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Transformation and Removal Pathways of Four Common PPCP/EDCs in Soil

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Abstract

Pharmaceutical and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs) enter the soil environment via irrigation with treated wastewater, groundwater recharge, and land application of biosolids. The transformation and fate of PPCP/EDCs in soil affects their potential for plant uptake and groundwater pollution. This study examined four PPCP/EDCs (bisphenol A, diclofenac, naproxen, and 4-nonylphenol) in soil by using ^{14}C-labeling and analyzing mineralization, extractable residue, bound residue, and formation of transformation products. At the end of 112 d of incubation, the majority of ^{14}C-naproxen and ^{14}C-diclofenac was mineralized to $^{14}CO_2$, while a majority of ^{14}C-bisphenol A and ^{14}C-nonylphenol was converted to bound residue. After 112 d, the estimated half-lives of the parent compounds were only 1.4 – 5.4 d. However a variety of transformation products were found and several for bisphenol A and diclofenac were identified, suggesting the need to consider degradation intermediates in soils impacted by PPCP/EDCs.

1. Introduction

As natural resources are stressed by population growth, urbanization, and climate change, previously under-utilized waste materials such as treated wastewater and biosolids from wastewater treatment plants (WWTPs) are increasingly being explored and used. For instance, about 3.6×10^9 cubic meters of treated wastewater is currently reused in the U.S. for purposes including agricultural and landscape irrigation, and water reuse is growing by 15% a year (Miller, 2006). Similarly, approximately 6×10^6 metric tons of biosolids are produced each year in the U.S., of which about 60% is applied to land (Water Environment Federation and NACWA, 2013). Regulations governing such reuses are mostly concerned

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with pathogens, nutrients, and heavy metals (U.S. Environmental Protection Agency, 2012, 2000). However, studies over the last two decades have shown that numerous anthropogenic chemicals, such as pharmaceutical and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs), are present in treated wastewater and biosolids (Anderson et al., 2010; Kinney et al., 2006a; Suárez et al., 2008; Xia et al., 2005). Many of these chemicals are known to have unintended biological effects on non-target organisms at low levels (Daughton and Ternes, 1999). Therefore, the beneficial reuse of these waste materials for irrigation or soil amendment introduces contaminants into the soil environment and may pose risks to terrestrial ecosystems and human beings through dietary exposure (Avisar et al., 2009; Chefetz et al., 2008; Dodgen et al., 2013; Kinney et al., 2006a; Topp et al., 2008b; Wu et al., 2010; Xia et al., 2010).

In general, the fate of a xenobiotic in soil includes complete mineralization (i.e., conversion to CO_2), conversion to transformation products, and formation of bound (non-extractable) residue (Gevao et al., 2000). Mineralization of a compound is viewed as complete detoxification, while formation of bound residue is also generally considered a decontamination process (Bollag and Loll, 1983; Verstraete and Devliegher, 1996). In soil, PPCP/EDCs may undergo microbially-mediated transformations, processes that are greatly influenced by both the soil microbial community and the physico-chemical properties of PPCP/EDCs (Kreuzig et al., 2003; Thiele-Bruhn, 2003). The formation of transformation products poses unknown risks as the new products may have biological activity (Celiz et al., 2009; Farré et al., 2008; Li et al., 2013; Lienert et al., 2007). However, to date, most studies on the fate of PPCP/EDCs in soil have only considered removal of the parent compound while ignoring fate pathways.

In this study, with the coupled use of ^{14}C-labeling and chromatographic separation, we quantitatively characterized mineralization and formation of bound residue, as well as disappearance of the parent compound and formation of transformation products, of four commonly occurring PPCP/EDCs, i.e., bisphenol A (BPA), diclofenac (DCL), naproxen (NPX), and nonylphenol (NP), under different soil conditions. Several transformation products of BPA and DCL were also identified. These PPCP/EDCs appear frequently in treated wastewater and biosolids (Anderson et al., 2010; Kinney et al., 2006b; McClellan and Halden, 2010), but little information is available on their complete fate in soil. More knowledge of the complete fate of PPCP/EDCs in soil may be used to improve risk evaluation for land application of treated wastewater and biosolids.

2. Materials and Methods

2.1. Chemicals

Bisphenol A (4,4′-(propane-2,2-diyl)diphenol), diclofenac sodium (2-[(2,6-dichlorophenyl)amino] benzeneacetic acid, monosodium salt), and naproxen ((S)-6-methoxy-a-methyl-2-naphthaleneacetic acid) labeled with ^{14}C and with 99% chemical purity were purchased from American Radiolabeled Chemicals (Saint Louis, MO). The specific radioactivities were 200, 55, and 55 mCi/mmol, respectively. Nonylphenol-111 (4-[1-ethyl-1,3-dimethylpentyl]phenol) labeled with ^{14}C (specific activity 75 mCi/mmol) was provided by Dr. Rong Ji at Nanjing University (Nanjing, China). Chemical structures,

including location of the ^{14}C label, are shown in Figure 1. Non-labeled standards were purchased from the following vendors: BPA, Sigma-Aldrich (St. Louis, MO); DCL, TCI America (Portland, OR); NPX and NP, Alfa Aesar (Ward Hill, MA); 2,6-dichlorobenzoic acid and 2,4-dichlorobenzoic acid, Santa Cruz Biotechnology (Dallas, TX); 4-hydroxybenzaldehyde, 4-hydroxyacetophenone, 4-hydroxybenzoic acid, and 3,5-dichlorobenzoic acid, Sigma Aldrich (St. Louis, MO); 5-hydroxydiclofenac and 4′-hydroxydiclofenac, Toronto Research Chemicals (Toronto, Ontario, Canada). Other chemicals (ACS grade or better) were from Fisher Scientific (West Chester, PA) or VWR (Visalia, CA, USA).

2.2. Soils

Agricultural soils were collected from the University of California's South Coast Research and Extension Center in Irvine, CA (San Emigdio fine sandy loam) and from the University of California's Hansen Agricultural Center in Ventura, CA (Salinas clay loam). A third soil was collected from a treated wastewater recharge basin at the Riparian Preserve at Water Ranch in Maricopa, AZ (Contine clay loam). Soils were collected from the surface layer (0 – 10 cm). After air-drying, soil was passed through a 2 mm sieve. To examine the effect of organic matter, a subsample of the Irvine soil was amended with sieved redwood compost (E. B. Stone Organics, Suisun, CA) at 50% (v/v) to create the Irvine Amended soil treatment. To understand the role of soil microorganisms, another subsample of Irvine soil was autoclaved at 121°C for 45 min on two consecutive days to create the Irvine Sterilized treatment. Soil texture and organic carbon content were determined using established methods (Albert Page et al., 1982; Arnold Klute, 1986). The field capacity of each soil was determined using the pressure chamber method, where -33 J/kg of hydraulic head was applied to saturated soil (Arnold Klute, 1986). Table 1 lists selected soil properties.

