Fate of bisphenol S (BPS) and characterization of non-extractable residues in soil: Insights into persistence of BPS

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A B S T R A C T

The environmental fate and persistence of bisphenol S (BPS), a substitute for bisphenol A (BPA), are unclear. This study used \textsuperscript{14}C-labeled BPS to examine the fate, biodegradation, and residue properties of BPS incubated in an oxic soil for 28 days. BPS dissipated quickly, with a half-life of 2.8 days. Most of the BPS was mineralized (53.6 ± 0.2% of initial amount by day 28) or transformed into non-extractable residues (NERs) (45.1 ± 0.3%), with generation of minor extractable residues (3.7 ± 0.2%) containing two metabolites. NERs were formed mainly via physico-chemical entrapment (51.1 ± 2.4% of the total NERs, consisting almost exclusively of BPS) and ester-linkages (31.5 ± 3.0% of the total NERs, consisting of both BPS and polar metabolites). When mixed with fresh soil, BPS-derived NERs became unstable and bioavailable. Subsequent mineralization was determined for 19.5 ± 1.1% of the total NERs and 35.5 ± 2.6% of the physico-chemically entrapped BPS. A fate model for 19.5 ± 1.1% of the total NERs and 35.5 ± 2.6% of the physico-chemically entrapped BPS. A fate model was used to describe the kinetics of NER formation, which indicated that microbial activity in soil could have strongly reduced the kinetic rate of the release of physico-chemically entrapped NERs into free form and therefore increased the stability of this type of NERs in soil. Our results provide unique insights into the fate of BPS in soil and suggest that while BPS is biodegradable, it includes the formation of large amounts of reversibly physico-chemically entrapped and covalently bound ester-linked NERs. The instability of these NERs should be considered in assessments on environmental persistence and risks of BPS. Our study also points out the environmental importance of NERs of agrochemicals.

1. Introduction

Bisphenol S (BPS; 4,4′-sulfonyldiphenol) is widely used in the production of polycarbonate plastics and resins. Regulations and restrictions on the use of BPA, a compound with estrogenic and androgenic activity at environmental concentrations (Goodman et al., 2009; Huang et al., 2012; Vom Saal and Hughes, 2005) have increased the demand for BPS as an alternative for BPA (Kinchen et al., 2015), even though BPS is also hormonally active and endocrine-disruptive (Kitamura et al., 2005; Rochester and Bolden, 2015). The growing use of BPS in “BPA-free” products (Simoneau et al., 2011) implies a greater potential for its release into the environment.

BPS has been frequently detected in various environmental matrixes and biological samples. In waters of the Meuse River, Netherlands, BPS concentration amounted to 3 μg L⁻¹ (Kienhuis and Geerdink, 2000). BPS was detected in 16% of 82 sediment samples obtained from the Detroit River, Chesapeake Bay, and several other rivers in the USA, Japan, and Korea [up to 1970 ng per g dry weight (dw)] (Liao et al., 2012a). BPS was also detected in 81% of 315 urine samples collected from the general population in the world (up to 21 μg L⁻¹) (Liao et al., 2012b). Human exposure to BPS may be mediated by dermal adsorption such as during contact with thermal receipt papers (13.8 ng g⁻¹–22.0 mg g⁻¹), currency bills (up to 6.26 μg g⁻¹), and canned foodstuffs (several tens of ng g⁻¹) (Liao et al., 2012c; Vinas et al., 2010). In addition, BPS accumulated in the fetal compartment after repeated maternal exposure (Grandin et al., 2018).

Previous studies showed the persistence of BPS in waters underoxic conditions. BPS was not degraded in seawater within 60 days (Danzl et al., 2009) nor in river water within 25 days (Ike et al., 2006). However, the latter study showed the dissipation of BPS in anoxic pond sediment and a removal of 60% within 80 days (Ike et al., 2006).

In contrast to water, in oxic soils BPS dissipated rapidly, with a short
half-life (< 1 day), as shown in two surface clay loamy soils (Choi and Lee, 2017). However, the environmental processes responsible for the rapid dissipation of BPS in soil are so far unknown. Three metabolites of BPS have been identified in oxic soils, being produced by hydroxylation and ortho- or meta-cleavage of one ring with subsequent degradation of the broken part (Choi and Lee, 2017). However, the quantity of such metabolites (including CO₂) produced in soil is still unclear, even though this information is important for environmental risk assessment of BPS as a BPA substitute (Choi and Lee, 2017).

