Occurrence of Synthetic Phenolic Antioxidants and Major Metabolites in Municipal Sewage Sludge in China

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S Supporting Information

ABSTRACT: Synthetic phenolic antioxidants (SPAs) are one group of widely used additive chemicals, which have not yet had focused attention except for a few compounds such as 2,6-di-*tert*-butyl-4-methylphenol (BHT). In this study, the occurrence and composition profiles of 12 frequently used SPAs and three BHT metabolites were investigated in fifty-six sludge samples collected from individual wastewater treatment plants in China. Eleven SPAs were positively found in the sludge samples, in which, to our knowledge, eight SPA compounds were identified for the first time in the environment. BHT, 4-*tert*-octylphenol (4-*t*OP), and 2,4,6-tri-*tert*-butylphenol (AO 246) were the most dominant SPAs in the sludge at mean concentrations of 4.14 μg/g, 374 ng/g, and 98.1 ng/g d.w. (dry weight). Meanwhile, three BHT metabolites, including 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (BHT-CHO), 2,6-di-*tert*-butyl-1,4-benzoquinone (BHT-Q), and 2,6-di-*tert*-butyl-4-hydroxy-4-methyl-2,5-cyclohexadienone (BHT-quinol), were also found in most of the samples (>98.2%) with mean concentrations of 141, 562, and 225 ng/g d.w., respectively. The activated sludge system (anaerobic, anoxic, and oxic tanks) of a wastewater treatment plant was further investigated for the removal efficiencies of the SPAs. High removal efficiencies (80.1–89.2%) were found for the six detected SPAs in the aqueous phase, while generation of large proportions of the three BHT metabolites was also observed.

INTRODUCTION

Synthetic phenolic antioxidants (SPAs) are a group of chemicals which present a basic common structure characterized by phenolic rings typically substituted with hindered alkyl groups in ortho-positions.¹ These chemicals are largely used in rubber, elastomers, plastic, cosmetic formulations, pharmaceuticals, and food applications to retard the oxidation process.^{2,3} It was reported that 2,6-di-*tert*-butyl-4-methylphenol (BHT), which is the most frequently used SPA, was added in various kinds of finished food and more than 1700 cosmetic formulations with contents up to 0.5%.³ The wide use of SPA compounds triggered an increasing concern on their potential toxicity effects. 4-*tert*-Octylphenol (4-*t*OP) was reported to have an estrogenic effect in *in vivo* and *in vitro* studies.^{4–6} Strong endocrine activity of 4,4'-butylidenebis(3-methyl-6-*tert*-butylphenol) (AO 44B25) was also found in the *in vivo* test.⁷ The International Agency for Research on Cancer (IARC) has evaluated 3-*tert*-butyl-4-methoxyphenol (BHA) and found sufficient evidence for its carcinogenicity in experimental

animals.^{8,9} Though the effect of BHT consumption on human health still remained controversial,^{10,11} some BHT metabolites may pose a health risk as they were reported to generate peroxides in mice and rats and may induce cellular DNA damage or have the capacity to be cancer initiators.¹²

Few studies have investigated the occurrence of SPA analogues in the environment, among which the pollution of BHT received the most attention. BHT was found in indoor dust, natural waters, and sewage influent and effluent with concentrations ranging from part-per-trillion (ppt) to part-per-million (ppm).^{13–16} Using time-of-flight mass spectrometry for nontarget screening of organic pollutants, BHT was positively identified in surface water and soil samples collected from a rice production area in Columbia.¹⁷ BHA and 4-*t*OP were also

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Figure 1. Chemical name, CAS registry number, and structure of SPA analogues and relevant metabolites.

Figure 2. Sampling locations and spatial distributions of the concentrations of SPA analogues and metabolites in municipal sewage sludge samples in China (mean concentration values of different SPA analogues and metabolites were present for sludge samples in the same provinces).

positively identified in house dust and various water matrices including sewage influent, surface water, and groundwater.^{18–22} Besides, migrations of BHT, BHA, and 2,2'-methylenebis(4-methyl-6-*tert*-butylphenol) (AO 2246) were found from plastic packaging materials to food simulates (distilled water, 3% acetic acid, 10% ethanol and oil), which were incubated at 40 °C for 10 days.²⁰ Nevertheless, as for other SPAs as well as relevant metabolites, little information was available on their occurrence and residue levels in environment compartments.

