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The fingerprints of dioxin-like bromocarbazoles and chlorocarbazoles in selected forest soils in Germany

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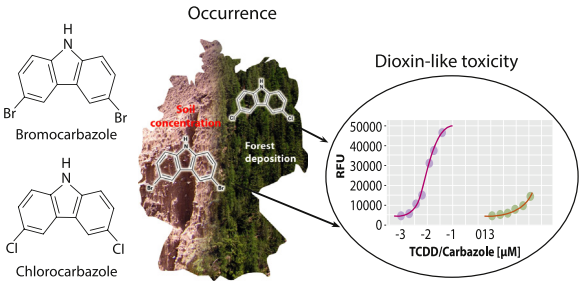
HIGHLIGHTS

- Occurrence of bromocarbazoles and chlorocarbazoles in forest soils in Germany.
- The halogenated carbazoles occur simultaneously with PCDDs, PCDFs and PCBs.
- Bromocarbazoles are here reported for the first time in soil.
- Bromocarbazoles and chlorocarbazoles possess dioxin-like toxicity.
- Their relative effect potency is directly related to the degree of halogenation.

GRAPHICAL ABSTRACT



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ABSTRACT

The occurrence of bromocarbazoles and chlorocarbazoles was studied in 86 forest soil samples from different regions in Germany. Carbazole, 3-chlorocarbazole, 3-bromocarbazole and 3,6-dibromocarbazole were qualitatively detected in the humic layer of 59 soil samples with bromocarbazoles reported here for the first time in soil. Furthermore, the halogenated carbazoles, PCDD/Fs and PCBs were detected in the humic and mineral soil horizons (0–5 cm and 5–10 cm) of a subset of 11 soil samples subjected to quantitative analysis. Concentrations ranged from 0.6 to 267.6 ng/g (carbazole); 0.2–7.2 ng/g (3-bromocarbazole); 0.0–9.1 ng/g (3-chlorocarbazole); 0.2–19.8 ng/g (3,6-dibromocarbazole); 0.4–67.6 ng/g (3,6-dichlorocarbazole); 0.0–0.7 ng/g (PCDDs); 0.0–0.3 ng/g (PCDFs) and 0.0–33.7 ng/g (PCBs). Concentrations decreased with depth and correlated positively to total organic carbon (TOC). When it was based on TOC%, an increase in concentration with depth was observed in most soil samples. With respect to dioxin-like toxicity, 3-bromocarbazole, 3-chlorocarbazole, 3,6-dibromocarbazole and 3,6-dichlorocarbazoles caused induction of CYP1A1-dependent EROD activity in HII4E rat hepatoma cell line. Their relative effect potency after 72 h exposure ranged from 0.00005 to 0.00013 and was directly related to the degree of halogenation with 2,3,7,8-tetrachlorodibenzo-p-dioxin as reference. Furthermore, their contribution to overall soil dioxin-like toxicity was not significant in comparison to PCDD/Fs

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and PCBs though the sum toxic equivalency was limited to three halogenated carbazole congeners. Bromocarbazoles and chlorocarbazoles are emerging dioxin-like toxic environmental contaminants with potential for wide distribution occurring simultaneously with PCDD/Fs and PCBs.

1. Introduction

Bromo- and chlorocarbazoles are a group of polycyclic heteroaromatic environmental contaminants. Their sources, environmental pathway and fate are not well known yet they could be occurring widely in the environment. Some congeners have been isolated from natural sources (Schmidt et al., 2012) although it has been suggested they could be anthropogenic in origin (Parette et al., 2015). They are resistant to degradation in soil (Mumbo et al., 2015; Tröbs et al., 2011) and possess dioxin-like toxicities (Riddell et al., 2015). Even though they possess attributes similar to persistent organic pollutants (POPs), they have not been categorized in a group of environmental pollutants. At the moment sediment and soil have been reported as the major sinks of the bromo- and chlorocarbazoles.

Mono-, di- and tri-substituted congeners have been detected in both soil and sediments. Tetra-substituted halogenated congeners have been detected only in sediments (Guo et al., 2014; Pena-Abaurrea et al., 2014). Bromocarbazoles have only been reported in aquatic sediments but not in soil (Guo et al., 2014; Peng et al., 2016; Grigoriadou and Schwarzbauer, 2011). Bromo- and chlorocarbazoles have both natural and anthropogenic sources. Mono- and di-substituted halogenated congeners could be from natural sources (Lee et al., 1999), while some from anthropogenic sources. For example, 2,7-dibromo-, 3,6-chloro- and 3,6-dichlorocarbazole are used as intermediates in producing polymers used in light emitting devices and other electrical devices (Karon et al., 2014; Boudreault et al., 2008; Morin et al., 2005). Haloperoxidase catalyzed halogenation of organic matter (Laturnus et al., 2005; Latumus et al., 1995; Asplund et al., 1993) could be responsible for the formation of chlorocarbazoles observed in the A- and upper-B horizons of forest soils and not in the organic layer (Reischl et al., 2005). Tetra-halogenated carbazoles have been suggested to be from anthropogenic sources (Parette et al., 2015; Kuehl et al., 1984).