Complete removal of contaminants from soil can be technically difficult and expensive. Instead, risk-based approaches to manage contaminated sites, which aim to minimize the exposure of contaminants to humans and wildlife, are gaining acceptance due to their feasibility (Umehe et al., 2017). This requires complete understanding of the non-extractable residues (NERs) of contaminants in soil. The formation of NERs, also previously called bound residues, is the major fate of phenolic compounds in soil (Kästner et al., 2014; Liu et al., 2013; Shan et al., 2011). NER formation strongly impacts the environmental behavior and effects of organic contaminants in soil, by decreasing their bioavailability (Kästner et al., 2014) and increasing their persistence. Hydrophobic contaminants like diclofenac could sorb to soils rich in organic matter and exhibit recalcitrance towards degradation (Lonappan et al., 2016). NERs can be formed by physico-chemical entrapment and ester-/ether-linkages in soil matrices (Kästner et al., 2014; Li et al., 2015). If the soil environmental conditions change, these compounds may be released and become bioavailable (Kästner et al., 2014; Liu et al., 2013). The need to include entrapped NERs in assessments on chemical persistence is well established (Schäffer et al., 2018). However, evidence for the presence of organic contaminants and/or their metabolites in NERs so far exists only for a limited number of chemicals (Borns et al., 2007; Dec et al., 1997; Junge et al., 2011, 2012; Li et al., 2015; Riefer et al., 2013; Yao et al., 2020; Zhu et al., 2019). Accordingly, a comprehensive environmental risk assessment of BPS will allow evaluations of the various forms of BPS-derived NERs and their stability in soil.

In this work we used a 14C-radiotracer (1) to study the mineralization, metabolism, and NER formation of BPS in soil under oxic conditions and (2) to characterize the nature and stability of NERs of BPS in soil. The results provide unique information about the fate of BPS in soil and thus contribute to more accurate assessments of the potential risk posed by BPS in the environment.

2. Materials and methods

2.1. Chemicals

Uniformly 14C-ring-labeled phenol (14C-phenol) (2.4 × 10⁸ Bq mmol⁻¹) in petrol ether (boiling point 60–90 °C) was purchased from Moravek Biochemicals Inc. (Brea, CA, USA). Non-labeled phenol (99% purity) and BPS (98%) were purchased from Sigma Corp. (Shanghai, China). Other chemicals were of chromatographic or analytical grade and were obtained from Nanjing Chemical Reagent, Ltd. (Nanjing, China).

2.2. Soil

A soil was collected in March 2015 from a paddy rice field at the Changshu Experimental Station of the Chinese Academy of Sciences, Jiangsu, China. The soil had a pH of 7.03 and a cation exchange capacity of 23.4 cmol kg⁻¹. The content of soil organic carbon and total nitrogen was 36.5 and 2.75 g kg⁻¹ soil dry weight (dw), respectively. The soil contained 46.7% clay, 37.9% silt, and 15.4% sand (Shan et al., 2011). It was activated at 70% maximal water-holding capacity (MWHC) in the laboratory for 7 days, air-dried, and sieved through a 10-mesh sieve (2.0-mm pore). A sterilized soil control was prepared by autoclaving the soil thrice at 121 °C for 20 min on three consecutive days.

2.3. Synthesis of uniformly 14C-ring-labeled BPS

Uniformly 14C-ring-labeled BPS (14C-BPS) on two rings was synthesized in our laboratory from 14C-phenol. Briefly, 14C-phenol was reacted with concentrated sulfuric acid (98%) at 125 °C for 2.5 h and then heated at 175 °C for 3 h. Preparative thin layer chromatography was used to purify the product. The synthesized 14C-BPS had a specific radioactivity of 4.8 × 10⁸ Bq mmol⁻¹, a chemical purity > 98%, and a radiochemical purity of 98.8%. For details, see the Supplementary Information (SI).

2.4. Incubation experiment

Aliquots of an aqueous solution of 14C-BPS (9.4 kBq) were added to 5.0 g of soil (dw) in 50-mL flasks. The BPS concentration in the flasks was 2.0 mg (kg soil dw)⁻¹. The soil moisture was adjusted to 70% of MWHC using sterilized water. The flasks were closed with rubber stoppers and incubated at 25 °C in darkness. The 14CO₂ released from 14C-BPS during the incubation was trapped by 1.0 mL of NaOH (1 M) in a 6-mL plastic vial fixed to the bottom of the stopper. During the incubation, these flasks were opened for ~1 min every day to allow headspace exchange with fresh air. After 0, 2, 4, 6, 11, 19, and 28 days of incubation, three flasks were removed for the analysis of radioactivity in 14CO₂, extractable residues, and NERs (see below). Control treatments were performed using sterilized soil. All experiments were performed in triplicate.

2.5. Extraction and fractionation of soil samples

Soil samples from the incubation experiments were freeze-dried and extracted three times with methanol (25 mL) by repeated shaking (200 rpm, 1 h) and centrifugation (4000 g, 15 min). The supernatants (extracts) were combined and the radioactivity was determined by liquid scintillation counting (LSC, see below). Preliminary experiments showed that this extraction procedure recovered 100.1 ± 0.8% (n = 3) of the 14C-BPS in the soil. The radioactivity in the organic extracts represented the extractable residues of 14C-BPS in soil, including BPS and its metabolites. The organic extract was concentrated to ~1 mL and analyzed by high-performance liquid chromatography coupled to an online radioactivity detector (HPLC-14C-LSC) (see below) and by liquid chromatography coupled to a mass detector (LC-MS) (see SI). The radioactivity remaining in the soil after this exhaustive extraction was defined as NERs of 14C-BPS and determined using an oxidizer (see below).