Based on the hydrophobic property of the SPA compounds ($\log K_{ow}$ in the range of 3.50 (BHA) to 17.17 (1,3,5-trimethyl-

2,4,6-tris(3,5-di-*tert*-butyl-4-hydroxybenzyl)benzene, AO 330)),²³ sewage sludge in wastewater treatment plants (WWTPs) could be sufficient to track the occurrence of emerging SPAs at a large geographical scale. In the present work, municipal sewage sludge samples were collected from different Chinese cities to provide valuable information on the presence, contamination levels, and distribution patterns of SPAs in the ambient environment in China. Influent, effluent, and sludge samples of the activated sludge system in one WWTP were further collected and analyzed to give a first

impression on its biotransformation and removal behaviors of target SPA analytes.

MATERIALS AND METHODS

Materials. Analyte name, structure and other relevant data are shown in Figure 1. BHT-quinol was supplied by Frontier Scientific Inc. (Logan, USA). AO 22E46 was supplied by AccuStandard (New Haven, USA). Certified standards of other analytes were obtained from TCI (Tokyo, Japan). The purities for all the target analytes were >95%. Isotope-labeled 2,6-di(*tert*-butyl-d₉)-4-methyl(phenol-3,5,0-d₃) (BHT-d₂₁) and ¹³C-tetrabromobisphenol A (¹³C₁₂-TBBPA) were obtained from Cambridge Isotope Laboratories (Andover, USA). Stock standard solutions (1000 µg/mL) were individually prepared in methanol, and intermediate solutions were weekly prepared from the stock standard solutions by appropriate methanol dilution. Silica gel (100–200 mesh size, Merck, Darmstadt, Germany) was activated at 550 °C for 12 h and 5% water-deactivated prior to use. Anhydrous sodium sulfate (Na₂SO₄) was baked at 600 °C for 6 h and stored in a sealed desiccator. Pesticide residue grade dichloromethane (DCM), hexane (Hex), and methanol (MeOH) were purchased from J.T. Baker (Phillipsburg, USA). Ultrapure water (18.3 MΩ × cm) was generated by a Milli-Q purification system (Millipore, Billerica, USA).

Sample Collection. Fifty-six WWTP sludge samples were collected from 33 cities in 20 provinces in China during October 2010 to May 2011 (Figure 2). Approximately 500 g of freshly digested sludge was collected at the dewatering process, packed in aluminum foil, sealed in a polypropylene bag, and express-delivered to our lab immediately. All sludge samples were freeze-dried, homogenized, sieved through a stainless steel 100-mesh sieve, and then stored in −20 °C until analysis. Details are given in the Supporting Information (SI, Table S1). Influent and effluent samples of the secondary treatment step were taken from a WWTP everyday during March 24–26, 2014. The WWTP investigated in this work is located in Beijing, China and has a conventional activated sludge system (anaerobic, anoxic, and oxic tanks) with an average domestic wastewater flow of 200 000 m³/d. The 24 h composite sewage samples were collected using flow proportional samplers (Global Water Inc., TX). At each site, 500 mL of water was collected and stored in a brown glass bottle. All vessels were precleaned with MeOH and ultrapure water to avoid contamination. All water samples were extracted as soon as possible after centrifugation at 5000 rpm for 10 min to separate liquid/solid phases, and the whole remaining suspended solid matters were also gathered for further instrumental analysis. Sludge samples of secondary clarifier were collected from the WWTP in the three successive days and prepared as above (Table S7).

Sample Preparation and Instrumental Analysis. For sludge samples, approximately 0.5 g of the sample (spiked with 200 ng of BHT-d₂₁ and 10 ng of ¹³C-TBBPA) was mixed with 15 g of Na₂SO₄ and extracted with DCM/Hex (3:1, v/v) at 90 °C and 1500 psi in three static extraction cycles of 10 min using an accelerated solvent extractor (ASE 350, Dionex Inc., Sunnyvale, CA). The extracts were concentrated by rotary evaporation to ~2 mL and fractionated on an 8 g silica column. The silica column was preconditioned by 30 mL of Hex before loading, and all analytes were eluted with 120 mL of DCM/Hex (1:1, v/v) mixture. The eluates were then concentrated, solvent exchanged into 5 mL of MeOH, and transferred to LC vials.

