RESEARCH ARTICLE

Degradative fate of 3-chlorocarbazole and 3,6-dichlorocarbazole in soil

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Abstract

Background, aim, and scope 3-Chlorocarbazole and 3,6-dichlorocarbazole were isolated from Bavarian soils. The stereospecific formation of the isomers of these chlorinated carbazols can be explained by quantum mechanical calculations using the DFT method. It was shown that chlorination of carbazole and 3-chlorocarbazole respectively is preferred via the sigma-complexes 3-chlorocarbazole and 3,6-dichlorocarbazole as the most stable products. The dioxin-like toxicological potential of 3,6-dichlorocarbazole, determined by the Micro-EROD Test, is in the range of some picogram TCDD equivalents per milligram carbazole. The degradative fate of 3-chlorocarbazole and 3,6-dichlorocarbazole was analysed within a long-term study (57 days) in soil.

This publication is dedicated to Prof. Sandermann on the occasion of his death on August 18th, 2009.

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Materials and methods The soil was extracted by ASE (accelerated solvent extraction) and a further clean-up procedure with column chromatography and chromatography with C18-SPE stationary phases. Quantification of 3-chlorocarbazole and 3,6-dichlorocarbazole was performed employing the isotope-dilution method. The samples were measured with high-resolution GC/MS.

Results The degradation ($\ln(c/c_0)$ vs. time with best-fit line) showed in almost every storage condition a very small degradation (slopes (h^{-1}) in -10^{-4} range). However, the decay for the controls were two to three times ($-28°C$) and six times (with sodium azide) higher, than the decrease of 3-chlorocarbazole and 3,6-dichlorocarbazole in the samples of environmental conditions.

Discussion Especially because of the toxicological potential of 3-chlorocarbazole and 3,6-dichlorocarbazole the proven degradative fate is of large interest. The results show that the analysed carbazoles are not readily degradable in this time period.

Conclusions The expected results of exponential decay behaviour could not be proven.

Recommendation and perspectives Longer-lasting studies are expected to reveal more accurate half-lives, although it has been shown here, that the compounds are not readily degradable in their native soil environment.

Abbreviations

A horizon	Soil horizon; top soil
ASE	Accelerated solvent extraction
Au	Energy in atomic units; 1au=627.51 kcal/mol
B horizon	Soil horizon, first horizon after top soil

CHC	Chlorinated hydrocarbons
CIS	Cold injection system
DFT	(Standard) density functional theory
E	Enthalpy [au]
E_{rel}	Relative energy [kcal/mol]
EI	Electron ionisation
EROD	7-Ethoxyresorufin-O-deethylase
FW	Fresh weight
GC/MS	Gas chromatography and mass spectrometry
Go-Ah	24–39 cm Transfer horizon: mineral top soil horizon with accumulation of organic substances and groundwater-swayed mineral soil horizon, oxidative circumstances
OCP	Organochlorinated pesticides
PAH	Polycyclic aromatic hydrocarbons
PCDD/F	Polychlorinated dibenzo-p-dioxin and dibenzofuran
PCT	Pentachlorotoluene
PSE	Potential energy surface
PTFE	Polytetrafluoroethylene
SIM	Selective ion monitoring
SPE	Solid phase extraction
STD	Standard deviation
TCDD	2,3,7,8-Tetrachlorodibenzodioxine
TE	Toxicity equivalent

1 Background, aim, and scope

Carbazole and lower chlorinated carbazoles have been identified in various soils and sediments (Reischl et al. 2005). Today, there are about 2,300 chlorinated organic compounds, which have been isolated and identified as natural compounds (Gribble 1998, 2003). Carbazole and chlorinated carbazoles have been isolated recently from sediments and soils. 3-Chlorocarbazole and 3,6-dichlorocarbazole are the mostly detected chlorinated isomers of carbazole. These compounds have been located in several matrices of topsoil horizons of 200 locations of different use (for example in forestry). The highest concentration is not in the organic layer, but in the A horizon and in the upper B horizon (Reischl et al. 2005).