2.3. Soil Respirometer Incubation Experiments

Soil respirometers were constructed by suspending a 2 mL glass vial in a 40 mL amber glass bottle with a screw-cap lined with a septum. During incubation, 1.0 mL of 1M NaOH solution was deployed in the 2 mL vial to trap $^{14}CO_2$ from mineralization. A syringe needle was inserted through the septum to enable the sampling and refill of the NaOH solution to monitor mineralization kinetics. A working solution was prepared for each ^{14}C-PPCP/EDC in water. Air-dried soil, equivalent to 10 g dry weight, was placed in the amber bottle and spiked with 0.8 mL of a working solution containing about 3×10^5 dpm radioactivity, making an initial concentration in soil of 12.6 μg/kg for BPA, 69.3 μg/kg for DCL, 46.4 μg/kg for NPX, or 52.8 μg/kg for NP. Deionized water was added to reach field capacity in each soil, which equated to 35% of the total water capacity for Irvine soil and Irvine Sterilized soil, 21% for Irvine Amended soil, 47% for Maricopa soil, and 45% for Ventura soil. Each soil sample was manually mixed to achieve homogenization. The sample bottles were closed, and then NaOH solution was injected into each suspended vial. All soil respirometers were incubated at room temperature (about 22 °C). Respirometers were opened briefly on a weekly basis for aeration and deionized water was added gravimetrically as needed to maintain the soil water content.

On 1, 3, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, 102, and 112 d after the treatment, the NaOH solution in each respirometer was exchanged with new NaOH solution using a disposable syringe. The used solution was placed in a 7 mL glass scintillation vial and mixed with 4 mL of Ultima Gold Scintillation Cocktail (Fisher Scientific, West Chester, PA), followed by measurement of ^{14}C on a Beckman LS 5000TD Liquid Scintillation Counter (LSC) (Fullerton, CA). On day 0, 3, 14, and 112, three soil samples from each treatment were transferred into a freezer (-21 °C) for extraction and analysis of extractable and bound residues.

2.4. Soil Extraction and Combustion to Determine ^{14}C Residue

Soil samples were extracted using EPA Method 1694. In brief, soil samples were removed from the freezer and the thawed soil was transferred to a 50 mL polypropylene centrifuge tube. The soil was sequentially extracted with 35 mL of freshly prepared phosphate buffer (pH 2)-methanol (3:4, v/v) twice and 20 mL of methanol once. For each extraction cycle, the centrifuge tubes were mixed at 260 rpm for 1 h on a horizontal shaker and then centrifuged at 2300 rpm for 15 min. The supernatant was decanted into a 100 mL glass flask, from which a 3 mL subsample was removed for analysis on LSC to determine the total extractable ^{14}C residue. The remaining solvent extract was capped and stored at 4 °C until further analysis.

After the sequential solvent extraction, the soil was air-dried in the fume hood and then 1.0 g aliquots were combusted on an OX-500 Biological Oxidizer (R.J. Harvey, Hillsdale, NJ) at 900 °C for 4 min. The evolved $^{14}CO_2$ was trapped in 15 mL of Harvey Carbon-14 cocktail (R.J. Harvey, Tappan, NY), followed by measurement on LSC to determine the total bound ^{14}C residue. The recovery of ^{14}C in soil was determined to be 71-110% by combusting spiked soil samples and was used to correct for the actual amount of ^{14}C in soil.

2.5. Soil Extract Fractionation and Analysis

The soil extracts were prepared for analysis of parent and transformation compounds by a method modified from Wu et al. (2012). In brief, selected extracts were removed from the refrigerator and mixed with 1200 mL of deionized water, such that methanol was less than 5% of the total solution. The aqueous sample was then passed through a solid phase extraction (SPE) cartridge (HLB, 150 mg, 6 cc, Waters, Milford, MA) at a rate of 5 mL/min. The cartridge was pre-conditioned with 5 mL each of methylene chloride, methanol, and ultra-pure water. A 6 mL subsample of the filtrate that passed through the cartridge was collected and analyzed on LSC to determine the presence of any ^{14}C not retained on the solid phase. The cartridges were then dried under nitrogen gas and eluted with 7 mL methanol. The eluent was condensed to 250 μL under a gentle nitrogen flow and transferred to a 2 mL glass vial. The condensing vessel was rinsed with 200 μL of methanol and the rinsate was added to the eluent in the glass vial. A 50 μL aliquot of non-labeled parent standard stock solution (100 mg/L in methanol) was spiked into each vial to make the final sample volume to 500 μL.

To characterize the extractable residue, a 50 μL aliquot of the prepared extract was injected into an Agilent 1100 Series high performance liquid chromatography (HPLC) with an

ultraviolet (UV) detector. A Dionex Acclaim-120 C18 RP column (4.6 × 250 mm) was used for separation at a flow rate of 1.0 mL/min at 35 °C. Mobile phase A was ultra-pure water acidified with 0.2% acetic acid and mobile phase B was acetonitrile. The ratio of mobile phase A to B was 60:40 for BPA, 50:50 for DCL, 60:40 for NPX, and 25:75 for NP, with corresponding UV wavelengths of 280, 284, 278, and 280 nm, respectively, for positioning the parent compounds. The HPLC eluent was fractionated in 1 min increments using an automated fraction collector (LKB Bromma 2112 Redirac, Bromma, Sweden). Each fraction was mixed with 4 mL of cocktail for analysis of ^{14}C to monitor the distribution of ^{14}C as a function of run time.

To identify transformation products, extracts from BPA and DCL treatments were further analyzed on an ACQUITY ultra-performance liquid chromatography (UPLC) system (Waters, Milford, MA) using an ACQUITY UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 μm particle size, Waters) at 40 °C. Mobile phase A was 0.001% formic acid in water and mobile phase B was methanol. The following mobile phase program (0.2 mL/min flow rate) was used: 0–0.5 min, 5 – 50% B; 0.5 – 12 min, 50 – 100% B; 12 – 13 min, 100% B; 13 – 16 min, 5% B. Analysis was performed with a Waters Micromass triple quadrupole detector (MS/MS) equipped with an electrospray ionization (ESI) source in the negative mode. Parameters of MS/MS were as follows: source temperature, 120 °C; desolvation temperature, 350 °C; capillary voltage, 3.0 kV; cone voltage, 20 V; desolvation gas flow, 600 L/h; cone gas flow, 50 L/h. Standards were run in scan and daughter modes to identify the most robust transition pattern and cone voltage for each compound, and the optimized parameters are listed in Supplemental Table 1. Quantitative analysis was performed in the multiple reaction monitoring (MRM) mode. All data were processed using MassLynx 4.1 software (Waters, Milford, MA).

