Prediction of gas chromatographic retention indices of polychlorinated dibenzothiophenes on non-polar columns

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Abstract

Polychlorodibenzothiophenes (PCDTs) have been found in several kinds of environmental samples. The lack of reference compounds has meant that very little is known about their gas chromatographic behavior. Here we discuss the retention of 19 authentic PCDTs and their sulfones on the widely used gas chromatographic stationary phases DB-5 and DB-5ms. The retention order is different from that of the polychlorodibenzofurans. The data generated allowed us to carry out a multiple linear regression to generate parameters for predicting the retention indices of unknown congeners based only on their structural features.   © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Polychlorodibenzothiophenes (PCDTs), the sulfur analogues of polychlorodibenzofurans (PCDFs) (Fig. 1), were first reported to occur in environmental samples in 1988 [1]. An overview of these and subsequent samples and their origins is given in Ref. [2]. For several years the main technique for the identification of PCDTs was high-resolution gas chromatography (GC) coupled with high-resolution mass spectrometry (MS) because of the similar masses of the PCDTs and the polychlorinated dibenzodioxins (PCDDs). A resolution of ca. 20 000 is needed for the selective detection of these two substance classes [3]. Recently we published a

method that makes it possible to use low-resolution MS for the analysis of PCDFs, PCDDs and PCDTs in a sample. It relies on the oxidation of the PCDTs to the corresponding sulfones which can be separated by chromatography on activated alumina from the (unchanged) PCDDs and PCDFs [4]. The same workup procedures used to isolate PCDDs and
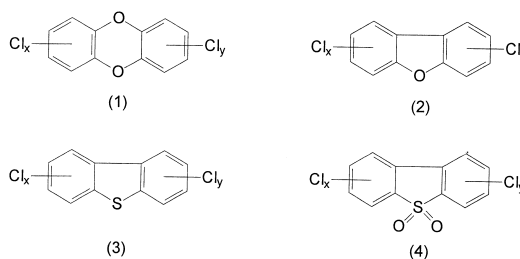

Fig. 1. Structures of polychlorinated dibenzodioxins (1), dibenzofurans (2), dibenzothiophenes (3) and dibenzothiophene sulfones (4).

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PCDFs from real-world samples are applicable to the sulfur heterocycles which will end up in the same fraction as their oxygen counterparts due to their very similar physico-chemical properties.

It is well known that PCDD and PCDF congeners substituted in the 2,3,7,8 positions exhibit a particularly high toxicity. It is not unreasonable to assume that some level of toxicity may be associated with the sulfur analogues, although 2,3,7,8-tetra-chlorodibenzothiophene (2,3,7,8-Cl$_4$-DBT) exhibited a lower enzyme-inducing effect in mouse hepatoma cell cultures than 2,3,7,8-tetrachlorodibenzodioxin [5]. In view of the several reports of PCDTs in environmental samples, an analytical method that allows for their identification and quantification should be of interest for this reason alone. Other congeners than the 2,3,7,8-substituted ones may be of interest to characterize the sources of these polychloro compounds as is the case for the oxygen analogues. This requires that individual components in complex mixtures can be identified. 2,3,7,8-Cl$_4$-DBT for instance was found in fly ash samples from municipal solid waste incinerators [6] and bleached pulp mill effluents [7]. In the latter sample, 3,4,6,7-tetrachlorodibenzothiophene was likewise identified. However, unlike the PCDDs and PCDFs, very few congeners of the sulfur heterocycles have been characterized so far.

One reason for the poor knowledge of PCDTs in different matrices is the lack of reference compounds. Published attempts to obtain reference compounds involve the synthesis of mixtures of PCDTs with subsequent liquid chromatographic separation into individual compounds or simple mixtures. A review of the literature on the synthesis of such reference mixtures is given in Ref. [8]. Recently we published a simple three-step synthesis based on the photocyclization of an appropriately substituted poly-chlorodiphenylsulfide to give PCDTs with a known substitution pattern [8]. Twelve PCDT congeners with between one and three chlorine substituents were obtained and characterized.

In the present work we have used these and some more recently synthesized substances and their corresponding sulfones to collect gas chromatographic data necessary for the analysis of real samples. To limit the synthetic work and the dealing with potentially toxic compounds, a major objective was to investigate whether it is possible to predict the gas chromatographic behavior of higher-chlorinated homologues based on that of the lower-chlorinated ones. If this is possible, there would be a tremendous saving in the synthetic work needed to prepare all 135 congeners possible. This approach has been taken for many other classes of compounds, including PCDFs [9].