3,6-Dichlorocarbazole has also been detected in one sample of a campaign of sediments of the Lippe River, Germany (Kronimus et al. 2004). Furthermore, 3,6-dichlorocarbazole as well as some other carbazole derivatives was detected in the compartment air of electrolysis halls of an aluminium smelter plant (Reischl et al. 2005). Recently, it succeeded to verify polychlorinated carbazoles in contaminated sites in Tokyo (Japan), Rheinfelden, and Lampertheim (Germany). Polychlorinated carbazoles were detected in part per billion level (Takasuga et al. 2009).

Currently, there exist only scarce information about the analysis and the environmental fate of these compounds.

3,6-Dichlorocarbazole is a white, solid substance with a melting point of 201°C (Ohashi et al. 1970a, b). Reischl et al. (2005) reported that by calculations, based on valuations with the help of EPI Suite v 3.10 of the US-EPA, a water solubility of about 0.3 mg/L (25°C) and vapour pressure of 1.1×10^{-3} Pa (25°C) was estimated. These properties suggest that the compound is formed in situ.

The UV-spectrum of 3-chlorocarbazole exhibits an absorption maxima of $\lambda_{max,1}=235$ nm (in 62% H_2SO_4+20 vol% EtOH) and $\lambda_{max,2}=259$ nm (in 96% H_2SO_4). The obtained absorption maxima for 3,6-dichlorocarbazole are $\lambda_{max,1}=239$ nm (in 62% H_2SO_4+20 vol% EtOH) and $\lambda_{max2}=258$ nm (in 90% H_2SO_4; Chen et al. 1971).

It has been suggested that 3-chlorocarbazole and 3,6-dichlorocarbazole originates in sediments and soils locally by natural processes and not by human activity or atmospheric deposition. In addition, the natural formation of 3-chlorocarbazole has been proven in animal carcass and for similar halogenated carbazole derivatives in algae (Reischl et al. 2005).

It has been shown, that if 5-chloro-1-(4-chlorophenyl) benzotriazole was photolysed and a 1,3-diradical is the intermediate and being 3,6-dichlorocarbazole the only product. Thereby, 5-chloro-1-(4-chlorophenyl)benzotriazole irradiated in acetonitrile with a low-pressure mercury lamp for 14 h gave 3,6-dichlorocarbazole (Ohashi et al. 1970a, b; Tsujimoto et al. 1972). The formation of carbazole from 1-phenylbenzotriazole by photolytic and thermolytic fragmentations is well documented (Ohashi et al. 1970a, b).

Next to the development by photolysis, 3-chlorocarbazole and 3,6-dichlorocarbazole are composed by chlorination of carbazole. Usually, carbazole and 9-methylcarbazole are chlorinated with sulfuryl chloride, N-chlorosuccinimide, or gaseous chlorine (Moskalev et al. 1985; Bonesi and Erra-Balsells 1997). Thereby mixtures of mono- and polychloro-derivatives are formed. With the use of phosphorus pentachloride in 1,2-dichloroethane as chlorinating agent carbazole and some of its derivatives leads predominantly to 3-chlorocarbazole and 3,6-dichlorocarbazole (Moskalev et al. 1985).

There is limited information about reactions of 3-chlorocarbazole and 3,6-dichlorocarbazole. 3-X-6-Y-carbazoles (X,Y=halogens) react in the presence of basic catalysts with vinyl esters simultaneously to 3-X-6-Y-N-(a-acyloxyethyl)carbazoles and the corresponding N-acylcarbazoles (Shekhirev et al. 1983). Furthermore, 3,6-dichlorocarbazole applied to Friedel–Crafts reaction conditions gave only N-acyl derivatives as product (Plant and Powell 1947). Generally, there is a high reactivity of aromatic rings of carbazole derivatives in electrophilic substitution (Bowyer et al. 1971).

There are no reports found about commercial or industrial use of these compounds in the literature.

In this study, the degradative fate of 3,6-dichlorocarbazole and 3-chlorocarbazole in Bavarian soil of a defined location was analysed and discussed. It was considered to analyse the soil in a long-term study with two different controls. It is suggested to obtain more information about the natural degradative properties of carbazoles and to make statements about their degradative kinetic fate.

Furthermore, some properties of 3,6-dichlorocarbazole were investigated. This includes the toxic potential of the compounds and their origin.