2.6. Silylation of soil

14C-BPS-derived residues physico-chemically entrapped in the soil matrices were released by the silylation of methanol-extracted soil, according to Wang et al. (2017) and Haider et al. (2000). Briefly, 2 mL of dimethyl sulfoxide (DMSO), 10 μL of pyridine, and 200 μL of trimethylsilyl chloride were added to 1.0 g of the dried methanol-extracted soil sample. The mixture was shaken at 220 rpm for 12 h in the dark. After centrifugation (3200 g, 30 min), 100 μL of the supernatant was analyzed by LSC (see below). The radioactivity in the supernatant represented the 14C-BPS-derived residues physico-chemically contained in the soil, whereas the remaining extracted solid consisted of covalently bound and biomass-incorporated NERs. Ten mL of deionized water was added to the supernatant and the mixture then extracted three times with ethyl acetate. The organic phases from the extractions were combined and then washed three times with saturated sodium chloride solution to remove the remaining DMSO. After concentration of the samples, the organic phase was analyzed using HPLC-14C-LSC (see below).
2.7. Cleavage of ester- and ether-linked NERs

NERs covalently bound via ester- and ether-linkage to soil organic matter were cleaved by alkaline hydrolysis according to Li et al. (2015). Briefly, the remaining soil (~1.0 g) after the silylation procedure (see above) was washed three times with methanol and then hydrolyzed with 1 M O2-free NaOH (5 mL) at 90 °C for 2 h. The hydrolysate was separated by centrifugation (3200 g, 30 min) and the precipitate was washed with distilled water (5 mL) to recover the residual hydrolysate from the precipitate. The hydrolysates were combined and the radioactivity, representing the radioactivity of NERs via the ester-linkage, was measured by LSC (see below). The hydrolysate was acidified with a few droplets of 6 M HCl to pH 1 and extracted with ethyl acetate. The extract was concentrated to 100 μL and analyzed by HPLC-14C-LSC (see below).

The remaining precipitate was further hydrolyzed with 4 M O2-free NaOH (4 mL) at 120 °C for 15 min. The radioactivity in this hydrolysate, representing the radioactivity of the NERs via ether-linkage, was measured by LSC (see below). Due to the low content of radioactivity, the extract was not further analyzed by HPLC-14C-LSC.

2.8. Incubation of NERs in fresh soil

Samples of the methanol-extracted soil (~0.40 g), containing NERs of 14C-BPS, from the active and sterilized treatments after 28 days of incubation were mixed with 2.4 g of freshly activated soil (the same soil as used for the first incubation in Section 2.4) in 50-mL flasks. The mixture was incubated for 40 days and the 14CO2 and organic extractable residues released from NERs were recovered and analyzed as described in Section 2.4.

2.9. HPLC-14C-LSC

HPLC-14C-LSC analysis was performed on an Eclipse XDB-C18 column (250 mm × 4.6 mm; Agilent Technology, Santa Clara, CA, USA) at 30 °C with an Agilent HPLC 1100 system (Agilent Technology, Santa Clara, CA, USA) equipped with an online radio-flow detector (Ramona Star; Raytest, Straubenhardt, Germany). The injection volume was set at 20 μL. The mobile phase consisted of water (A) and methanol (B) and ran at 1 mL min−1 using the following gradient program: isocratic with 40% B for 0–2 min, linear increase to 95% B for 2–12 min, isocratic with 95% B for 12–16 min, linear decrease to 40% B for 16–18 min, and isocratic with 40% for 18–20 min. The scinitillation cocktail (Gold Flow Multipurpose; Meridian Biotechnologies Ltd., Epsom, UK) ran at 2 mL min−1. The UV signal was recorded at 220 and 254 nm.

2.10. Determination of radioactivity

The radioactivity was quantified on a liquid scintillation counter (LS6500; Beckman Counter, USA). The radioactivity of the 14CO2 trapped in NaOH was determined by mixing 1 mL of NaOH with 3 mL of scintillation cocktail (Gold Star Multipurpose; Meridian Biotechnologies Ltd., UK), and that of the organic extracts by mixing 1 mL of the extracts with 3 mL of the cocktail. To estimate the radioactivity in the ester- and ether-linked NERs, 200 μL and 2 mL of the alkaline extracts were mixed with 10 mL of cocktail, respectively. For the radioactivity in NERs, 0.25 g of solid pellets was combusted using a biological oxidizer (OX-500; Zinsser Analytic, Germany) and the generated 14CO2 was absorbed with 10 mL of an alkaline cocktail (Oxysolve C-400; Zinsser Analytic) and counted by LSC. For the radioactivity in the silylation mixture (DMSO), 100 μL of the mixture was added to 18 mL of cocktail.