2.6. QA/QC and Data Analysis

All experimental treatments were in triplicate. Non-spiked soils were included as treatment blanks. Pure methanol was analyzed in each HPLC and UPLC/MS/MS run as solvent blanks. From preliminary experiments, the average extraction recovery of ^{14}C from freshly spiked soil samples was 65.6% for BPA, 61.7% for DCL, 74.5% for NPX, and 75.6% for NP. The average recovery from SPE extraction was determined to be 92.5% for BPA, 89.3% for DCL, 91.9% for NPX, and 77.2% for NP. The mass balance calculated as the sum of ^{14}C from mineralization, extractable residue, and bound residue was 93.9 ± 14.0% for BPA, 85.4 ± 9.7% for DCL, 92.2 ± 6.5% for NPX, and 73.8 ± 25.6% for NP. Statistical analysis of data was performed with R (R Development Core Team, 2008) using Student's t-test, ANOVA, and post-hoc Tukey's Honestly Significant Difference test. Significance was assigned at $p \leq 0.05$.

3. Results and Discussion

3.1. Formation of Extractable and Bound Residues

The extractable fraction of xenobiotics is often used to represent the bioavailable fraction that may illicit biological effects (Ehlers and Luthy, 2003). Incubated soil samples were extracted with solvents to determine the extractable residue of spiked ^{14}C-PPCP/EDCs.

Figure 2 depicts the extractable residue of treatments after 112 d of incubation. For all compounds in all soils, the extractable residue decreased over the incubation period. For example, in Irvine soil spiked with DCL, the extractable ^{14}C decreased to only 6.6 ± 0.2 % at 112 d. The abundance of extractable ^{14}C varied among the PPCP/EDCs, and the general order was NP > BPA > DCL ≥ NPX. For example, in Ventura soil at 112 d, the extractable fraction was 12.9 ± 0.8% for NP, 9.8 ± 0.3% for BPA, 6.8 ± 0.4% for DCL, and 5.6 ± 0.1% for NPX (Figure 2). The level of extractable residue was generally similar among Irvine, Maricopa, and Ventura soils. After sterilization, the level of extractable residue was consistently higher than in the non-sterilized treatment, suggesting that the dissipation of extractable residue was largely due to microbially-mediated transformations. In addition, compost amendment slightly increased the level of extractable residue in Irvine soil.

In Fent et al. (2003), no ^{14}C was detectable in the extract of soil treated with ^{14}C-BPA after 120 d, which was in agreement with the present study, where extractable residue in the unmodified soils (i.e., without sterilization or compost amendment) was low at the end of incubation (8.5 – 11.8%). In a clayey silt soil and a silty sand soil, Kreuzig et al. (2003) reported 5% and 43% extractable ^{14}C after 102 d of incubation following ^{14}C-DCL treatment; the difference between soils was attributed to indigenous microbial activity In this study, only 6.6 – 8.1% of ^{14}C-DCL residue was extractable at the end of incubation. Lin and Gan (2011) found that after 84 d of incubation, 5% and 40% of the spiked NPX (non-labeled) were recovered as the parent compound from a sandy soil and medium loam soil, respectively, while the extractable fraction was only 3.1 – 5.6% in the current study. Topp and Starratt (2000) showed that about 10% of ^{14}C-NP was extractable at 40 d, which was in general agreement with the current study (about 25% at 40 d).

The formation of bound residue is considered a decontamination process, as the chemical (or its transformation products) has become an integral part of the soil matrix. In this study, bound residue was quantified by combustion of extracted soil samples. Figure 2 shows the fractions of bound residue in the incubated soils at 112 d. The levels of bound residue were significantly different among the PPCP/EDCs and followed the overall order BPA > NP > DCL ≥ NPX. For example, at 112 d of incubation in Maricopa soil, bound residue accounted for 66.2, 36.3, 29.6, and 14.9% of the spiked ^{14}C-labeled BPA, NP, DCL, and NPX, respectively (Figure 2). However, no significant difference was noted among the different soils, except for the sterilized Irvine soil, which had significantly reduced levels of bound residue for most compounds. For example, at 112, bound residue for NP was 45.4 ± 16.3% in Irvine soil, 43.9 ± 8.2% in the compost amended Irvine soil, 17.1 ± 6.5% in the sterilized Irvine soil, 36.3 ± 1.2% in Maricopa soil, and 34.8 ± 4.6% in Ventura soil (Figure 2).

Few studies have examined bound residues of PPCP/EDCs, as such analysis requires the use of ^{14}C-labeling and combustion of solvent-extracted samples. In Fent et al. (2003), 79% was determined to be in the form of bound residue following incubation of ^{14}C-BPA for 120 d, which was slightly higher than that found in this study (53.0 – 66.2%). Kreuzig et al. (2003) measured the bound residue at 44 – 78% of the spiked ^{14}C-DCL after 102 d of incubation in two soils, which was greater than in the current study for ^{14}C-DCL (15.2 – 29.6%). Such differences may be attributed to the different soil properties, such as organic carbon content, and to the specific ^{14}C-labeling positions among the studies.

The tendency of an organic compound to become bound is strongly affected by the hydrophobic partitioning to soil organic matter (Gevao et al., 2000; Thiele-Bruhn, 2003). Since the soils used in this study had similar low organic content (Table 1), this may explain why the levels of bound residue for a compound were similar among soils. Due to this partitioning process, compounds with higher hydrophobicity typically become more bound than compounds with lower hydrophobicity (Adams, 2009; Gevao et al., 2000). DCL and NPX are both ionizable compounds, with pK_a values of 4.0 and 4.19, respectively, suggesting that they were partly ionized under the experimental conditions used (Stevens-Garmon et al., 2011). Ionic compounds are much more polar than neutral compounds like BPA and NP, which may explain why NP and BPA had more bound residue and less extractable residue than DCL and NPX.