To get more information about the chemical and toxicological properties of 3,6-dichlorocarbazole, a Micro-EROD bioassay has been accomplished (Schwirzer et al. 1998). Since these compounds show similar structures compared to PCDD/F (dioxin–like compounds) this test seems appropriate.

2 Stereoselectivity in chlorination of carbazole, formation pathways of isomers of mono- and dichlorocarbazole

It was suggested that 3-chlorocarbazole and 3,6-dichlorocarbazole (Fig. 1) are the most abundant structures of natural occurring chlorinated carbazoles (Reischl et al. 2005).

It has been shown that chlorination of natural aromatic structures is formed mainly by enzymatically controlled oxidation processes of chloride ions yielding electrophilic chlorine as reactive species (Gribble 2004). Electrophilic substitution, such as chlorination of benzene derivatives, shows typical stereoselective behaviour as demonstrated by preferred formation of special positional isomers (Taylor 1990; Ben-Daniel et al. 2003). According to present theory of electrophilic substitution such as halogenation reactions it is not the stability of the formed products which control the formation of the specific isomer, but the stability of the intermediate sigma-complex, which is the most important point of the PSE of this reaction (Smith and March 2006).
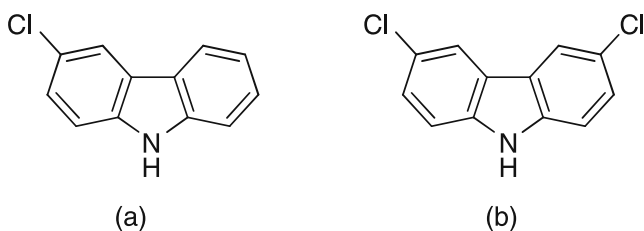
Carbazole is a nearly planar heterocyclic aromatic molecule with C_{2v} symmetry. The numbering of the nucleus is different from that of dibenzofuran (Fig. 2).

3 Materials and methods

3.1 Materials

To simulate environmental conditions, a climate chamber (Phytotron) of Heraeus–Vötsch, Heraeus–Vötsch-test rig for simulation of environmental conditions with Interface IEEE 488/RS 232 Typ VRSU, 20 C, 49% air humidity is used. The homogenization is carried out with a Retsch grindomix GM 200, Haan. For extraction, an Accelerated Solvent Extractor ASE 200 from Dionex, Idstein is used.

For the gas chromatography and mass spectrometry (GC/MS) measurement, a GC/HRMS-system Finnigan MAT 95S (mass spectrometer Finnigan MAT 95S, gas-phase chromatograph Hewlett–Packard Agilent 6890, computer Dell Optiplex GX 270, laser printer Digital DEClaser 3500, cooler Julabo FC-600) is used. The GC/MS conditions are presented in Table 1.

The solvents were obtained from Promochem. Hydromatrix was purchased from Varian.

3,6-Dichlorocarbazole was quantified with reference to aldrin, octachlorostyrene, and mirex. The added 3,6-dichlorocarbazole was purchased from Sigma (Taufkirchen, Germany).

Two standards are added to the samples: OCP standard (internal standard for quantification, mixture of ^{13}C-marked CHC standards) and Pentachlorotoluene (PCT standard (recovery standard)).

The "Bayerisches Landesamt für Umwelt" supplied four different samples representing the horizons of the soil from Lower Bavaria (Tröbs 2009). The horizon Go-Ah, which is at the geothermal gradient of 24–39 cm, contains the highest concentration of 3-chlorocarbazole and 3,6-dichlorocarbazole, and is therefore used in this study. It is a strongly humic, dark brown, clay soil.