A second path to the peak assignment in GC without recourse to the pure compounds would be to correlate the gas chromatographic properties of the PCDTs with those of the PCDFs, i.e., to ascertain whether the retention order in dependence on the substitution pattern is the same within the two classes of compounds. The retention indices for the PCDFs have been published [9] so that a comparison should be simple to perform. This should be a reasonable assumption since the only difference between the two classes of compounds is the identity of the hetero atom, oxygen or sulfur, which in a first approximation can be assumed to lead to a somewhat longer retention time for the sulfur heterocycles, due to their lower vapor pressure, but should have little influence on the relative elution order of the chlorinated derivatives. A third approach to the prediction of retention indices might be to compare the retention order of the methyldibenzothiophenes and the PCDTs, as well as those of their sulfones, since a chlorine atom and a methyl group have about the same steric demand, although they show opposite electronic properties. This comparison is also carried out in this work.

2. Experimental

2.1. Materials and reference compounds

Most chemicals used for synthesis were from Aldrich (Steinheim, Germany) and Lancaster (Mühlheim, Germany). Solvents were from Fluka (Buchs, Switzerland). The chromatographic alumina (neutral) was from Merck (Darmstadt, Germany). The gas chromatographic columns DB-5ms and DB-5 were from J&W Scientific (Folsom, CA, USA). The PCDT mixture (catalog number ''PCDBT'') was obtained from Astec (Münster, Germany). It was

synthesized by direct chlorination of dibenzothiophene. For details, see Ref. [4].

2.2. Instrumentation

The high-resolution GC–MS instrument was a Finnigan MAT/GCQ system with a split–splitless inlet (275°C). The column was a DB-5ms 30 m× 0.25 mm I.D. coated with a 0.25 μm film. Helium (purity 4.6) was used as carrier gas at a constant velocity of 40 cm s^{-1}. The oven temperature was held at 80°C for 2 min and then programmed at 8°C min^{-1} to 320°C and held for 5 min. This temperature program was used for all determinations of retention indices of PCDTs with aromatic retention markers. The investigations with the column DB-5 25 m× 0.25 mm I.D. coated with a 0.25 μm film were done with a Hewlett-Packard 5890 II chromatograph equipped with a split–splitless inlet (270°C) and a flame ionization detection (FID) system (300°C). Hydrogen (purity 4.6) at an average linear velocity of 53 cm s^{-1} was used as carrier gas. The oven temperature was held at 80°C for 2 min and then programmed at 15°C min^{-1} to 295°C and held for 1 min. It was used for the determination of the Kovats retention indices with n-alkanes as markers (according to Ref. [9]). The injection volume was usually 1 μl (splitless mode for 60 s) with toluene as solvent. The retention times were determined at least three times with a deviation from each other of less than 2 s. The retention indices are calculated using the equation for temperature programmed indices [9,10] and are the mean values from three or four injections.

The standard deviations of the I values range from 0 to 0.275 and are independent of the substitution pattern and/or the degree of chlorination.

The multiple linear regressions of Eq. (1) were done with STATISTICA/$_w$ 5.0 on a computer running under Windows 98. Further analyses were done with Microsoft Excel.

2.3. Synthesis of PCDTs, PCDTO$_2$s and MeDBTO$_2$s

The synthesis of most of the polychlorinated dibenzothiophenes is described elsewhere [8]. The syntheses of a mixture of 1,4- and 3,6-dichlorodibenzothiophene by photochemical reaction of 2,2′,5-trichlorodiphenylsulfide and of a mixture of 1,6- and 3,6-dichlorodibenzothiophene by photochemical reaction of 2,3′,6-trichlorodiphenylsulfide has been done in the same manner.

2,3′,6-Trichlorodiphenylsulfide: ^{1}H-NMR (C^{2}HCl$_3$): δ=7.45 (m, 2H, H-3 and H-5); 7.27 (m, 1H, H-4); 7.13 (m), 7.07 (m), 6.98 (m) (4H, H-2′, H-4′, H-5′ and H-6′) ppm.