3.2. Mineralization to $^{14}CO_2$

Microbially-mediated mineralization represents the complete breakdown of an organic compound and is therefore regarded as an environmentally beneficial decontamination process. Throughout the soil incubation in this study, the mineralized $^{14}CO_2$ was continuously sequestered in NaOH solution and periodically measured. Figure 3 shows the cumulative ^{14}C mineralization for each compound in the different soil treatments. The final mineralized fractions were significantly different among the different PPCP/EDCs in the same soil and followed the order NPX > DCL > BPA > NP. For example, in Irvine soil the cumulative fraction mineralized at the end of 112 d of incubation reached 74.8 ± 2.4% of the initially spiked amount for NPX, 65.7 ± 3.6% for DCL, 22.9 ± 0.1% for BPA, and only 9.2 ± 3.7% for NP. These differences were likely related to the extractable fraction of each compound that may be bioavailable for microbial metabolism (discussed above). This relationship was supported by the fact that mineralization followed the same order as the abundance of the extractable fraction. In addition, it must be noted that the location of the ^{14}C-label may have also contributed to the different mineralization rates. As shown in Figure 1, ^{14}C was present on a substituent group in DCL and NPX, while the aromatic ring was labeled for BPA and NP.

The mineralization rate differed among the soil treatments. For BPA and DCL, mineralization in Maricopa soil was more limited in comparison to Irvine or Ventura soil (Figure 3). For example, at the end of the 112 d incubation, the cumulative mineralized fraction for ^{14}C-DCL in Maricopa soil was 49.8 ± 3.9%, lower than that in Irvine (65.7 ± 3.6%, $p < 0.01$) or Ventura soil (68.3 ± 4.1%, $p < 0.01$). On the other hand, mineralization for NPX and NP was generally similar among the three soils. For example, the fractions of ^{14}C-NP mineralized after 112 d were 9.2 ± 3.7%, 10.0 ± 0.6%, and 10.7 ± 0.8% for Maricopa, Irvine, and Ventura soils, respectively. Since the extractable fraction was not different among the soils for a compound, the differential mineralization rates suggested that the variation was likely due to differences in microbial population and activity in these soils. Both Irvine and Ventura soils were from agricultural fields not previously exposed to treated wastewater, while Maricopa soil was from an uncultivated area used as a groundwater recharge basin for over 10 years. It is likely that agricultural cultivation and exposure to treated wastewater, respectively, may have led to the establishment of specific microbial communities in these soils, resulting in preferential transformations of some compounds in a

given soil. Sterilization of Irvine soil significantly decreased mineralization of PPCP/EDCs ($p < 0.001$), confirming the role of soil microorganisms in the transformation of these PPCP/EDCs. In addition, amendment of compost to the Irvine soil generally resulted in decreased mineralization, with the exception of NPX which was quickly mineralized in all non-sterilized soils. For example, mineralization of BPA decreased from 22.9 ± 0.1% in Irvine soil to 17.5 ± 0.3% after compost addition (Figure 3).

The mineralization of PPCP/EDCs was previously examined only in a few studies. In Fent et al. (2003), 13.1 – 19.3% of the spiked ^{14}C-BPA was mineralized after 120 d in four soils, which was in good agreement with the 14.2 – 22.9% range observed for unmodified soils in this study. Mineralization of ^{14}C-DCL was monitored for 102 d in Kreuzig et al. (2003) and was found to be 13% of the spiked amount, which was substantially smaller than that in the current study (49.8 – 68.3%). This reduced mineralization may be attributed to the difference in the ^{14}C labeling position between the two studies. Topp et al. (2008a) reported that about 50% of ^{14}C-NPX was mineralized after 27 d of incubation, and the rapid mineralization was in agreement with the current study (59 – 67% at 28 d). In a separate study, Topp and Starratt observed that 40% of the initially spiked ^{14}C-NP was mineralized after 40 d of incubation at 30 °C in a sandy soil (2000), which was greater than that measured in this study (6 – 8% at 49 d). On the other hand, Shan et al. (2011) reported that only 5% of spiked ^{14}C-NP was mineralized after 58 d of incubation at room temperature. Since ^{14}C-NP was labeled on the aromatic ring in all these studies, the higher mineralization in Topp and Starratt (2000) may be partly attributed to the use of a higher incubation temperature in that study.

Mineralization was the major loss pathway for DCL and NPX, which amounted to 49.8 – 68.3% and 69.2 – 78.3% of the initially spiked ^{14}C, respectively. In comparison, formation of bound residue appeared to be the predominant dissipation pathway for BPA and NP in the soils considered in this study, accounting for 53.0 – 66.2% and 34.8 – 45.4% of the initially spiked ^{14}C, respectively. At the end of 112 d of incubation, the extractable fraction for each compound was consistently smaller than the mineralized or bound residue fraction, suggesting that these PPCP/EDCs were mostly removed in three months through mineralization or formation of bound residue. Concurrently, the potentially bioavailable extractable residue greatly diminished.

3.3. Formation of Degradation Intermediates

Residues extracted from Irvine, Maricopa, and Ventura soils were further analyzed to characterize the composition of extractable ^{14}C after 14 d and 112 d of incubation. Extracted ^{14}C was identified as the parent compound, transformation products appearing during the HPLC run, and transformation products appearing in the SPE filtrate. The results for Irvine soil are shown in Figure 4. Extensive transformation of parent PPCP/EDCs was evident in all soils for most compounds. For example, after 112 d of incubation, parent compounds accounted for only ND – 13.8% of the extractable ^{14}C for BPA and 2.4 – 8.4% for NP. The remaining extractable ^{14}C was in the form of transformation products recovered early in the HPLC run (i.e., before the parent compound) or in the SPE filtrate, suggesting that transformation led to the formation of intermediates more polar than the parent.

Differences among soil types were also evident. For example, while no parent compound was detected at the end of the incubation for DCL and NPX in Maricopa soil, the majority of the extractable residue was found as the parent for DCL (41.7%) and NPX (about 100%) in Ventura soil.

A first-order decay model was used to fit the dissipation of parent compounds in the different treatments. The calculated half-lives ranged from 1.4 to 5.4 d for all PPCP/EDCs in the unmodified soils (Table 2). The test compounds were relatively more persistent in Ventura soil, and less persistent in Maricopa soil, likely reflecting differences in the native microbial communities. The half-lives of BPA and NP in this study were generally similar to those previously reported (Topp and Starratt, 2000; Xu et al., 2009; Ying and Kookana, 2005; Yu et al., 2013). However, the half-lives calculated for DCL (1.4 – 4.3 d) and NPX (3.0 – 5.4 d) were somewhat shorter than those reported by Xu et al. (2009) (3.1 – 20.4 d and 5.7 – 16.8 d, respectively) or Lin and Gan (2011) (4.8 – 29.6 d and 17.4 – 69.3 d, respectively). This difference may be caused by the different soils and experimental conditions used. Overall, none of the PPCP/EDCs considered in this study exhibited significant persistence in soil as the parent compound.