3.2 Sample preparation

The already homogenized soil is prepared and stored in three different ways. The first part of the soil is stored at

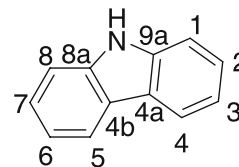Fig. 1 a 3-Chlorocarbazole and b 3,6-dichlorocarbazole

Fig. 2 Carbazole with numbering (Bonesi et al. 2005)

Table 1 GC/MS parameter for the isomer specific detection of organochlorine pesticides and chlorocarbazoles

GC	Type	Agilent 6890
	Column	Rtx-Dioxin2, 40 m, 0.18 mm ID, 0.18-μm film thickness (Restek)
	Temperature program	60°C, 1.5 min, 25°C min^{-1}, 140°C, 8°C min^{-1}, 300°C, 20 min
	Carrier gas	helium, constant flow: 1.3 mL/min
	Injector	Cold injection system CIS 4 (Gerstel)
	Temperature program injector	120°C, 12°C s^{-1}, 280°C, 5 min
	Temperature transferline	300°C
	Autosampler	A200S (CTC)
	Injection volume	0.5 μL pulsed spitless
MS	Type	MAT 95 S (Thermo)
	Ionisation mode	EI, 50 eV, 260°C
	Resolution	>9,000
	Detection	SIM mode

20°C without any additions (environmental conditions), the second part is stored at −28°C also without any additions, and the third part is stored at 20°C after addition of 15 g sodium azide per 10 g soil to inhibit microbial activity. The second and third lots are used as controls.

In each case, 10 g soil is weighed in a jar, placed at the adequate temperature and is frozen to −70°C after corresponding sampling times of hours and then days. The entire long-term study encompasses 57 days. Each sampling and preparation was executed in triplicate. Thereby the whole study represents 69 samples. Moreover, three blank values are included, one at the beginning, one in the middle, and one in the end of the study.

The extraction and the clean-up of each sample and blank sample are equal and exemplarily explained as follows.

3.3 ASE-200- extraction

The extraction cell is filled with a cellulose filter, 1 cm silica gel and a second cellulose filter. On the top of this setup, 30 μL OCP standard is spiked. Ten grams of soil is mingled with hydromatrix and funnelled into the extraction cell. The extraction cell is filled up with hydromatrix and is completed with another cellulose filter.

The soil is extracted with method 2 (Table 2). Approximately 40 mL eluent are used for extraction depending on the packing and packing-density of the cell.

The extract is transferred into a round beaker and the extraction vial is rinsed three times with approximately 1 mL solvent (hexane/dichloromethane 1:1).

3.4 Preparation of the sample for measurement

The clean-up with silica gel and Alumina B with 3% water for OCP and PAH analysis and the clean-up with C18 is carried out following the procedure published previously by Wang et al. (2009).

After adding 30 μL PCT standard (in n-nonane) and 20 μL nonane, the sample is reduced with a gentle stream of nitrogen onto 50 μL.

The sample is stored in a freezer (−28°C) until measurement.

3.5 Measurement and reporting

The measurement is carried out with GC/MS. The parameters used for the isomer specific detection of organochlorine pesticides, which includes 3-chlorocarbazole and 3,6-dichlorocarbazole, are given in. The criteria for the identification and quantification of 3-chlorocarbazole and 3,6-dichlorocarbazole are shown in Table 3. The quantification of 3-chlorocarbazole and 3,6-dichlorocarbazole is carried out based on the pseudo-internal standards aldrin, octachlorostyrene, and mirex. The calculation of the GC/MS results is correct, if the ratio-limit for the isotope ratio (two different masses) is located in the predefined range.

3.6 Micro-EROD bioassay

EROD bioassay using rat liver cell line (HII4E) expressing cytochrome P4501A1 upon exposure to AhR agonists was

Table 2 ASE 200 – extraction – method 2

Eluent n-hexane/acetone	75:25
Extraction temperature	120°C
Extraction pressure	120 bar
Heating-up period	5 min
Pre-heating-up period	0 min
Static extraction period	10 min
Static cycles	2
Rinsing (eluent)	70%
Rinsing (nitrogen)	300 s

Table 3 Criteria for the identification and quantification of 3-chlorocarbazole and 3,6-dichlorocarbazole

Compound	Retention time [min]	Quantifying mass [g/mol]
Aldrin [13]C12 STD	17.71	269.8305–269.9305
Aldrin (native)	17.50	262.8070–262.9070
3-Chlorocarbazole	19.85	200.9845–201.0845
3,6-Dichlorocarbazole	22.91	234.9496–235.0456

performed according to Donato et al. (1993), with modifications such as described in Schwirzer et al. (1998). The EROD induction caused by 3,6-dichlorocarbazole after 24 h (all compunds able to elicit a response) and 72 h of incubation (persistent compounds) were compared to the dose–response curve obtained with standards of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Results are given as picogram TCDD toxicity equivalent values (TE values) per milligram carbazole.