Finally, 20 µL of the sample was injected into the instrument for quantification analysis. The water samples were extracted using a liquid–liquid extraction method. Briefly, a 400 mL sample (spiked with 200 ng of BHT-d₂₁ and 10 ng of ¹³C-TBBPA) was mixed with 100 mL of DCM and shaken for 5 min. Then the DCM was transferred to another glass bottle, and the extraction was repeated three times. After extraction, the DCM phases were combined, concentrated by rotary evaporation to ~2 mL, fractionated on the silica gel packed column, and further quantitatively analyzed as described for sludge samples.

An Alliance 2695 high performance liquid chromatograph interfaced with a Quattro Premier XE triple-quadrupole mass spectrometer (HPLC-MS/MS, Waters, Milford, MA) was used for the determination of the SPAs. A 150 × 2.1 mm SymmetryShield 5 µm C18 analytical column (Waters, Milford, USA) was chosen for chromatographic separation. Column temperature was set at 40 °C. MeOH (A) and ultrapure water (B) were chosen as mobile phases. The gradient was initiated at a composition of 25:75 (A:B, v/v) and was first held for 5 min. Then the gradient elution program was optimized with composition A linearly increased to 100% in 10 min and then held for 10 min. After immediately returning to the initial composition of 25:75, the column was allowed to re-equilibrate for 3 min, which gave a total analysis time of 23 min. Flow rate for the separation procedure was set at 0.3 mL/min. The mass spectrometer was operated in negative electrospray ionization (ESI) mode. The source and desolvation temperature were set at 120 and 450 °C, respectively. Desolvation gas flow was 800 L/h, cone gas flow was 50 L/h, while argon pressure in the collision cell was kept at 3.8×10^{-3} mbar for MS/MS measurement. Detailed information on the optimized parameters and monitored ion transitions for each analyte is given in Table S3.

Quality Assurance/Quality Control. For the evaluation of extraction efficiency, 10 sludge samples and 2 water samples were randomly selected for a fourth extraction after the first three extraction cycles. No quantifiable amounts of target compounds were detected in the fourth extractions, which suggested high efficiencies of the first three extractions for both sludge and water samples. Relative recoveries of the target analytes in matrix-spiked sewage water (100 ng/L) and sludge samples (200 ng/g) were 61.0–105% and 65.0–103%, respectively (Table S4). Matrix effects for the target analytes were calculated by the percentage of signal response in sample matrix versus the signal of the same concentration in MeOH. No severe ionization suppression/enhancement was found for all the analytes in sewage water and sludge matrices, with matrix effects within the range of 85.3 (4-tOP)–110% (AO 2246). The relative standard deviations (RSD, *n* = 5) for the water and sludge matrices were all less than 17.0%. One procedural blank of 15 g of Na₂SO₄ (for solid samples) or 400 mL of ultrapure water (for water samples) was included in every batch of samples (*n* = 8) to monitor for background contamination levels. All analytes in the blanks were under the quantification limits. Quantification of the analytes was based on an external calibration curve, and two internal standards were used to correct the possible target losses during the sample preparation. Instrumental calibration was verified by injection of 7 calibration standards (ranging from 20 to 2000 ng/mL for BHT, 0.5 to 100 ng/mL for others), and the linearity of the calibration curve (*r*²) was >0.99. For samples with analyte concentrations beyond the linear dynamic range, MeOH

Table 1. Descriptive Statistics of the Measured Concentrations (ng/g, d.w.) of SPAs and Relevant Metabolites in Sludge Samples

compounds	GM ^a	median	range	DR ^b (%)	averaged proportion (%)
BHT	4.14e3	2.35e3	51.7–30.3e3	100	83.4
BHA	3.58	2.44	<MQL–17.4	66.1	0.07
4-tOP	317	85.4	<MQL–8.57e3	92.8	6.39
AO 246	374	244	9.28–1.70e3	100	7.54
DTBSBP	98.1	77.8	2.32–461	100	1.98
AO 2246	3.53	0.75	<MQL–91.3	53.5	0.07
AO 22E46	0.43	0.14	<MQL–5.50	21.4	0.01
AO 330	11.4	4.33	<MQL–262	89.3	0.23
AO 425	0.11	0.07	<MQL–2.04	8.93	0.002
AO 4426	2.86	1.13	<MQL–97.9	1.78	0.06
AO 44B25	11.0	3.92	<MQL–174	69.6	0.22
BHT-CHO	141	112	<MQL–660	98.2	c
BHT-Q	562	345	15.5–5.13e3	100	c
BHT-quinol	225	221	28.9–848	100	c

^aGM: geometric mean. ^bDR = detection frequency. ^cBHT metabolites were not included in the calculation of composition proportions.