Information on environmental fate and pathways of halogenated carbazoles is limited. Previous studies have reported them as unknown, non-target or unintentional compounds assessed during soil analysis (Guo et al., 2014; Pena-Abaurrea et al., 2014; Grigoriadou and Schwarzbauer, 2011) but information on their local or regionalized occurrence is rarely provided. Therefore limited information is available on the method and extent of their environmental distribution given their persistence and dioxin-like toxic properties. Germany has experienced previous historic industrial activities whose emissions have been suggested to be responsible for occurrence of environmental contaminants and forest acidification in some federal states (Gocht et al., 2007; Steinbrecher et al., 2000; Steinberg et al., 1988). Direct evidence of long range atmospheric transport of POPs from source to deposition on pine needle has been reported (Chen et al., 2012; Klánová et al., 2009; Eriksson et al., 1989). This is not known with regard to halogenated carbazoles. Polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) have been extensively studied in soil (Aichner et al. 2013, 2015). They are ubiquitous with both natural (Hoekstra et al., 1999) and anthropogenic sources (Aichner et al., 2013). They are persistent and toxic, attributes shared with halogenated carbazoles yet no correlation in this respect including their occurrence, sources, environmental pathways and fate have been reported.

In this study, the occurrence of bromo- and chlorocarbazoles was investigated in soil samples collected from different forest regions under the second National Forest Soil Inventory of the Federal Republic of Germany (BZE II). The vertical variations in the soil horizon of these compounds were assessed including PCDDs, PCDFs, and PCBs for a comparative analysis. The contribution of halogenated carbazoles to overall soil dioxin-like toxicity levels in relation to the PCDDs, PCDFs, and PCBs was determined. The soil profile concentrations of bromo- and chlorocarbazoles in relation to soil organic matter were evaluated. It is expected that knowledge on their likely sources, occurrence, toxicity potentials and relationship to other POPs shall be obtained from this study.

2. Material and methods

2.1. Chemicals and reagents

Pure compounds and standards of carbazole [≥95%-Gas Chromatography (GC)], 3-bromocarbazole (≥97%-GC), 3-chlorocarbazole, 3,6-dibromocarbazole (≥97%) and 3,6-dichlorocarbazole were purchased from Sigma Aldrich (Taufkirchen, Germany). Silica gel (SiO_2), sodium sulphate (Na_2SO_4) and all organic solvents used were obtained from LGC Standards (Wesel, Germany). Alumina B (Al_2O_3) was supplied by MP Biomedicals (Eschwege, Germany) while octadecyl-C18-modified silica gel by Macherey-Nagel (Düren, Germany). Hepatoma rat cells H4IIEC3/T (H4IIE) were obtained from Deutsche Sammlung von Microorganismen (DSZM, Braunschweig, Germany). H4IIE cells were cultured in Dulbecco's modified Eagle's medium (DMEM) bought from Biochrom AG (Berlin, Germany). Fetal Bovine Serum (FBS), L-Glutamin were also supplied by Biochrom while 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) by Gibco. The BCA protein assay kit consisting of bicinchoninic acid (BCA) solution, bovine serum albumin (BSA) standard solution and 4% cupric sulphate solution (Cu_2SO_4) was bought from Novagen (Darmstadt, Germany). 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) standard was obtained from LGC Standards (Wesel, Germany). All reagents were of analytical grade and solvents were of picograde quality.

2.2. Study area, soil and sample selection

As part of the second National Forest Soil Inventory in Germany (BZE II) a subset of 86 forest soil samples was selected from a set of 474 samples taken from a 16 km × 16 km grid covering the Republic of Germany (Aichner et al., 2013) in a sampling campaign that was carried out in the years 2006–2008. These samples did not include sampling points out of forest areas. Within the analysis of organochlorine pesticides in the humic layer, the samples were checked for the presence of 3,6-dichlorocarbazole (identified by retention time, exact mass, isotope ratio) in 86 samples. Out of the 59 positive results, 11 samples with higher concentration of that compound were selected in the way that nearly every federal state in Germany was represented; specifically, Brandenburg, Bavaria, North Rhine-