When compared to chromatograms of the parent compound, many transformation products were evident in the soil extracts, and the relative presence of transformation products in the extractable ^{14}C generally increased over time (Figure 4). For example, in Maricopa soil treated with ^{14}C-NPX, 62.1% of the extractable ^{14}C was associated with transformation products at 14 d and the fraction increased to about 100% at 112 d. For NP treatments, ^{14}C was also detected in the SPE filtrate. Since preliminary experiments showed that ^{14}C-NP was quantitatively retained by the SPE cartridge, the ^{14}C in the SPE filtrate may be assumed to be polar compounds not adsorbed by the cartridge sorbent. The extensive transformation of spiked PPCP/EDCs in soil extracts suggests the importance of considering degradation intermediates in addition to the parent compound (Li et al., 2013; Unold et al., 2009).

Samples from BPA and DCL treatments were further analyzed on UPLC/MS/MS to tentatively identify degradation intermediates. Authentic standards were used to verify the identity by matching retention time and mass transitions of the isolated peaks (Supplemental Table 1). In solvent extracts from soil treated with BPA, 4-hydroxyacetophenone (HA), 4-hydroxybenzaldehyde (HBA), and 4-hydroxybenzoic acid (HBacid) were detected as transformation products (Figure 5). While information on BPA degradation in soil is very limited, it was suggested by Spivack et al. (1994) that BPA may undergo oxidative rearrangement to form 1,2-bis(4-hydrozyphenyl)-2-propanol, which is then dehydrated to 4,4′-dihydroxy-a-methylstilbene. Oxidative cleavage may then result in HBA and HA, and further oxidation of HBA forms HBacid.

At 14 d, extracts of Maricopa and Ventura soils treated with DCL showed the presence of 5-hydroxydiclofenac (5HD), as well as 2,6-dichlorobenzoic acid (26DCB) in Ventura soil only. A small amount of 2,4-dichlorobenzoic acid (24DCB) was detected in Irvine soil. At the end of 112 d of incubation, 5HD was detected in all soils, while 24DCB and 26DCB were found in Irvine soil, 24DCB and 3,5-dichlorobenzoic acid (35DCB) in Maricopa soil, and 24DCB in Ventura soil. It is likely that oxidation of DCL led to the formation of 5HD,

and both DCL and 5HD may serve as precursors to DCB through *N*-dealkylation of the biphenyl compounds followed by carboxylation (Figure 5) (Blum et al., 1996; Pérez and Barceló, 2008). 4′-Hydroxydiclofenac was analyzed for, but not detected in any sample, in contrast to other observations made using microbial culture or human metabolic enzymes (Bort et al., 1999; Webster et al., 1998).

Only a few previous studies examined the transformation products of PPCP/EDCs in soil, sediment, or sewage. In a soil incubated with gram negative bacteria, Spivack et al. (1994) identified some of the same intermediates of BPA as in this study. However, even though degradation of DCL was evaluated in sewage (Pérez and Barceló, 2008), sediment (Gröning et al., 2007), and in fungal cultures (Webster et al., 1998), no effort was made to identify the specific dichlorobenzoic acid isomers. Little information is available about the toxicity of these transformation products as compared to their parent forms. The oral LD_{50} in mice was found to be similar for BPA and its products (2200-2400 mg/kg), but lower for the product HA (1500 mg/kg) (U.S. National Library of Medicine, 2013). The transformation products of DCL for which LD_{50} values were available generally had higher LD_{50} values. However, it must be noted that these threshold values were for acute exposures and may have little relevance to effects at low levels that are typical of environmental contamination.

The coupled use of ^{14}C labeling and chromatographic analysis in this study allowed a comprehensive investigation of transformation and removal pathways of four common PPCP/EDCs in soil. The results showed that the primary decontamination mechanisms may vary with compounds. In this study, formation of bound residue was the predominant removal process for BPA and NP, while mineralization was significant for DCL and NPX. In addition, extractable residues consisted of both the parent compound and multiple transformation products, and the relative contribution of the parent varied with compound and incubation time. The abundance of transformation products detected in all soil treatments highlights the importance of a more comprehensive evaluation of PPCP/EDC transformation and fate processes, in order to improve risk assessments of ecosystem and human health effects due to the reuse of treated wastewater and biosolids.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- The comprehensive fate and loss pathways of four common PPCP/EDCs were studied.
- Mineralization and bound residue formation were important removal processes.
- Parent compounds had half-lives of only 1.4 – 5.4 d.
- Multiple transformation products were detected.