Figure 3. Percentage profiles of individual SPA in each sludge samples.

dilution was carried out. The method quantification limits (MQLs), which were calculated as a signal-to-noise ratio of 10, were from 0.1 to 25 ng/L and 0.1 to 15 ng/g for sewage water and sludge matrices, respectively (Table S4).

Statistical Analysis. Geometric mean, median, and concentration ranges were used to describe the results. All data were reported on a dry weight (d.w.) basis. Analyte concentrations under MQLs were set as MQL divided by the square root of 2. Statistical analyses were conducted using SPSS V17.0 for Windows Release (SPSS Inc., 2009). The correlations among the log-transformed concentrations of different SPA compounds were assessed by the Pearson's correlation analysis. AO 22E46, AO 425, and AO 4426 were excluded in the statistical analysis due to their low detection frequencies. All the statistical significant levels were set at $p < 0.05$, unless otherwise mentioned. The total concentration of SPAs was the sum concentrations of all the target antioxidants analyzed in this study, whereas the BHT metabolites were not included. The composition profiles of the target SPAs were calculated as

geometric mean concentration of individual SPA divided by the $\sum$ SPAs concentration.

RESULTS AND DISCUSSION

Concentrations and Composition Profiles of the Target SPAs in Sludge. The concentrations of the target SPAs in the sludge samples are presented in Table 1 and the SI (Table S1), while the percent stacked columns of the detected SPA congeners are shown in Figure 3. Except for AO ZKF, the 11 other SPAs could be positively identified in the sludge samples with concentrations ranging from <MQLs to 30.3 μ g/g d.w. As shown in Figure 2, no obvious geographic trends were found for the target analytes. Total concentrations of SPAs (sum of the 11 SPAs, $\sum$ SPAs) in sludge samples ranged from 183 ng/g to 41.0 μ g/g, with an average value of 4.96 μ g/g. BHT was the most dominant SPA analogue, which constituted 28.7–97.7% (average 83.4%) of $\sum$ SPAs. It was followed by AO 246 (average 7.54%), 4-tOP (average 6.39%), DTBSBP (average 1.98%), AO 330 (average 0.23%), AO 44B25 (average

0.22%), and BHA (average 0.07%). BHT, AO 246, and DTBSBP were detected in all the investigated samples, while the detection frequencies for 4-*t*OP, AO 44B25, AO 330, BHA, and AO 2246 were 92.8%, 69.6%, 89.3%, 66.1%, and 53.5%, respectively. The high detection frequencies of these SPAs demonstrated their wide usage in China. Low detection frequency (12/56) was found for AO 22E46. AO 425 was only detectable in five samples, while AO 4426 was only found in one sample, which indicated that both of the compounds might only have limited production and usage in China.