3.7 DFT calculations of the stability of sigma-complexes of mono- and dichlorocarbazoles

We have calculated the eight sigma-complexes by standard density functional theory (DFT) methods using a B3LYP 6–31G/d as basis set (Foresman and Frisch 1996; Frisch et al. 2004). The relevant results are summarized in Table 9 und Table 10.

4 Results

4.1 Degradation experiment

A comparison of the 24 given standards of the OCP standards showed, that aldrin with recoveries of approximately 30–120% is the best pseudo-internal standard to calculate the concentrations of 3-chlorocarbazole and 3,6-dichlorocarbazole by isotope-dilution method. Also, octachlorostyrene and mirex showed acceptable properties as pseudo-internal standards. With the samples obtained, two different measurement campaigns were carried out.

Several measurements of the pseudo-internal standards and averaging of the obtained response factors and ^{13}C-concentration resulted in the data in Table 4. The results

differ for the two measurement campaigns due to the status of the instrumentation.

The amount of 3,6-dichlorocarbazole is approximately 20 times higher than the concentration of 3-chlorocarbazole in the soil. The concentration of 3,6-dichlorocarbazole is approximately 800 ng/g_{FW} and the concentration of 3-chlorocarbazole is approximately 35 ng/g_{FW}.

Regressions of $\ln(c/c_0)$ versus the time with error bars and best-fit line were carried out for the two campaigns and the data originated by using different pseudo-internal standards. The obtained slopes, axis intercepts, and coefficients of determination are summarized in Table 5 and Table 6.

The coefficients of determination show, that most of the points do not contact the best-fit lines. Especially the hit rate at 20°C, with coefficients of determination of 0.2103 (3-chlorocarbazole) and 0.1181 (3,6-dichlorocarbazole), is low. The R^2 values of samples treated with sodium azide at 0.7644 (3-chlorocarbazole) and 0.8035(3,6-dichlorocarbazole) are highest.

The slopes of almost all best-fit lines are negative or close to zero.

Results with aldrin as pseudo-internal standard in the first measurement campaign show almost identical slopes and axis intercepts at 20°C in comparison to the slopes and the axis intercepts of the controls (−28°C, with sodium azide).

4.2 Validation of the analytical methods

Validation of the test method was restricted by the fact that no ^{13}C-labeled 3-chlorocarbazole and ^{13}C-labelled 3,6-dichlorocarbazole was available for full execution of isotope-dilution method. Furthermore, there is no certified soil reference material.

Table 4 Response factors and ^{13}C-concentration of pseudo-internal standards

		Response factor	^{13}C-concentration [pg/µL]
Campaign 1	Aldrin	1.72	796
	Octachlorostyrene	2.56	396
	Mirex	1.09	460
Campaign 2	Aldrin	1.89	773
	Octachlorostyrene	1.89	343
	Mirex	0.97	453

Table 5 Results of 3-chlorocarbazole

		Storage condition	Slope (a) [h^{-1}]	Axis intercept (b)	Coefficient of determination R^2
Measurement 1	Aldrin	20°C	−0.0001	−0.0523	0.2103
		−28°C	−0.0003	0.2132	0.4589
		NaN$_3$	−0.0006	0.2917	0.7644
	Octachlorostyrene	20°C	−0.0004	−0.0560	0.5002
		−28°C	−0.0006	0.2061	0.6530
		NaN$_3$	−0.0010	0.4758	0.7807
	Mirex	20°C	−0.0003	−0.0897	0.3533
		−28°C	−0.0005	0.1675	0.6112
		NaN$_3$	−0.0009	0.4026	0.7829
Measurement 2	Aldrin	20°C	0.0001	0.2397	0.1536
		−28°C	0.0001	−0.0527	0.1559
		NaN$_3$	−0.0001	0.0837	0.2044
	Octachlorostyrene	20°C	0.0002	0.4671	0.1520
		−28°C	0.0003	−0.0826	0.1766
		NaN$_3$	5×10^{-5}	0.1216	0.0096
	Mirex	20°C	0.0002	0.3586	0.1753
		−28°C	0.0002	−0.0297	0.1973
		NaN$_3$	4×10^{-7}	0.0673	9×10^{-7}

