DBPs	Cl_2	chlorine
C-DBPs	carbonaceous by-products	PA	phloretic acid
CF	chloroform	GC/MS	gas chromatography/mass spectrometry
DCAN	dichloroacetonitrile	UPLC/DAD	ultra-performance liquid chromatography/diode array detector
TCAN	dichloroacetonitrile	Q-TOF-MS	Quadrupole-Time of Flight Mass Spectrometer
CH	chloral hydrate	TKN	Total Kjeldahl nitrogen
CP	chloropicrin	$TEQ_{4\text{-}NQO}$	Toxicity Equivalent Quotient
DCP	1,1-dichloropropanone	HOCl	hypochloric acid
SD	Supplementary data		