As shown in Table 1, the concentrations of BHT ranged from 51.7 ng/g to 30.3 μ g/g (GM: 4.13 μ g/g, median: 2.35 μ g/g). BHT has been used as an antioxidant since the 1950s, and approximately 40 countries permit the use of BHT as a food additive.³ A few studies have already proved the presence of BHT in an aquatic environment in Germany with concentrations of 7–791 ng/L.¹⁶ A study on indoor dust in Sweden showed that BHT was among the highest concentration contaminants with the mean concentration of 70 μ g/g.¹³ The consumption of BHT by food intake and other ways led to its occurrence in the body fat at the mean concentration of 0.23 μ g/g in 11 residents in UK and at 1.30 μ g/g in 12 residents in the USA.²⁴ The concentrations of AO 246 varied from 9.28 ng/g to 1.70 μ g/g (GM: 374 ng/g, median: 244 ng/g) in the investigated sludge samples. AO 246 can be used as a primary antioxidant or as an intermediate in the synthesis of primary antioxidants.²⁵ It was reported with high bioconcentration factors (4320–23200 in fish, exposed to 1 μ g/L for 8 week)²⁶ and was found in vegetables, animal meat, liver, and fish at nanogram per gram level in Japan.²⁵ DTBSBP, which is an isomer of AO 246, was also detected with concentrations from 2.32 to 461 ng/g (GM: 98.1 ng/g, median: 77.8 ng/g). DTBSBP was identified as a high priority for assessment of ecological risk in Canada because it meets the ecological categorization criteria for persistence, bioaccumulation potential, and inherent toxicity to aquatic organisms.²⁷ Also, both AO 246 and DTBSBP are in the list of Work Plan Chemicals for Assessment During 2013 and 2014 together with bis(2-ethylhexyl)-3,4,5,6-tetrabromophthalate, tri(2-chloroethyl)-phosphate, and 2-ethylhexyl-2,3,4,5-tetrabromobenzoate by U.S. EPA for ecological risk evaluation.²⁸ To our knowledge, there was no empirical data on measured concentrations of AO 246 and DTBSBP in environmental media previously. 4-*t*OP, though largely used to produce octylphenol polyethoxylate surfactant, can also be used as primary antioxidant. 4-*t*OP has been detected in WWTP effluent and sludge samples with concentrations up to μ g/L and μ g/g levels, respectively.^{21,29} Comparable concentrations of 4-*t*OP (GM: 317 ng/g, median: 85.4 ng/g) were also found in this study. BHA was previously reported in surface water, groundwater, and reclaimed water in southern California with concentrations at ng/L levels.²² In the present study, BHA was detected in the samples with concentrations ranging from <MQL to 17.4 ng/g (GM: 3.58 ng/g, median: 2.44 ng/g). A weak estrogenic effect of BHA was reported *in vitro* study.³⁰ Besides, BHA is included in the Contaminant Candidate List 3 (CCL 3) by the U.S. EPA.³¹

Other SPAs with more than one phenolic ring (AO 44B25, AO 330, AO 2246, AO 22E46, AO 425, and AO 4426) were also detected in the collected samples with varying concentrations (<MQLs–262 ng/g). AO 44B25, which was reported to have androgenic effect and genetic toxicity,⁷ was detected with concentrations from <MQL to 174 ng/g (GM: 11.0 ng/g, median: 3.92 ng/g). The concentrations of AO 330 ranged

from <MQL to 262 ng/g (GM: 11.4 ng/g, median: 4.33 ng/g). AO 2246 was detected with GM and median concentration of 3.53 and 0.75 ng/g, respectively. AO 22E46, AO 425, and AO 4426 were detected with concentrations varying from <MQLs to dozens of ng/g level. To our knowledge, no previous data in literature was available on their concentrations in environmental matrices for AO 44B25, AO 330, AO 2246, AO 22E46, AO 425, and AO 4426.

The six sludge samples collected from different WWTPs in Beijing showed different contamination levels with $\sum$ SPAs varying from 3.61 to 9.76 μ g/g, indicating that many factors including daily wastewater processing volume and biological treatment techniques might impact the concentrations of SPAs. Besides, the six WWTPs in Beijing serve different districts, which might have different SPA emissions. The observed composition pattern in the sludge samples was well corresponded to the SPA production volumes in China. BHT dominated the SPA concentration profile (83.4% of $\sum$ SPAs) in the investigated sewage sludge samples. This is quite consistent with the BHT usage, which was reported to account for 80% of the total SPAs with a production volume of 23.3 kt/a in 1998 in China.³² Pearson correlation analysis was performed on the log-transformed concentrations of SPA compounds. Table S5 shows that BHT was significantly correlated with AO 2246, 4-*t*OP, and AO 44B25 ($r = 0.357$ – 0.784 , $p < 0.01$). 4-*t*OP was significantly correlated with AO 2246 ($r = 0.497$, $p < 0.01$) and AO 44B25 ($r = 0.399$, $p < 0.01$). AO 246 showed a similar relationship with DTBSBP ($r = 0.741$, $p < 0.01$) and AO 44B25 ($r = 0.435$, $p < 0.01$). AO 2246 was significantly correlated with AO 44B25 ($r = 0.350$, $p < 0.01$) and AO 330 ($r = 0.394$, $p < 0.01$), and a similar relationship was found between DTBSBP and AO 44B25 ($r = 0.399$, $p < 0.01$). Strong correlation between concentrations of different environmental contaminants might suggest common sources and/or similar environmental fates.