2.11. Data analysis

The kinetic model for fate of BPS in the soil (Fig. 1) was based on the four-pool model used in Matthies et al. (2008) and Loos et al. (2012) with modifications. Briefly, parent compound (P) is transformed to metabolites (M) and further mineralized to volatile products (V), i.e., CO2 in our study. NERs are divided into two parts: physico-chemically entrapped NERs (Nent), i.e., type 1 NERs according to Kästner et al. (2014), and other NERs (Noth). Nent are formed from P and can release back to P, because in our study showed that physico-chemically entrapped NERs were not stable and contained almost completely the parent BPS. Noth are formed from P and M (Fig. 1). All the reactions were assumed as first-order kinetics according to Matthies et al. (2008) and Loos et al. (2012). Other models we tested are shown in SI.

The model can be fully expressed in a system of linear differential equations (Eqs. (1)–(5)):

\[
dP(t) \frac{dt}{dt} = -(k_{PM} + k_{PNe} + k_{PNe})P(t) + k_{NPt}N_{ent}(t)
\]

\[
dM(t) \frac{dt}{dt} = k_{PM}P(t) - (k_{MNo} + k_{MV})M(t)
\]

\[
dN_{ent}(t) \frac{dt}{dt} = k_{PNe}P(t) + k_{MNo}M(t)
\]

\[
dN_{oth}(t) \frac{dt}{dt} = k_{PNe}P(t) - k_{MNo}N_{ent}(t)
\]

\[
dV(t) \frac{dt}{dt} = k_{MV}M(t),
\]

where \(P(t), M(t), N_{ent}(t), N_{oth}(t),\) and \(V(t)\) are state variables in % of initially applied radioactivity and \(k_{XY}\)’s (day−1) are first-order reaction rate constants. Parameter estimation for the differential equations was performed using deSolve and minpack.lm packages in the R environment (RStudio Inc., MA, USA). Starting values for M and V were set to 0, while the starting values for P, Nent, and Noth were set to the measured values at initial experiment point. The goodness of fit was evaluated by calculating coefficient of determination (\(R^2\)), root-mean-squared error (RMSE), and correlation between the observed results and predicted results. Dissipation half-life (t1/2) of BPS was obtained as

\[t_{1/2} = (\ln 2)/(k_{PM} + k_{PNe} + k_{PNe}).\]
The mineralization of NERs in fresh soil was fitted to Eq. (7):

\[ M_t = M_{\text{Max}} \left(1 - e^{-k_m t}\right), \]  

where \( M_t \) is the cumulative \(^{14}\text{CO}_2\) (% of initial NERs) released from the soil at time \( t \), \( M_{\text{Max}} \) is the maximum mineralizable amount (% of initial NERs), and \( k_m \) is the mineralization rate (day\(^{-1}\)) of NERs. The data were fitted to the equation using the MATLAB R2019a software (MathWorks, Natick, MA, USA). Single first-order and two-compartment first-order models were fitted using MATLAB.

The estimations of microbial yield of BPS and amount of biogenic NERs were based on the Microbial Turnover to Biomass (MTB) model proposed by Trapp et al. (2018). An efficiency of microbial catabolism of 40% and an energy requirement for microbial biomass growth of 5 g mol\(^{-1}\) adenosine 5'-triphosphate (ATP) were used for the estimation by MTB model, according to Trapp et al. (2018).