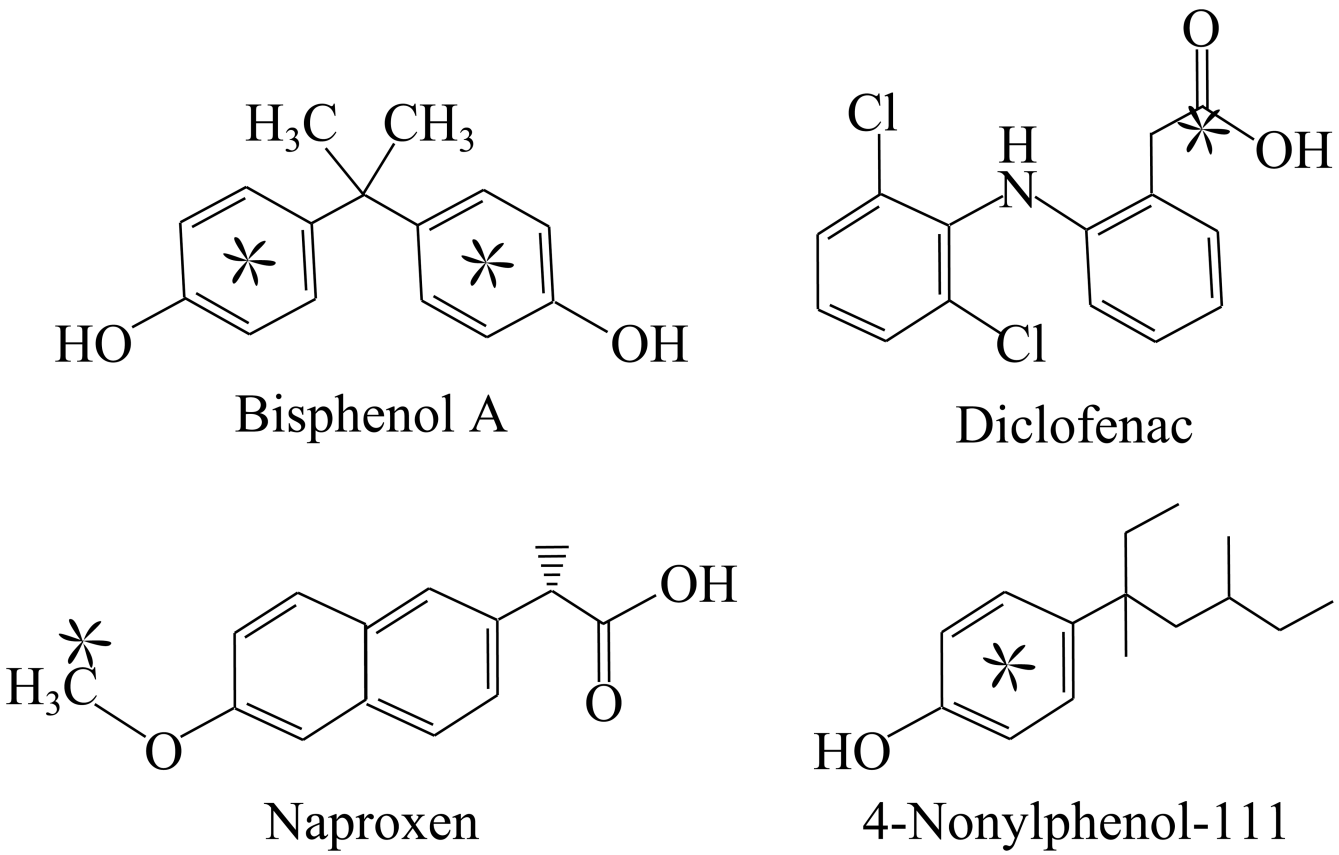

Figure 1.
Chemical structures of PPCP/EDCs considered in this study, with * indicating the location of the ^{14}C label.

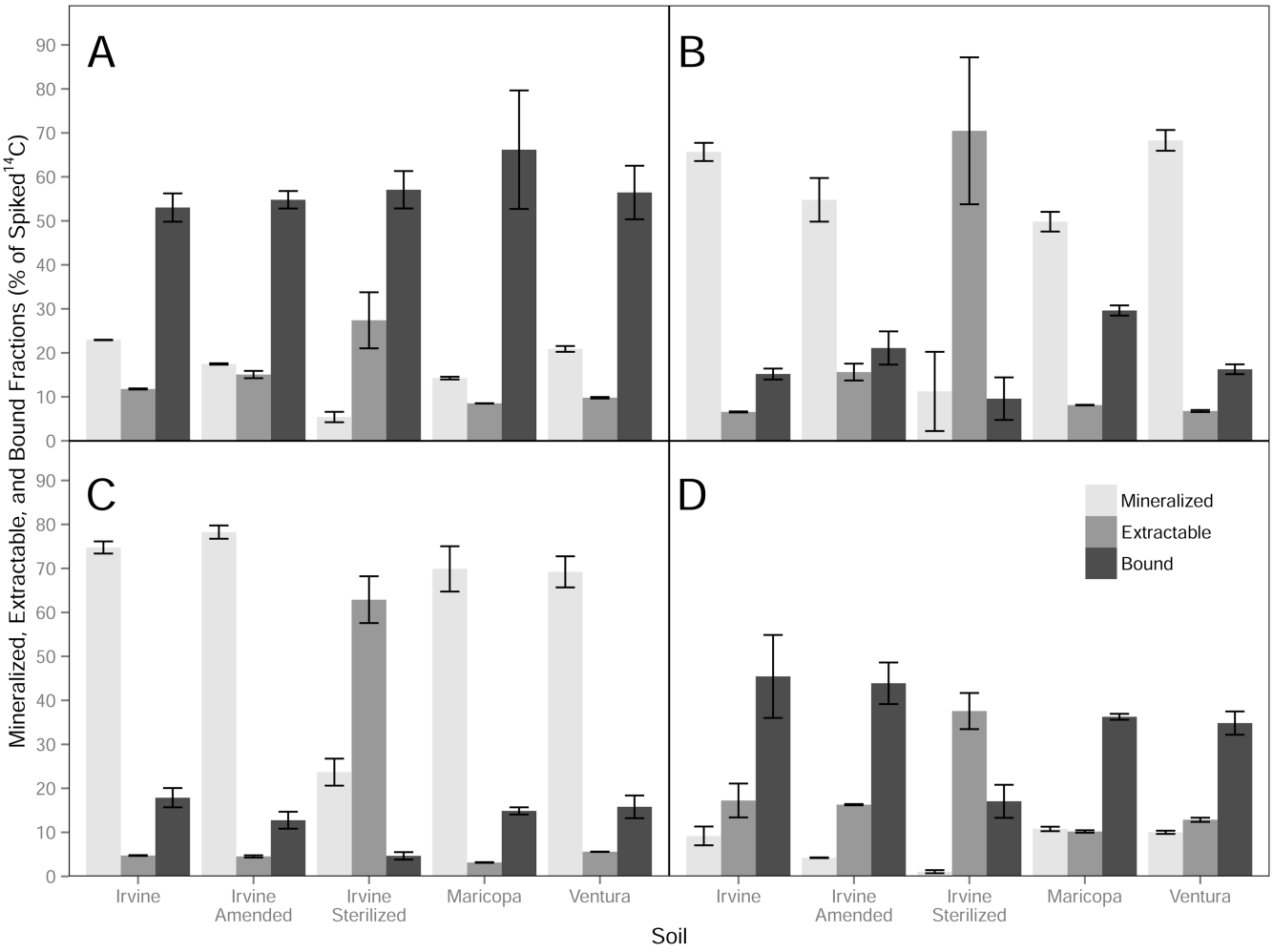

Figure 2.

Distribution of ^{14}C among mineralized, extractable residue, and bound residue fractions in soils spiked (A) ^{14}C-bisphenol A, (B) ^{14}C-diclofenac, (C) ^{14}C-naproxen, and (D) ^{14}C-nonylphenol after 112 d of aerobic incubation at room temperature. Data are expressed as percent of spiked ^{14}C ± standard deviation (n=3).