More Toxic BHT Degradation Metabolites in Sludge.

In vivo and *in vitro* metabolism studies of BHT revealed two main metabolic processes which include oxidation of both the alkyl substituent and aromatic ring system.³³ Oxidation of the alkyl substituent will lead to BHT-CHO, while oxidation of the aromatic ring of BHT leads to BHT-Q and BHT-quinol. BHT-CHO has also been identified as BHT photoinduced transformation product.

Though the toxicities of BHT metabolites have been largely investigated, a few studies have reported their occurrence in the environment.^{12,14} In the present study, BHT-CHO was detected in 98.2% of the samples with concentrations from 0.67 to 660 ng/g (GM: 141 ng/g, median: 112 ng/g), which was 3.4% of BHT in mean concentration. BHT-CHO was detected along with BHT in river, rain, and groundwater samples from Germany with concentrations from several tens to hundreds ng/L.¹⁶ Another BHT metabolite, BHT-Q, was identified in all the investigated samples with concentrations varying from 15.5 ng/g to 5.13 μ g/g (GM: 562 ng/g, median: 345 ng/g), which was 13.6% of BHT in mean concentration. The mean concentration of BHT-Q in the investigated sludge was higher than all the detected SPAs except for BHT, which ranked the second order in concentration. The mean concentration of BHT-Q is 4 times as high as that of BHT-CHO. BHT-Q was once detected in WWTP influents in Spain with the mean concentration of 771 ng/L, which was also higher than BHT-CHO in the same samples.¹⁴ The highest concentrations of BHT-CHO and BHT-Q were both found in

Table 2. Concentrations (Mean $\pm$ SD) of SPAs and Relevant Metabolites in Sewage and Sludge Samples Collected in the Investigated WWTP

compounds	concns (ng/L) in aqueous phase		concns (ng/g, d.w.) in solid phase		removal efficiency (%)
	influent	effluent	TSS in influent	excess sludge	
BHT	2.42e3 $\pm$ 419	262 $\pm$ 24.7	2.46e3 $\pm$ 171	4.43e3 $\pm$ 464	89.2
BHA	14.5 $\pm$ 0.76	2.3 $\pm$ 0.38	8.37 $\pm$ 1.10	16.5 $\pm$ 0.75	83.9
4-tOP	155 $\pm$ 27.0	30.9 $\pm$ 7.30	78.7 $\pm$ 13.4	196 $\pm$ 21.3	80.1
AO 246	26.0 $\pm$ 4.50	3.2 $\pm$ 0.40	90.7 $\pm$ 2.89	160 $\pm$ 7.21	87.8
DTBSBP	12.3 $\pm$ 0.29	1.63 ^a	21.7 $\pm$ 3.79	57.7 $\pm$ 5.68	86.8
AO 2246	0.78 $\pm$ 0.03	0.14 ^a	5.43 $\pm$ 0.42	6.20 $\pm$ 0.61	82.1
BHT-Q	23.2 $\pm$ 8.21	108 $\pm$ 17.7	47.3 $\pm$ 8.97	137 $\pm$ 20.7	−365
BHT-quinol	200 $\pm$ 49.1	556 $\pm$ 191	37.7 $\pm$ 3.81	160 $\pm$ 26.6	−178
BHT-CHO	156 $\pm$ 16.6	534 $\pm$ 171	37.3 $\pm$ 6.33	173 $\pm$ 10.5	−242

^aConcentration <MQL.