4.3 Isotope-dilution method

Ten grams soil was extracted by ASE without OCP standard. Four equal parts (3 mL, equates 1.5 g) of the extract were applied to the clean-up procedure (each with 30 µL OCP standard). Further purifications and the measurements were carried out as described in Section 3.

The results of the measurements are summarized in Table 7. There are no large variations in the concentrations of 3-chlorocarbazole and 3,6-dichlorocarbazole

Table 6 Results of 3,6-dichlorocarbazole

		Storage condition	Slope (a) [h^{-1}]	Axis intercept (b)	Coefficient of determination R^2
Measurement 1	Aldrin	20°C	−0.0001	−0.0779	0.1181
		−28°C	−0.0002	0.2013	0.3698
		NaN$_3$	−0.0006	0.2642	0.8035
	Octachlorostyrene	20°C	−0.0004	−0.0858	0.4000
		−28°C	−0.0006	0.1952	0.6175
		NaN$_3$	−0.0010	0.4556	0.8010
	Mirex	20°C	−0.0002	−0.1192	0.2541
		−28°C	−0.0005	0.1572	0.5747
		NaN$_3$	−0.0009	0.3834	0.8044
Measurement 2	Aldrin	20°C	3×10^{-5}	0.2370	0.0079
		−28°C	0.0002	0.0043	0.1965
		NaN$_3$	−0.0002	0.1454	0.3253
	Octachlorostyrene	20°C	0.0001	0.4621	0.0495
		−28°C	0.0003	−0.0287	0.2043
		NaN$_3$	-2×10^{-5}	0.1798	0.0024
	Mirex	20°C	9×10^{-5}	0.3543	0.0472
		−28°C	0.0003	0.0165	0.2209
		NaN$_3$	-7×10^{-5}	0.1255	0.0328

Table 7 Concentration of 3-chlorocarbazole and 3,6-dichlorocarbazole in a sample which has been divided into four parts after extraction

	3-Chlorocarbazole [ng/g_{FW}]	3,6-Dichlorocarbazole [ng/g_{FW}]
1	7.8	146.2
2	5.7	101.2
3	8.3	149.0
4	6.8	119.4
STD	1.2	24.2

in the four parts of one sample. Also the standard deviation is with 1.2 ng/g_{FW} for 3-chlorocarbazole and 24.2 ng/g_{FW} for 3,6-dichlorocarbazole in an acceptable range.

4.4 Check of linearity of application of mass percentages to height percentages of aldrin

A dilution series of aldrin has been prepared, each dilution was measured with GC/MS and the mass-ratios of ^{12}C to ^{13}C are calculated and all points are located close to the linear best-fit line, which crosses the point of origin. The coefficient of determination is 0.9992. Linearity check was positive. All obtained and calculated results are published by Tröbs (2009).

5 Discussion of the degradation experiment

It has been expected, that 3-chlorocarbazole and 3,6-dichlorocarbazole exhibit an exponential degradative fate, when they are stored at environmental conditions. The controls at −28°C and with sodium azide were expected to show no or a less degradative fate of 3-chlorocarbazole and 3,6-dichlorocarbazole. These expectations could be proven within this study because only minor degradation could be observed and thus the correlation coefficients are very weak, indicating no significant decay during the test period of nearly two months.

In comparison to the samples at 20°C, the kinetic coefficients of the samples stored at −28°C are two and three times higher. The coefficients with sodium azide are up to six times higher in comparison to the samples at 20°C.

Decay kinetics is in the range of slopes of −10^{-4} and −10^{-5} and too small to distinguish, between the samples at 20°C and the controls. No clear decay could be observed at the conditions given during the test period

Due to the persistence and toxicity of the chlorocarbazole, further extended studies are required and degradative pathways under aerobic and anaerobic conditions of 3-chlorocarbazole and 3,6-dichlorocarbazole should be investigated.