3. Results and discussion

3.1. Fate of BPS in soil

The fate of \(^{14}\text{C}\)-BPS in oxic soil during 28 days of incubation included formation of \(^{14}\text{CO}_2\), extractable residues, and NERs (Fig. 2). The reaction rates of different processes as well as statistical parameters for the fitted model are shown in Table 1, showing goodness of the fitting. The main fate of BPS in the active soil was mineralization and NER formation, each accounting after 28 days for approximately half of the initial application (53.6 \(\pm\) 0.2%) and 44.9 \(\pm\) 2.9%, respectively) (Fig. 2A). BPS dissipation was attributed to biodegradation and NER formation, with a half-life \((t_{1/2})\) of 2.8 days. The dissipation rate was slightly lower than that in a clay loamy soil at 22 \(\pm\) 2 °C, in which the \(t_{1/2}\) was \(<\) 1 day (Choi and Lee, 2017). The dissipation \(t_{1/2}\)'s of BPS in soils were similar to those of BPA in other oxic soils at 18–25 °C (0.81–7 days) (Li et al., 2013; Xu et al., 2009; Ying and Kookana, 2005). Mineralization and NER formation were rapid, without a lag phase, and maximum NER formation was reached before maximum mineralization (after \(~6\) vs. \(~11\) days, respectively) (Fig. 2A). The rate-limiting step for mineralization of BPS in the soil was BPS biodegradation to metabolites, with the degradation constant \(k_{\text{d}}\) of 0.186 \(\pm\) 0.231 day\(^{-1}\) (Table 1), while mineralization of the metabolites was more rapid (\(k_{\text{m}}\) of 0.773 \(\pm\) 0.281 day\(^{-1}\), Table 1), indicating that BPS is utilized by soil aerobic microorganisms as a substrate for energy production, in contrast to the prolonged persistence of BPS in oxic seawater and river water (Danzl et al., 2009; Ike et al., 2006), which was probably attributed to the relative low microbial activity in waters compared to in soil. Mineralization was not detected in the sterilized soil and NERs were present in significantly lower amount (28.8 \(\pm\) 0.7%) (Fig. 2B), indicating that microbial activity was responsible for the enhanced formation of NERs \((~38\%)\) of BPS in the active soil, although abiotic processes are clearly of importance as well. The rate constants of the formation of physico-chemically entrapped NERs (\(k_{\text{PNe}}\)) in active soil and sterilized soil were similar (0.060 \(\pm\) 0.007 vs. 0.063 \(\pm\) 0.004 day\(^{-1}\), Table 1), indicating that the entrapping rate of BPS in soil in the presence and absence of microorganism were the same. The gradual increase of NERs in the sterilized soil was actually the abiotic aging of BPS in soil (Bergeron et al., 1994; Gevao et al., 2000). The abiotic formation of NERs seems to be a general process for polar organic contaminants in soil and also has been observed for dicamba (Gevao et al., 2005), nonylphenol (Shan et al., 2011), tetrabromobisphenol A (TBBPA) (Li et al., 2015; Liu et al., 2013; Sun et al., 2014), and bisphenol F (Guo et al., 2019) in oxic or anoxic soils. Abiotic aging processes include adsorption and diffusion (Semple et al., 2003). Thus, abiotic NER formation of BPS may have been due to BPS adsorption onto soil matrixes and entrapment in the pores of soil organic matter, organo-mineral complexes, and interlayers of clay minerals. As a dissociable compound, adsorption of BPS could also be affected by soil cation exchange capacity as in the case of active pharmaceutical ingredients (Lees et al., 2016). Since day 11, the amount of NERs in active soil started to decrease (Fig. 2A), which indicated the instability of NERs formed by BPS, but the release kinetic constant \((k_{\text{NeP}})\) of physico-chemically entrapped NERs to BPS (0.022 \(\pm\) 0.007 day\(^{-1}\)) was much lower than the formation constant \((k_{\text{NeP}})\) of BPS to NERs (0.060 \(\pm\) 0.007 day\(^{-1}\)) (Table 1). Surprisingly, the fate model demonstrated a much higher \(k_{\text{NeP}}\) value in the sterilized soil (0.158 \(\pm\) 0.014 day\(^{-1}\)) than in the active soil, indicating that microorganisms could have increased the stability of NERs during their formation by inhibiting the release of physico-chemically entrapped NERs. Sterilization of the soil could be another reason for the accelerated release of physico-chemically entrapped NER by changing the soil conditions.
3.2. Metabolites of BPS in soil

The organic extracts of the soil samples were analyzed using HPLC-14C-LSC, which revealed two major metabolites (Fig. 3), one (M1) with a polarity higher than that of BPS [retention time (RT) of 3.5 min] and the other (M2) with a polarity lower than that of BPS (RT of 11.0 min). The kinetics of BPS degradation and metabolite formation are shown in Fig. 4. In contrast to the rapid dissipation of BPS, the total amount of its metabolites in soil was always below 1.8 ± 0.5%, in agreement with the fast rates of BPS mineralization and NER formation (Fig. 2A) and indicative of the rapid transformation of BPS metabolites (Choi and Lee, 2017). The metabolites M1 and M2 already appeared on day 2 and their amounts increased until day 6. Thereafter, M1 remained stable whereas M2 decreased rapidly and was no longer detectable at the end of the incubation (Fig. 4). The dissipation of M2 might have resulted from its further degradation to CO₂ and/or the formation of NERs.

3.3. Physico-chemically entrapped (type I) NERs of BPS in soil

Physico-chemical entrapment in soil organic matrixes resulted in the formation of considerable amounts of NERs (Fig. 5). Thus, at the end of the incubation NERs formed by this route accounted for 23.5 ± 0.5% and 21.1 ± 1.2% of the initial BPS in the active and sterilized soil (Fig. 5A) and for 51.1 ± 2.4% and 73.2 ± 5.2% of the total BPS-derived NERs (Fig. 5B), respectively. HPLC-14C-LSC analysis showed that the physico-chemically entrapped NERs (type I NERs, Kästner et al., 2014) in the active soil revealed the same chromatographic properties as the parent compound BPS (92.4–100%), except for a small amount of a polar metabolite (M3, Fig. 6A). By contrast, physico-chemical entrapment in the sterilized soil yielded only 14C-BPS and no metabolites (data not shown).