2,2′,5-Trichlorodiphenylsulfide: ^{1}H-NMR (C^{2}HCl$_3$): δ=7.51 (m, 1H, H-3′); 7.36 (d, J=8.58 Hz, 1H, H-3), 7.31 (m, 3H, H-4′, H-5′, H-6′); 7.14 (dd, J=8.58 Hz and 2.4 Hz, 1H, H-4); 6.92 (d, J=2.37 Hz, 1H, H-6) ppm.

The mass spectra of the two diphenylsulfides were similar to the spectra described in the literature [11] and are not shown here.

The assignment of the different isomers of the PCDTs was possible since both mixtures contained only two components, one of which was common to both mixtures.

A mixture of 2,3,6,8-tetrachloro- and 1,2,4,7,8-pentachlorodibenzothiophene was obtained by photochemical cyclization of 2,2′,4,4′,5,5′-hexachlorodiphenylsulfide. The tetrachlorodibenzothiophene was formed by loss of one chlorine atom in the 1-position of the pentachlorodibenzothiophene during the reaction. The photochemical lability of the 1-position is well known for the PCDFs [12] and polychloronaphthalenes [13].

Because of the different amounts of the two isomers it was possible to assign the ^{1}H-NMR signals of the mixture to the different isomers.

2,3,6,8-Tetrachlorodibenzothiophene: ^{1}H-NMR (C^{2}HCl$_3$): δ=8.16 (s, 1H, 1-H); 7.97 (s, 1H, 4-H); 7.97 (d, J=1.89 Hz, 1H, 9-H); 7.51 (d, J=1.89 Hz, 1H, 7-H) ppm.

1,2,4,7,8-Pentachlorodibenzothiophene: ^{1}H-NMR (C^{2}HCl$_3$): δ=9.10 (s, 1H, 9-H); 7.98 (s, 1H, 6-H); 7.64 (s, 1H, 3-H) ppm.

The mass spectra are similar to the ones described in the literature [6].

4,6-Dichlorodibenzothiophene was a by-product (ca. 1%) of the photochemical synthesis of 4-chlorodibenzothiophene from 2,2′-dichlorodiphenylsulfide. The mass spectrum of this isomer was similar to the mass spectra of the other dichlorodibenzothiophenes.

Oxidation of the PCDTs and the methyldibenzo-

thiophenes (MeDBT) to the sulfones: a few milligrams of the PCDTs or the MeDBTs dissolved in 2 ml dichloromethane is treated with an excess of *meta*-chloroperbenzoic acid for 3 to 10 h. The solvent is changed to toluene and the reaction mixture is treated with a saturated sodium sulfite solution to remove remaining *meta*-chloroperbenzoic acid. The organic phase is then chromatographed on activated alumina [4]. The volume of the solution is reduced in some cases prior to the gas chromatographic investigations. The PCDT mixture was oxidized using the method described in Ref. [4].

3. Results and discussion

The most useful way to compare retention data in gas chromatography is to use their retention indices. All retention index (*I*) systems are based on certain substances which are used to define the intervals of the *I* scale. These substances should be closely related to the compounds to be investigated so that small changes in conditions have a similar influence on the retention markers and the analytes. The *I* values of the PCDTs on the DB-5ms column used in this work have been determined with dibenzothiophene, benzonaphtho[2,1-*d*]thiophene and benzophenanthro[2,1-*d*]thiophene as markers. This system has been used for methylated polycyclic aromatic sulfur heterocycles [10] so that the comparison of the retention behavior of PCDTs and the similarly substituted methyldibenzothiophenes should be straightforward.

The retention indices of the PCDFs have been published but are based on alkanes as retention markers [9]. In order to compare the retention of PCDTs with that of PCDFs, we had to determine the *I* values of the PCDTs on the DB-5 column using

Table 1
Retention data (*I* values) of PCDTs, PCDTO$_2$s, MeDBTs, MeDBTO$_2$s and PCDFs measured according to Ref. [10]