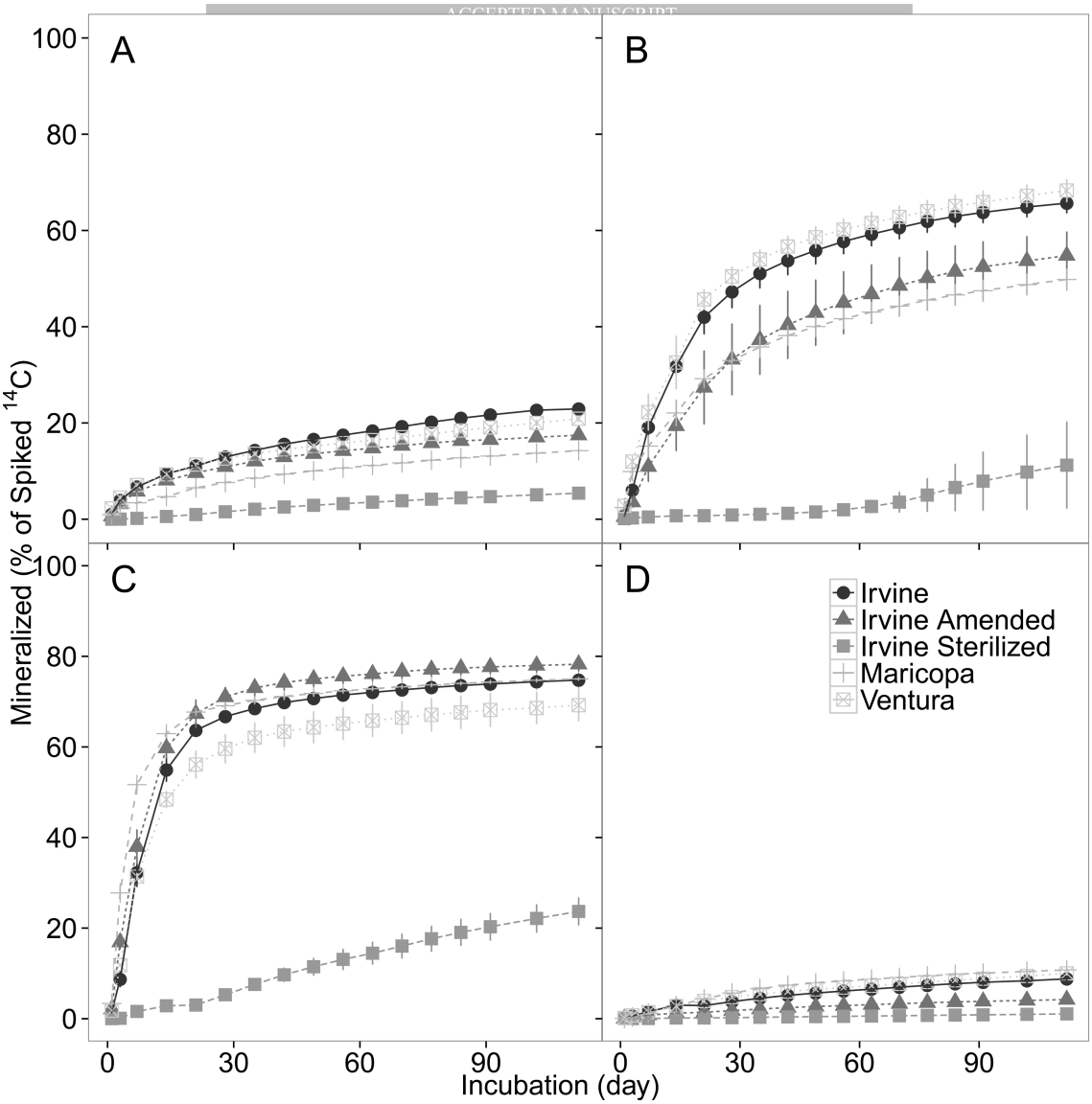

Figure 3.

Cumulative mineralization of (A) ^{14}C-bisphenol A, (B) ^{14}C-diclofenac, (C) ^{14}C-naproxen, and (D) ^{14}C-nonylphenol in different soils during aerobic incubation at room temperature. Data are expressed as percent of spiked ^{14}C ± standard deviation (n ≥ 3).

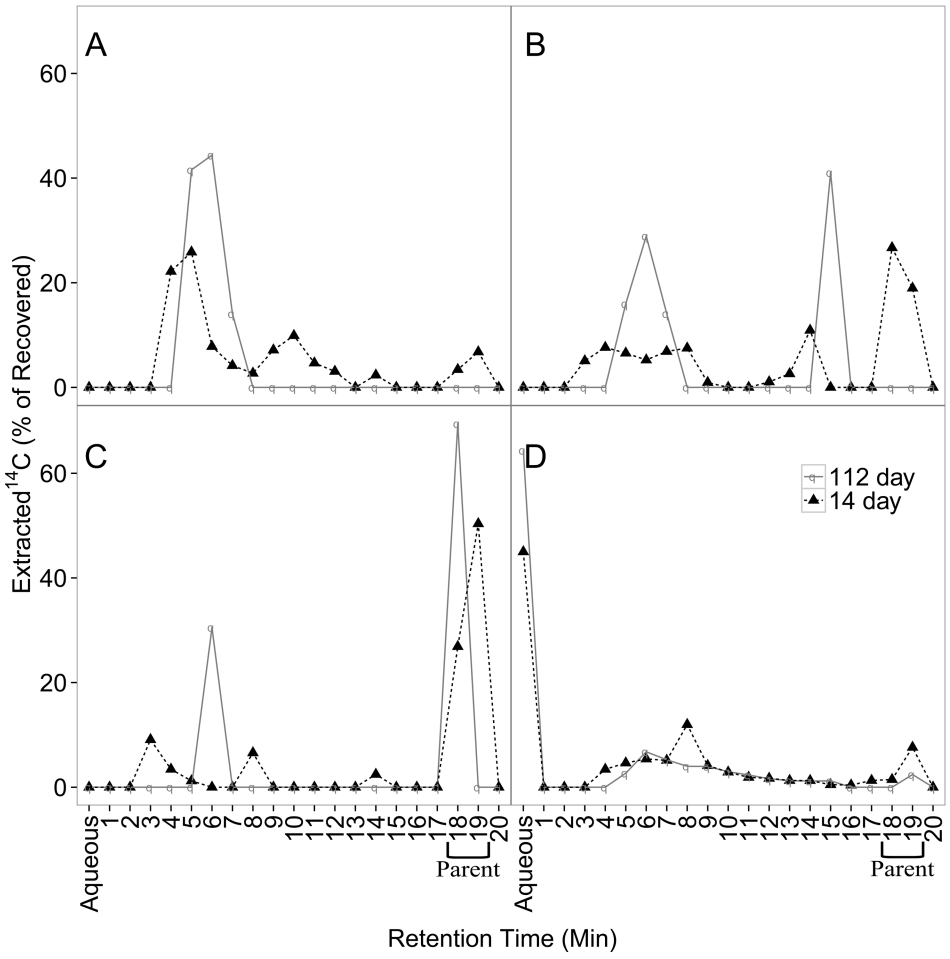

Figure 4.