the sample of NB-2, which also contained maximum BHT. BHT-Q can cause DNA damage through H₂O₂ and oxygen radical generation, which leads to internucleosomal DNA fragmentation. The lowest concentration of BHT-Q causing DNA cleavage *in vitro* was quite low (10^{−6} M).³⁴ Besides, BHT-Q and BHT-CHO were demonstrated to be stable during the WWTP process even when high concentration of chlorine was used.³⁵ BHT-quinol, the environmental occurrence of which was not reported previously, was positively identified in all the sludge samples. The concentrations of BHT-quinol varied from 28.9 to 848 ng/g (GM: 225 ng/g, median: 221 ng/g), which accounted for 5.4% of BHT in concentration. Significant correlation ($r = 0.459$, $p < 0.01$) between concentrations of BHT and sum of the three metabolites was also observed in the sludge samples. As shown in Table S6, the predicted overall persistence of the target SPAs by OECD overall persistence and long-range transport potential fugacity screening tool (Pov-LRTP tool)³⁶ ranged from 106 to 519 d, which implied their environmental persistency. The calculated overall persistence of BHT-Q (172 d), BHT-quinol (170 d), and BHT-CHO (108 d) was longer than or equal to their parent compound BHT (108 d). The high environmental persistency and universal occurrence of these BHT metabolites might imply their nonignorable environmental or human health risk. To our knowledge, this is the first study to report the occurrence of BHT metabolites in sludge in China.

Removal of the Detected SPAs by Activated Sludge System. WWTPs in China usually comprise only primary and secondary treatments, with the latter mainly based on conventional activated sludge.³⁷ Therefore, investigation on the removal of pollutants through the activated sludge system is important. As shown in Table 2 and Table S7, nine target analytes (six SPAs: BHT, BHA, 4-tOP, AO 246, DTBSBP, and AO 2246; three BHT metabolites: BHT-CHO, BHT-Q, BHT-quinol) were detected in total suspended solids (TSS) of the influent with mean concentrations from 5.43 ng/g to 2.46 μ g/g. The concentrations of these compounds in excess sludge (6.20 ng/g–4.43 μ g/g) were higher than that in TSS. The nine analytes, which were detected in solid samples, were also found in the collected water samples with concentrations ranging from <MQLs to 2.42 μ g/L.

Removal efficiencies (R) were calculated on the base of target SPA concentrations in the aqueous phase of influent and effluent samples using the following equation: $R = (C_{\text{influent}} - C_{\text{effluent}}) / C_{\text{influent}} \times 100\%$, where C_{influent} and C_{effluent} are the target concentrations measured in influent and effluent samples of the activated sludge system, respectively.³⁸ As shown in

Table 2, the removal efficiencies of the six detected SPAs in the aqueous phase were 80.1–89.2%, which indicated that the SPA contaminants released into the recipient river from secondary effluent might be minor. Similar phenomenon was found for the hydrophobic benzotriazole ultraviolet stabilizers through the WWTP activated sludge system with removal efficiencies larger than 72%.³⁹ The removal efficiencies of BHT metabolites in the aqueous phase were opposite to those of other SPA analytes (Table 2), which indicated the generation of large proportions of these BHT metabolites (178% for BHT-quinol to 365% for BHT-Q) in the activated sludge system of the WWTP.

Environmental Implications. In this work, 11 SPAs and three BHT metabolites were positively identified in 56 sewage sludge samples collected from different WWTPs in China, which suggested the prevalent usage of SPA-containing products and widespread occurrence of emerging SPA anthropogenic chemicals in China. This is the first time to report the occurrence of BHT-quinol, AO 246, DTBSBP, AO 2246, AO 22E46, AO 425, AO 44B25, AO 4426, and AO 330 in environmental matrices. Other targets including BHT, BHT-CHO, BHT-Q, and BHA were reported for the first time in sludge samples in China. Attention should be paid to simultaneous exposure to several SPAs as a synergistic effect was found in the induction of apoptosis between BHT and BHA.⁴⁰ Moreover, the conventional secondary treatment (anaerobic, anoxic, and oxic tanks) of a WWTP was demonstrated to be efficient in removal of SPAs in aqueous phase. However, the concentrations of BHT metabolites in the secondary effluent were much higher than that of in influent, which indicated the generation of BHT metabolites through the activated sludge system. Further studies are needed to discern the release pathways and spatial and temporal distributions and to investigate if additional SPA analogues could also be present in the environment. As the prevalence of more toxic BHT metabolites was reported in the present study, more detailed research is warranted regarding the fate of other SPAs during wastewater treatment procedures as well as their impact on environmental compartments.

■ ASSOCIATED CONTENT

● Supporting Information

Additional tables containing information on the sampling sites and data analysis not presented in the main text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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