Substitution	Number according to Ref. [14] (PCDF)	PCDT (DB-5ms)	PCDTO$_2$ (DB-5ms)	MeDBT (DB-5ms) [15]	MeDBTO$_2$ (DB-5ms)	PCDT (DB-5)	PCDF (DB-5) [9]
1-	1	334.09	378.69	322.86	383.92	1989	1739
2-	2	335.12	381.48	319.21	380.42	1989	1749
3-	3	335.19	383.01	319.51	378.23	1990	1749
4-	4	332.70	394.30	316.05	368.52	1979	1760
1,3-	6	360.69	394.53	341.81	400.49	2141	1884
1,4-	7	360.87	409.89	339.51	n.d.[a]	2139	1913
1,6-	8	362.83	412.06	339.74	n.d.	2154	n.d.
2,3-	12	369.20	405.95	344.94	403.87	2192	1939
2,4-	13	359.27	409.35	334.62	387.51	2127	1912
2,6-	14	364.27	415.40	335.47	n.d.	n.d.	1946
2,8-	16	367.76	402.89	338.86	n.d.	n.d.	1935
3,6-	18	364.94	416.65	335.90	n.d.	2163	1944
4,6-	20	362.36	429.04	332.15	n.d.	n.d.	1953
1,3,6-	28	386.70	427.01	n.d.	n.d.	2318	2072
1,3,7-	29	390.08	414.46	360.82	419.02	2341	2057
2,3,8-	42	399.05	425.76	n.d.	n.d.	2401	2132
2,4,6-	43	385.55	442.01	350.18	395.40	2312	2101
2,4,8-	45	388.53	428.46	353.70	407.41	2324	2097
2,3,6,8-	78	419.48	451.01	n.d.	n.d.	n.d.	2297
1,2,4,7,8-	108	446.13	467.66	n.d.	n.d.	n.d.	2469
1,2,3,4,6,7,8-	131	497.27	522.87[b]	n.d.	n.d.	n.d.	2898
1,2,3,4,6,7,9-	132	495.93	515.61[b]	n.d.	n.d.	n.d.	2913
1,2,3,4,6,8,9-	133	495.18	513.80[b]	n.d.	n.d.	n.d.	2922
1,2,3,4,7,8,9-	134	506.18	510.91[b]	n.d.	n.d.	n.d.	2986
Octa-	135	524.17[b]	541.01[b]	n.d.	n.d.	n.d.	3147

[a] n.d.: Not determined.
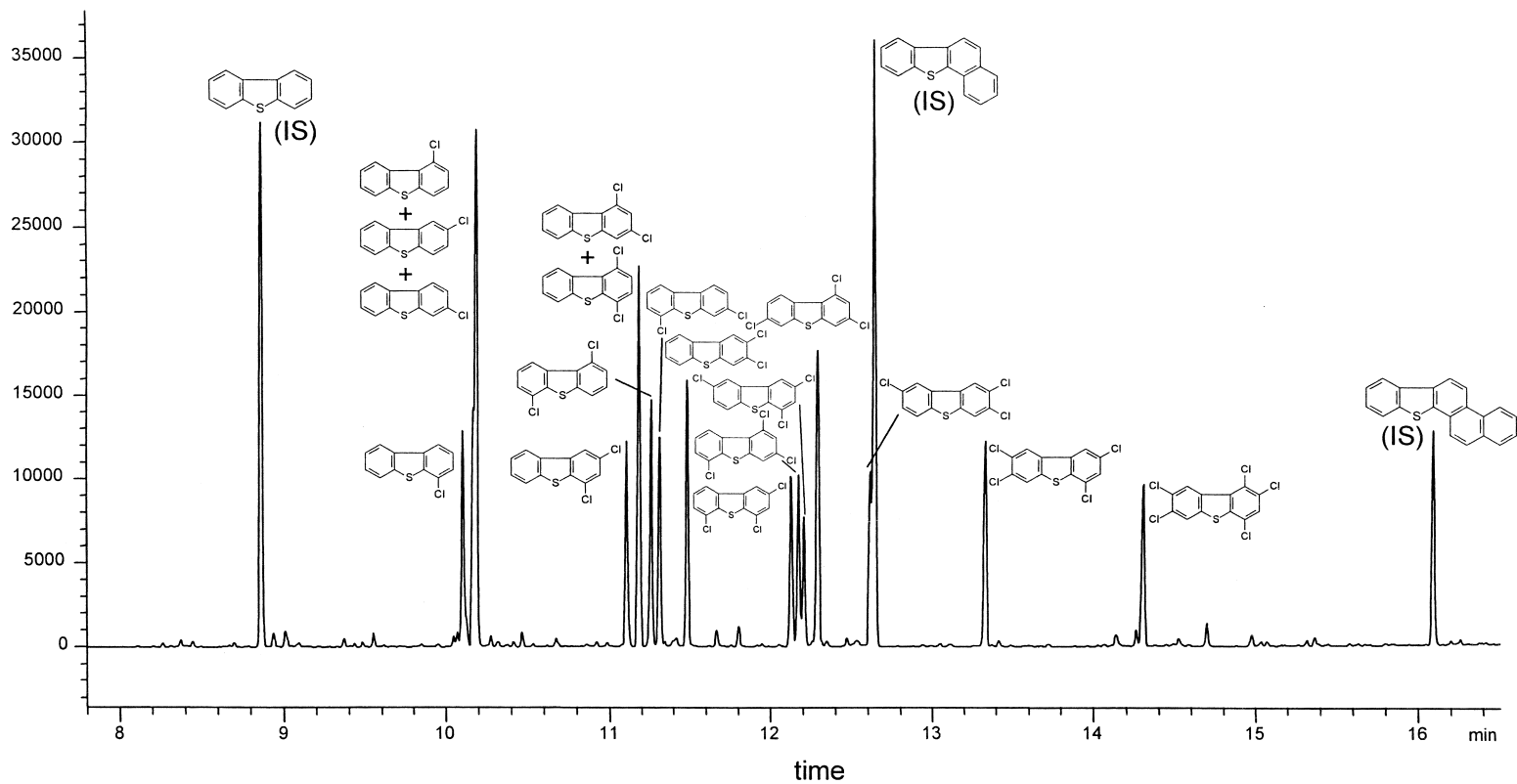[b] Congener elutes in the isothermal range of the chromatogram.