6 Micro-EROD bioassay

The results (Table 8) show that a dioxin-like toxicological potential is present in 3,6-dichlorocarbazole.

After 24 h, the toxicological potential equates averaged 535.7 pg TCDD equivalents/mg carbazole. 3,6-Dichlorocarbazole is able to exhibit a response. After 72 h, the mean value was 94.8 pg TCDD equivalents/mg carbazole. The analysed compound is persistent.

7 Results of DFT calculations, stereoselectivity of chlorination of carbazole

There are four isomeric monochlorinated derivatives with which are related to four positively charged sigma complexes leading to each of the final products. They are summarized in Table 9.

There are 16 isomeric dichlorocarbazoles, all these correspond to sigma-complexes. The following approach was selected: (a) we start with the most stable monoisomer and introduce a second chlorine atom, (b) the second chlorine is not introduced into the same ring but into the opposite ring of carbazole nucleus. Sigma complexes with chlorine in the same ring are expected to be destabilized substantially, since the positive charge is strongly destabilized by the strong-I effect of neutral chlorine. Therefore, the four most likely sigma complexes of dichloroisomers are summarized in Table 10.

Relative energies of the sigma complexes (Table 9 and Table 10) are significantly different in either row. For the monochlorinated complexes, the 3-chlorocomplex is the most stable one, for the dichlorinated complexes the 3,6-dichloro-complex is the most stable one out of the set of four isomers.

Table 8 Results of the Micro-EROD bioassay of 3,6-dichlorocarbazole

	TE-value pg TCDD/mg carbazole	
	24-h incubation	72-h incubation
	533.5	85.7
	553.0	87.1
	533.6	85.0
	543.4	85.3
	518.6	110.1
	531.5	100.4
	536.4	106.0
		98.9
Mean	535.7	94.8
STD	10.6	10.3

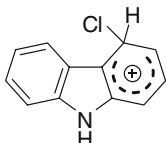**Table 9** Sigma-complexes which are leading to the monochlorinated products of carbazole
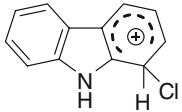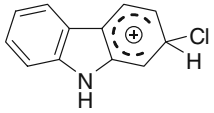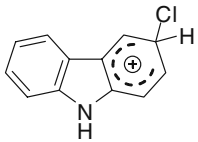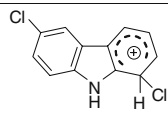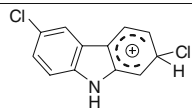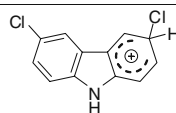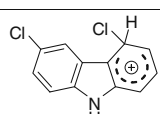
	chlorinated position	E [au]	E_{rel} [kcal/mol]
sigma-1-chlorocarbazole	1	-977.39668	3.13
sigma-2-chlorocarbazole	2	-977.39311	5.40
sigma-3-chlorocarbazole	3	-977.40167	0.00
sigma-4-chlorocarbazole	4	-977.39110	6.63

Table 10 The four most likely sigma complexes of dichloroisomers of carbazole

	E [au]	E_{rel} [kcal/mol]
3-chlorinesigma-1-chlorocarbazole	-1436.98609	3.07
3-chlorinesigma-2-chlorocarbazole	-1436.99097	0.23
3-chlorinesigma-3-chlorocarbazole	-1436.99099	0.00
3-chlorinesigma-4-chlorocarbazole	-1436.97975	7.20

Both structures of isomers have been suggested by Reischl et al. (2005) for the isolated species from soil.

Chlorination of carbazole in the natural environment proceeds likely by a type of electrophilic chlorination that is an enzymatically controlled oxidative chlorination, probably catalysed by oxyhalogenases (Gribble 2004).

8 Conclusions

Expected results of exponential degradative fate of 3-chlorocarbazole and 3,6-dichlorocarbazole could not be proven within this study. Especially because of the large number of contradictions of the results and the involved interesting conclusions, more attention should be paid due to the persistence and dioxin-like toxicity of these compounds.