Between day 1 and day 6 of the incubation, the amount of physico-chemically entrapped NERs in active soil was markedly higher than in sterilized soil, in agreement with the higher equilibrium constant value between BPS and NERs (ratio of \( k_{PM} / k_{MNe} \)) in the active soil (Table 1), clearly indicating a role for microorganisms in the formation of type I NERs. The mechanisms for such enhanced formation of type I NERs of BPS need further study. During the incubation, the amount of physico-chemically entrapped NERs increased continuously in the sterilized soil (Fig. 5A), suggesting that type I NER formation was a diffusion-limited process. In active soil, the amount of these NERs decreased after 6 days, suggesting their release from the entrapment sites. The surprising detection of large amounts of BPS in physico-chemically entrapped NERs underlines the importance of including NERs in environmental risk assessments of contaminants.

3.4. Ester- and ether-linked NERs

In the active soil, a considerable amount of ester-linked NERs (15.0 ± 1.4% of initial BPS) had formed after 28 days of BPS incubation (Fig. 7A), while ether-linked NERs accounted only for 0.85 ± 0.02% of the initial amount of BPS (Fig. 7C). The formation of both ester- and ether-linked NERs was rapid, without a lag phase, and the maximum amount was detected by day 11 (Fig. 7A, C), i.e., later than the active formation of ester-linked NERs in sterilized soil. The dissipation of both types of NERs was rapid, indicating the importance of including NERs in environmental risk assessments of contaminants.

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### Table 1

| Constant or statistical parameter | Active soil | Sterilized soil |
|----------------------------------|-------------|-----------------|
| \( k_{PM} \) (day⁻¹)             | 0.186 ± 0.231 | –               |
| \( k_{PM} \) (day⁻¹)             | 0.060 ± 0.007 | 0.063 ± 0.004   |
| \( k_{MNe} \) (day⁻¹)            | 0.000 ± 0.231 | –               |
| \( k_{MNe} \) (day⁻¹)            | 1.784 ± 3.222 | –               |
| \( k_{MNe} \) (day⁻¹)            | 0.773 ± 0.281 | –               |
| \( k_{NeP} \) (day⁻¹)            | 0.022 ± 0.007 | 0.158 ± 0.014   |
| \( R^2 \)                        | 0.980       | 0.980           |
| RMSE (%)                         | 2.1         | 1.0             |
| Correlation                      | 0.994       | 0.999           |

* Data unavailable.

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Fig. 3. HPLC radiochromatograms of organic extracts of active soil incubated with 14C-BPS for 0, 6, and 28 days. Two metabolites (M1 and M2) occurred during the degradation. The peak at 12 min was an impurity of 14C-BPS.

Fig. 4. Amounts of extractable BPS, total metabolites (left y-axis), and the metabolites M1 and M2 (right y-axis, see Fig. 3) during a 28-day incubation of BPS in active soil. No metabolite was detected in sterilized soil. The data are presented as the mean values of three individual experiments; bars indicate standard deviations. Solid lines for BPS and total metabolites are predicted data using the fate model (Fig. 1). Dashed lines are predicted data using two-compartment first-order model fitted to the observed data.

Fig. 5. HPLC radiochromatograms of organic extracts of active soil incubated with 14C-BPS for 28 days. Two metabolites (M1 and M2) occurred during the degradation. The peak at 12 min was an impurity of 14C-BPS.
than the 6 days needed for total NERs to reach a maximum (Fig. 2A). The difference was consistent with the decrease in the amount of physico-chemically entrapped NERs after day 6 (Fig. 5A) and suggested the transformation by day 6 of some of those NERs to ester- and/or ether-linked NERs. Given the almost complete absence of ester- and ether-linked NERs in the sterilized soil (Fig. 7A, C), the formation of ester- and ether-linked NERs can clearly be attributed to microbial activity, which degraded BPS into reactive intermediates, especially phenolic intermediates, able to bind to soil organic matter via ester and ether bonds. Both types of bonds also occur in the formation of TBBPA-derived NERs in soil, but they are less important than in the case of BPS (see below) (Li et al., 2015). Recently, Yao et al. (2020) showed that one congener of the apolar polybrominated diphenyl ether, BDE3 can be bound to organic matter via ester-linkage after hydroxylation in soil.

The contribution of ester-linked NERs was much larger than that of ether-linked NERs to the total NERs of BPS (Fig. 7B, D). The relative amounts of both increased until day 11 and remained essentially constant thereafter. At the end of the incubation, ester-linked NERs accounted for 21.5 ± 3.0% of the total NERs (Fig. 7B) as well as 64.8% of the sum of covalently bound NERs (type II NERs) and NERs in microbial biomass (biogenic NERs, Kästner et al., 2014), indicating that ester-linkage was the main covalent bonding type in the formation of NERs of BPS in the soil. Ether-linked NERs accounted for only 0.10–1.74% of the total NERs formed during the incubation, suggesting a negligible role of ether-linkage in BPS-derived NER formation. In the case of TBBPA, the contribution (~10%) of ester- and ether-linked NERs was much smaller relative to the total NERs of TBBPA at steady state (Li et al., 2015), being probably attributed to steric hindrance by the bromine atoms of TBBPA and its metabolites, which prevents a reaction with soil organic matter. Nonetheless, ester- and ether-linkages did not account for all of the covalently bound NERs of BPS (Fig. 7B, 7D); thus, other covalent bonds, such as C-C and C-N bonds, or metal bridges with humic macromolecules, via ligand-exchange, may also be important in the formation of NERs from BPS. Further studies are needed to elucidate the contributions of these other chemical bond types to BPS-NER formation.