Fig. 2. GC–FID chromatogram of 17 authentic polychlorinated dibenzothiophenes on DB-5. Temperature program: 80°C held for 2 min, then programmed at 15°C min^{-1} to 295°C, held for 1 min (I.S.: internal standard).

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alkane markers. All retention indices used for the discussions in this work are summarized in Table 1.

First the influence of the chlorine substitution pattern on the *I* values of the PCDTs and their sulfones on the DB-5ms column will be discussed. Within a given degree of chlorination, the largest influence on the elution of a PCDT comes from a chlorine atom in the 4-position and leads to a lower *I* in all cases. 4-Cl-DBT elutes before the other monochlorodibenzothiophenes. For Cl_2-DBTs having one chlorine atom in each ring, the retention data show that isomers bearing a chlorine atom in the 4-position elute before the one that does not, namely 2,8-Cl_2-DBT. This is suggestive since a similar tendency toward faster elution when the 4-position is substituted is discernible for higher-chlorinated dibenzothiophenes also. Furthermore, it seems to be a general phenomenon that two chlorine atoms in a 1,3-position (*meta*) to each other lead to a shorter *I* than calculated based on non-interacting substituents. The two Cl_2-DBTs with this substitution pattern (1,3- and 2,4-Cl_2-DBT) elute before the other isomers. Likewise an 1,4-effect (*para*) can be observed and it also shortens the retention time but less so than the *meta*-effect. In comparison to these two attenuating effects, the *ortho*-effect leads to increased *I* values. For instance, 2,3,8-Cl_3-DBT elutes after all other Cl_3-DBTs under investigation here which all lack an *ortho* substitution pattern. Similar intra-ring interactions have been described previously for the polychlorinated dibenzofurans [9] and xylenes [16]. Finally, all PCDTs elute according to the number of chlorine atoms. This is known for the PCDDs and PCDFs on non-polar columns, too [17].

The oxidation of the PCDTs to the corresponding sulfones, an integral part of our analytical method for PCDTs in environmental samples [4], leads to a totally different gas chromatographic elution pattern. Now the 4-position contributes to an increased *I* so that e.g., 4-Cl-DBT sulfone elutes after the other isomers. This fact is particularly obvious for the Cl_3-DBT sulfones where 2,4,6-Cl_3-DBT sulfone elutes after all the other investigated isomers. The *ortho*-, *meta*- and *para*-effects described for the PCDTs can be observed here as well. Furthermore, the elution order does not strictly follow the chlorination degree. Thus 1,3,7-Cl_3-DBT sulfone elutes before the sulfones of 2,6-, 3,6- and 4,6-Cl_2-DBT.