References

Ben-Daniel R, De Visser SP, Shaig S, Neumann R (2003) J Am Chem Soc 125:12116–12117

Bonesi SM, Erra-Balsells R (1997) J Heterocycl Chem 34:877–889

Bonesi SM, Ponce MA, Erra-Balsells R (2005) J Heterocycl Chem 42:867–875

Bowyer PM, Iles DH, Ledwith A (1971) J Chem So C Org 2775

Chen HJ, Hakka LE, Hinman RL, Kresge AJ, Whipple EB (1971) J Am Chem Soc 93:5102–5107

Donato MT, Gomez-Lechon MJ, Castell JV (1993) Anal Biochem 213:29–33

Foresman JB, Frisch A (1996) Exploring chemistry with electronic structure methods second edition. Gaussian Inc, Pittsburgh

Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Montgomery JA, Vreven T, Kudin KN, Burant JC, Millam JM, Iyengar SS, Tomasi J, Barone V, Mennucci B, Cossi M, Scalmani G, Rega N, Petersson GA, Nakatsuji H, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajiama T, Hona Y, Kitao K, Nakai H, Klene M, Li X, Knox JE, Hratchian HP, Cross JB, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pompelli C, Ochterski JW, Ayala PY, Morokuma K, Voth GA, Salvador P, Dannenberg JJ, Zakrzewski VG, Dapprich S, Daniels AD, Strain MC, Farkas O, Malick DK, Rabuck AD, Raghavachari K, Foresman J, Ortiz JV, Cui Q, Baboul AG, Clifford S, Cioslowski J, Stefanov BB, Liu G, Liashenko A, Piskorz P, Komaromi I, Martin RL, Fox DJ, Keith T, Al-Laham MA, Peng CY, Nanayakkara A, Challacombe M, Gill PMW, Johnson B, Chen W, Wong MW, Gonzales C, Pople JA (2004) Gaussian 03 (Revision C.02). Gaussian, Inc, Wallingford

Gribble GW (1998) Acc Chem Res 31:141–152

Gribble GW (2003) Chemosphere 52:289–297

Gribble W (2004) 'Natural organohalogens' science dossier euro chlor. Dartmouth College, Hanover, New Hampshire

Kronimus A, Schwarzbauer J, Dsikowitzky L, Heim S, Littke R (2004) Water Res 38:3473–3484

Moskalev NV, Sirotkina EE, Ogorodnikov VD (1985) Chem Heterocycl Compd 21(5):542–543

Ohashi M, Tsujimoto K, Yonezawa T (1970a) J Chem Soc. D Chem Commun 1089-1099

Ohashi M, Tsujimoto K, Yoshino A, Yonezawa T (1970b) Org Mass Spectrom 4:203–210

Plant SGP, Powell J F (1947) J Chem Soc 937–939

Reischl A, Joneck M, Dumler-Gradl R (2005) UWSF-Z- Umweltchem Ökotox 17:197–200

Schwirzer SMG, Hofmaier AM, Kettrup A, Nerdinger PE, Schramm K-W, Thoma H, Wegenke M, Wiebel FJ (1998) Ecotoxicol Environ Saf 41:77–82

Shekhirev YP, Lopatinskii VP, Sutyagin VM, Tuzovskaya SA (1983) Chem Heterocycl Compd 19(11):1192–1197

Smith MB, March J (2006) Advanced organic chemistry, reactions, mechanisms, and structure, 6th edn. Wiley, New York

Takasuga T, Takemori H, Yamamoto T, Higashino K, Sasaki Y, Weber R (2009) Organohalogen Compd 71:2239

Taylor R (1990) Electrophilic aromatic substitution. Wiley, New York

Tröbs L (2009) 'The degradative fate of 3,6-dichlorocarbazole in soils', Bachelor Thesis, Institut für Ökologische Chemie des Helmholtz Zentrum München, Lehrstuhl für Analytische Chemie der Technischen Universität München – Department Chemie – Institut für Wasserchemie und Chemische Balneologie

Tsujimoto K, Ohashi M, Yonezawa T (1972) Bull Chem Soc Jpn 45:515–519

Wang J, Bi Y, Pfister G, Henkelmann B, Zhu K, Schramm K-W (2009) Chemosphere 75:1119–1127