Composition of ^{14}C in extracts of Irvine soil spiked with (A) ^{14}C-bisphenol A, (B) ^{14}C-diclofenac, (C) ^{14}C-naproxen, and (D) ^{14}C-nonylphenol after aerobic incubation at room temperature. Data are expressed as percent of recovered ^{14}C.

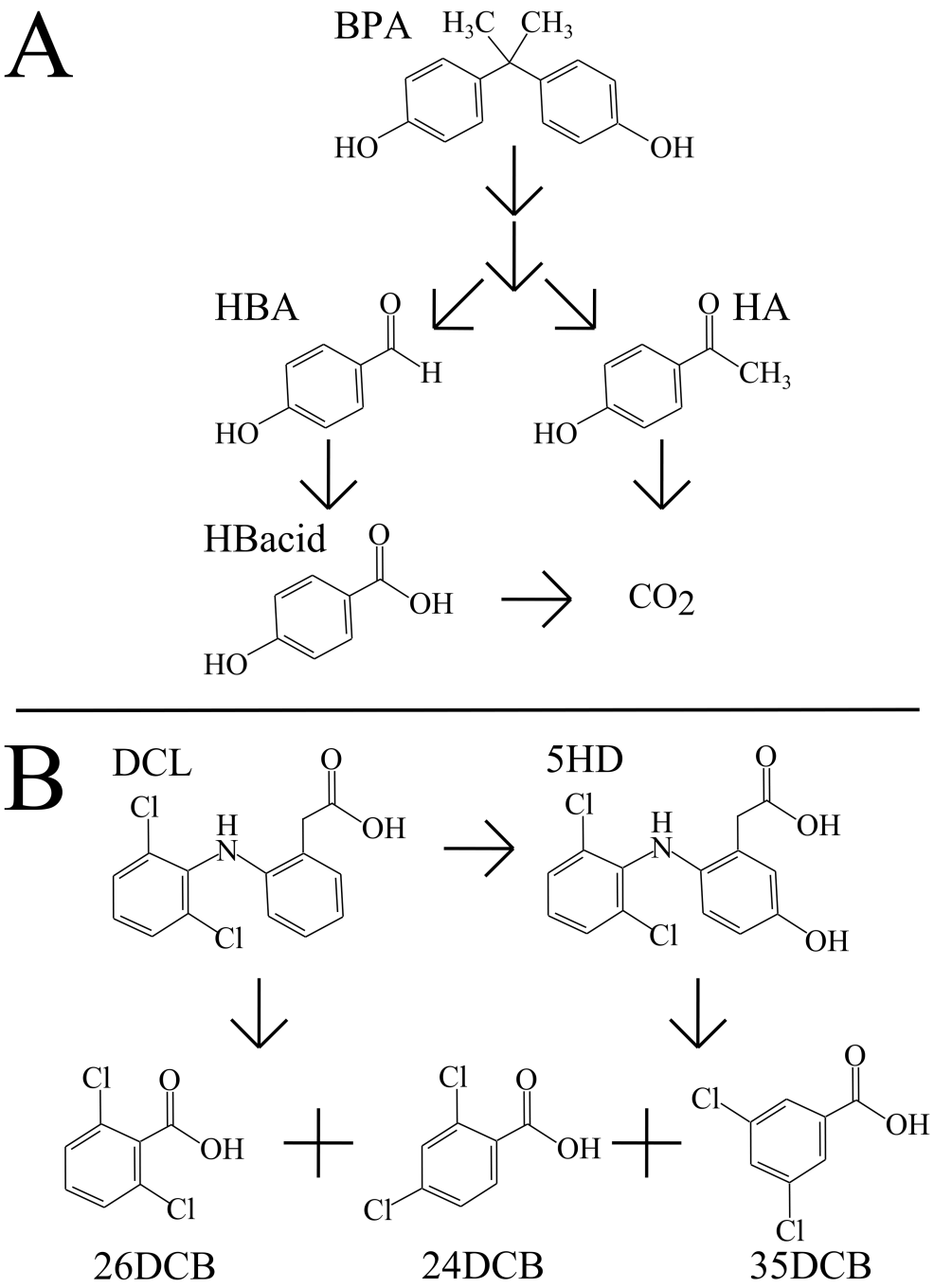

Figure 5.

Tentative transformation pathways based on the identified transformation products. A – Bisphenol A (BPA) and B – Diclofenac (DCL).

Table 1

Select properties of soils used in this study.

Soil	Sand (%)	Silt (%)	Clay (%)	Organic Carbon (%)
Irvine	55.1	20.5	24.4	0.58
Ventura	4.5	51.0	44.6	0.89
Maricopa	31.4	18.0	50.6	0.51
Irvine Amended	---	---	---	1.31

Table 2

First-order rate constants and half-life values calculated from the dissipation of parent compound in different soils.

Compound	Soil	Rate constant (d^{-1})	Half-life (d)
Bisphenol A	Irvine	0.35 ± 0.16	2.20 ± 0.99
	Maricopa	0.33 ± 0.01	2.09 ± 0.09
	Ventura	0.21 ± 0.01	3.33 ± 0.11
Diclofenac	Irvine	0.17 ± 0.05	4.25 ± 1.20
	Maricopa	0.51 ± 0.00	1.36 ± 0.01
	Ventura	0.33 ± 0.27	3.18 ± 2.60
Naproxen	Irvine	0.14 ± 0.00	4.88 ± 0.07
	Maricopa	0.24 ± 0.07	3.04 ± 0.89
	Ventura	0.13 ±0.01	5.44 ± 0.62
Nonylphenol-111	Irvine	0.24 ± 0.01	2.87 ± 0.11
	Maricopa	0.18 ± 0.03	3.86 ± 0.67
	Ventura	0.19 ± 0.01	3.61 ± 0.22