HPLC-14C-LSC analysis showed that BPS and its three polar metabolites (M1 at RT 3.5 min, M4 at RT 5.0 min, and M3 at RT 7.0 min) were released from ester-linked residues by alkaline hydrolysis (Fig. 6B). The dominant ester-linked residues during the incubation were M1 and BPS. Metabolites M3 and M4 formed between day 6 and day 19 of the incubation (Fig. 8). However, after 28 days, M1 was the most dominant ester-linked residue, accounting for 9.7% (Fig. 8). This result indicated that the ester-linked, i.e., covalently bound, residues of BPS were not stable in soil and their proportions changed during the incubation.

According to the results of the fate model (Table 1), “other NERs” could have been formed mainly from metabolites instead of parent BPS ($k_{p0} = 0$). This could indicate that covalently bound NERs (type II NERs) and biogenic NERs (type III NERs) were attributed to the binding of metabolites formed during the incubation, which was not consistent with the observation that BPS was one of the dominant ester-linked NERs (Fig. 8). This disagreement could be explained by the large uncertainties for some of the rate constants calculated from the model.
3.5. Estimation of biogenic NERs

The amount of biogenic NERs was estimated according to Trapp’s MTB model (Trapp et al., 2018). BPS has a molar mass of 250.26 g mol⁻¹ with eight C–H bonds. The standard Gibbs free energy of BPS formation is −145.4 kJ mol⁻¹, calculated online at http://equilibrator.weizmann.ac.il, according to Noor et al. (2013). The calculated anabolic yield of BPS was 0.31 g dry microbial biomass g⁻¹ BPS. This value was in the range of measured yields (0.05–0.71 g per g substrate) for chemicals of environmental concerns (Trapp et al., 2018).

The amounts of estimated biogenic NERs over time was shown in SI Table S5 and SI Fig. S9. On day 28, the estimated biogenic NERs accounted for 23.7% of the initial amount applied (SI Table S5), corresponding to 53.2% of the total NERs formed. However, based on this calculation, the sum of physico-chemically entrapped NERs (Fig. 5), ester- and ether-linked NERs (Fig. 7), and estimated biogenic NERs was above the experimentally measured amount of total NERs (62.1% vs. 45.1% at the end of the incubation). This means that the amount of biogenic NERs estimated using MTB model was overestimated, probably owing to that the anabolic yield of microbial utilizing BPS was overestimated. The microorganisms could have a catabolic efficiency for BPS turnover lower than 40%. Assuming that the remaining NERs non-identified in this study was biogenic NERs, an efficiency of microbial catabolism of BPS was estimated to be 13.2%. The overestimated biogenic NERs of BPS in soil could also be owing to possible mineralization of the biogenic NERs during incubation. Overestimation of microbial anabolic yield was also found for compounds like phenanthrene and linuron (Adam et al., 2014; Helbling et al., 2014). More information is needed to calibrate the parameters in the MTB model. This model predicts that 9.7% of initial BPS would be incorporated, via biogenic NERs, into soil organic matter after long-term incubation (> 100 days).
and BPS-derived NERs in soil under oxic conditions, calculated according to Eq. (7). This result was consistent with the relative instability of physico-chemically entrapped NERs of BPS (Xu et al., 2009).

Mineralization of the BPS-derived NERs depended on the soil conditions under which they had formed. The NERs that formed in sterilized soil were composed solely of physico-chemically entrapped BPS, and their mineralization was slower but more complete \( (k_m = 0.101 \pm 0.020 \text{ day}^{-1}, M_{\text{max}} = 35.5 \pm 2.6\%) \) than that of NERs from the active soil \( (k_m = 0.186 \pm 0.038 \text{ day}^{-1}, M_{\text{max}} = 19.5 \pm 1.1\%) \) (Table 2). This result was consistent with the relative instability of physico-chemically entrapped NERs of BPS \( (K_{\text{ned}} \text{ values in Table 1}) \) and their likely release into the immediate surroundings, where they became bioavailable. The entrapped BPS mineralized at a slower rate than did freshly amended BPS \( (k_m = 0.142 \pm 0.015 \text{ day}^{-1}) \), which indicated the greater resistance in soil of physico-chemically entrapped NERs than of the parent compound.

![Cumulative release of \(^{14}C\)CO\(_2\) (%) of initial NERs](image)

**Fig. 9.** Mineralization of NERs of \(^{14}C\)-BPS during 28 days of incubation in freshly activated soil. The data are presented as the mean values of three individual experiments; bars indicate standard deviations. Lines are predicted data using Eq. (7).