From an analytical point of view the transformation of the PCDTs to their corresponding sulfones in several cases has the advantage of a better resolution. While, for example, three of the monochlorodibenzothiophenes coelute on the DB-5 column, their sulfones are all resolved. The improved resolution of the sulfones is well known for methylbenzothiophene sulfones [10] and -dibenzothiophene sulfones [18].

All the facts discussed for the DB-5ms column are also valid for the DB-5 column with subtle differences only (1,3-dichlorodibenzothiophene elutes after 1,4-dichlorodibenzothiophene on DB-5). In Fig. 2, a chromatogram on DB-5 of most of the PCDTs available to us, including the three compounds necessary for the calculation of the *I* values [10], is shown. Fig. 3 depicts a chromatogram of the corresponding sulfones on the same stationary phase.

3.1. Comparison of retention indices with other substance classes

One way of identifying the PCDTs in environmental samples is the assumption that the GC behavior of the PCDTs is similar to that of the PCDFs. The assignment of peak identities to PCDTs with the help of the data known from the PCDFs was described in some cases, for example for the identification of Cl_4-DBTs from the reaction of hexachlorobiphenyls with sulfur [19]. No experimental basis was given to verify this assumption. With the congeners now in hand, we see that the retention of the monochlorodibenzothiophenes is totally different from that of the monochlorodibenzofuranes on DB-5 (Table 1). The elution order of the PCDTs is 4<1/2<3 and for the PCDFs it is 1<2/3<4. The elution order for the di- and trichlorinated substances differs as well. Furthermore, in Ref. [7] it is shown that three Cl_4-DBTs elute on the non-polar phase HP-5, which is similar to DB-5, in the order 3,4,6,7<1,2,7,8<2,3,7,8 which is different from the elution order of the PCDFs: 1,2,7,8<2,3,7,8<3,4,6,7. Consequently, it does not seem possible to conclude from the retention behavior of the PCDFs to that of the PCDTs. In Fig. 4 this fact is demonstrated graphically. The measured *I* values of the 14 PCDTs of known structure available to us are plotted against the *I* values of the corresponding PCDFs. The scatter within the chlorination degrees immediately shows that the two classes of
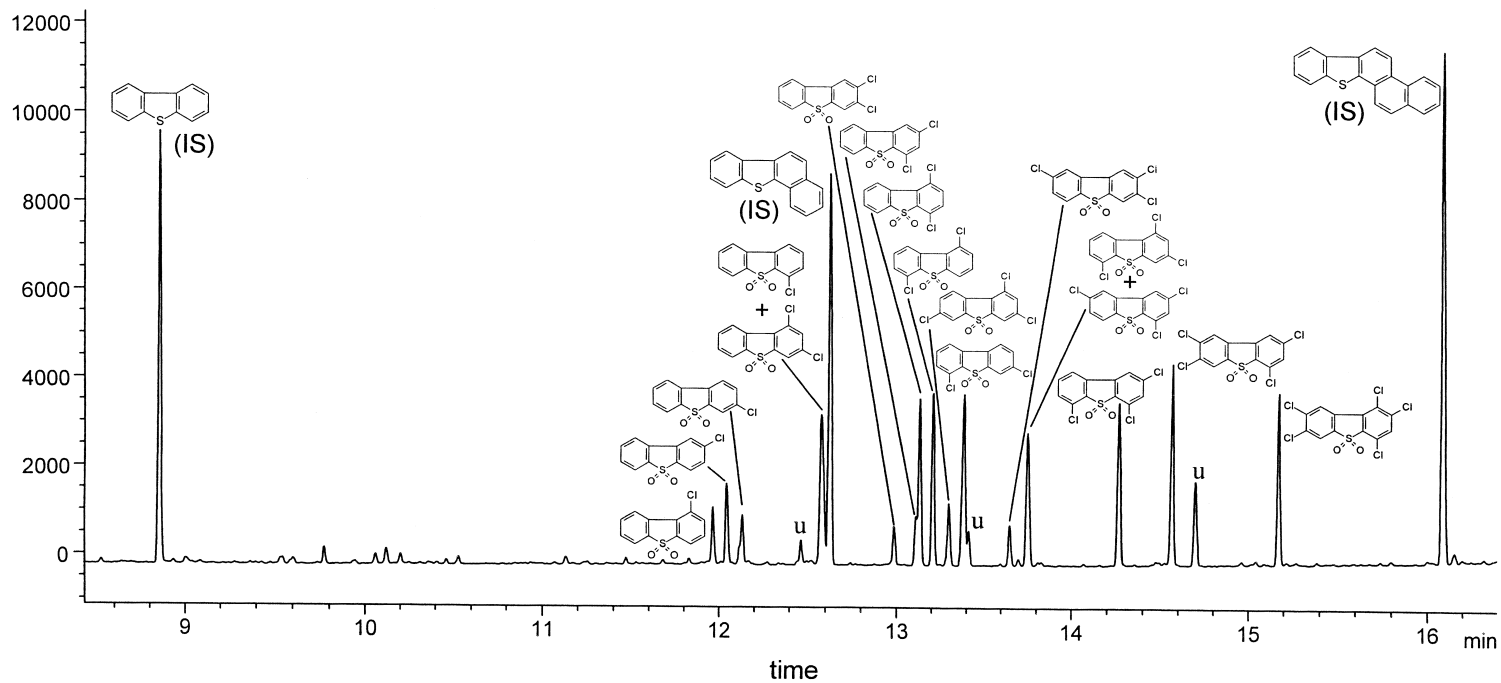
Fig. 3. GC–FID chromatogram of 17 authentic polychlorinated dibenzothiophene sulfones on DB-5. Temperature program: 80°C held for 2 min, then programmed at 15°C min^{-1} to 295°C, held for 1 min; (I.S.: internal standard; u: unknown).
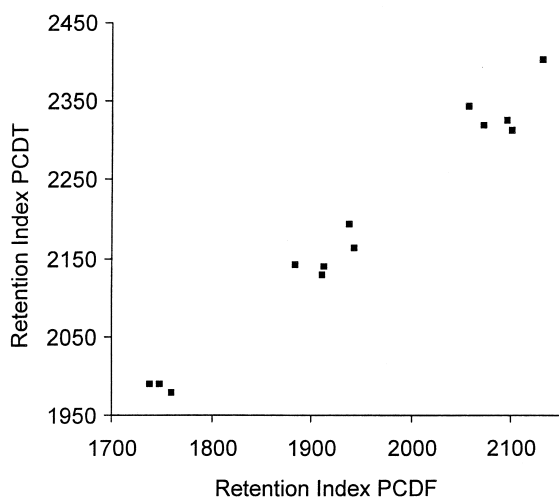
Fig. 4. Retention indices of PCDTs vs. those of the identically substituted PCDFs.