### 3.6. Stability of NERs

When mixed with fresh active soil, the NERs of BPS that had previously formed in active and sterilized soil \( (i.e., \text{in soil with and without microbial activity}) \) underwent rapid mineralization, without a lag phase (Fig. 9), and were thus bioavailable for microbial degradation. The manual mixing of the fresh soil with soil pellets containing NERs may have disrupted soil aggregates and thereby promoted the bioavailability of physico-chemically entrapped NERs, by, e.g., increasing the diffusion or desorption of sequestered compounds (Xu et al., 2009).

Table 2

| Substance | Mineralization kinetic constants | Mineralization nature of BPS |
|-----------|---------------------------------|-----------------------------|
| BPS       | \( k_m = 0.142 \pm 0.015 \) \text{ day}^{-1} \ | \( M_{\text{max}} = 35.5 \pm 2.2\% \) \ | \( R^2 = 0.992 \) |
| BPS-derived NERs, formed in active soil | \( k_m = 0.186 \pm 0.038 \) \text{ day}^{-1} | 19.5 \pm 1.1 \ | 0.990 |
| BPS-derived NERs, formed in sterilized soil \( (i.e., \text{physico-chemically entrapped BPS}) \) | \( k_m = 0.101 \pm 0.020 \) \text{ day}^{-1} | 35.5 \pm 2.6 \ | 0.994 |

After 40 days of incubation, \( 3.60 \pm 0.04\% \) and \( 8.2 \pm 0.1\% \) of the initial NERs derived from the active and sterilized soils, respectively, were released and became extractable, but \( 76.8 \pm 5.1\% \) and \( 51.1 \pm 3.0\% \) of the initial NERs, respectively, remained in the soil as non-extractable. Together, our results indicate that NERs formed in active soil are more stable than those formed in sterilized soils, owing to the formation of chemically bound residues and their incorporation into biomass during the transformation of BPS by soil microbes. The nature of the chemically bound residues merits further studies.

### 3.7. Environmental implication

As a substitute for BPA, BPS has found application in many industrial products. Our study provides the first insights into the fate and NER formation of BPS in oxic soil. The high mineralization rate of BPS in soil contrasts with the persistence of BPS in aquatic environments (Danzl et al., 2009; Ike et al., 2006) and suggests that BPS is a readily available substrate for soil microorganisms. However, BPS-derived NERs were detected in large amounts in soil, with \~50\% consisting of physico-chemically entrapped BPS and its metabolites. This finding provides direct evidence that organic contaminants in their parent form may become enclosed in soil matrices. Although NER formation is considered a detoxifying process for organic contaminants, the NERs of BPS in soil were unstable, such that a change in the soil environmental conditions could result in the release of BPS and its metabolites from physico-chemical entrapment and their subsequent accumulation in plants and animals. The release of these NER-linked compounds would be especially pronounced in specific soil environments, such as in the rhizosphere, and in the guts of soil macrofauna \( (e.g., \text{earthworms}) \). Alternatively, the released residues may be rapidly degraded and mineralized by microorganisms. The toxicity and structure of BPS metabolites in soil porewater and sequestered in NERs are still unknown. A prerequisite of a reliable environmental assessment on the risks of BPS as a substitute of BPA is an overall understanding of the fate of BPS, the nature and stability of BPS-derived NERs in various environments, and the quantity and toxicity of BPS metabolites, all of which require further study. Our finding that NERs, especially type I NERs, contain parent substance and primary metabolites, should also be kept in mind in assessment on the environmental persistence and risk of agrochemicals that are deliberately released into the environment.

### 4. Conclusions

This study investigated the environmental fate and NER nature of BPS in an oxic soil for 28 days \( (S1 \text{ Fig. S10}) \). BPS dissipated fast in the oxic soil via two major pathways: mineralization \( (53.6\% \text{ of initial}) \) and NER formation \( (45.1\% \text{ of initial}) \), with a dissipation half-life of 2.8 days at \( 25^\circ \text{C} \). More than half of the NERs were present in soil via physico-chemical entrapment and around one third were formed via ester-linkages. Formation of most NERs, especially ester-linkages, were attributed to microbial activities, while abiotic aging played a role in formation of physico-chemical entrapped NERs. Such NERs were unstable and became bioavailable when mixed with fresh soil. The findings suggest that formation of large amounts of reversible NERs in soil should be taken into account when evaluating the environmental persistence and risks of BPS, which questions the safety of BPS substitution for BPA use in consumer products.

### CRediT authorship contribution statement

**Siqi Cao:** Investigation, Validation, Methodology, Formal analysis, Visualization, Writing - original draft. **Songfeng Wang:** Resources, Investigation. **Yingying Zhao:** Investigation. **Lianhong Wang:** Resources. **Yini Ma:** Resources, Methodology. **Andreas Schäffer:** Resources, Writing - review & editing. **Rong Ji:** Conceptualization, Writing - review & editing, Supervision, Funding acquisition.
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105908.

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