compounds have different GC properties and *that the indices of the thiophenes cannot be predicted from the indices of the furans.*

A comparison was also made between the *I* values of the PCDTs and the polymethyldibenzothiophenes based on the assumption that the similarities of the substituents chlorine and methyl should lead to similar retention orders. That this is not the case is immediately obvious from the data in the third and the fifth column of Table 1.

Is it on the other hand possible to find a direct relationship between the elution of the sulfones of the PCDTs and the sulfones of the MeDBTs? This would be helpful if the method described in Ref. [4] is used for the determination of PCDTs in environmental samples since in the last cleanup step the PCDTs are oxidized to the corresponding $PCDTO_2$s. However, the elution of the $PCDTO_2$s is again completely different from that of the $MeDBTO_2$s as can be seen from the retention indices in Table 1.

As a result of the investigations so far we must conclude that it is *not* possible to estimate the *I* values of the PCDTs and $PCDTO_2$s from the known retention data of the PCDFs or of the MeDBTs and $MeDBTO_2$s. Therefore the next step will be to attempt to predict the *I* values of the PCDTs and $PCDTO_2$s *within* the two substance classes, based on a system established for PCDFs.

3.2. Calculation of I values

The system for prediction of *I* values for PCDFs was developed on the non-polar phase DB-5 [9] and is based on an equation that describes the relationship between retention characteristics and molecular structural features, such as the substitution position of the chlorine atoms and the inter- and intra-ring interactions of the substituents. Intra-ring interactions are the well known *ortho-*, *meta-* and *para-*effects; inter-ring interactions occur when two chlorine atoms are in the 1- and 9-position and/or in the 4- and 6-position. With these parameters it was possible to calculate *I* values for PCDFs in good agreement with the measured *I* values.

The first attempt was aimed at the prediction of retention indices of PCDTs on the non-polar phase DB-5ms. For this purpose we calculated the *I* values with the retention of the dibenzothiophene marker set at 0 and benzophenanthro[2,1-*d*]thiophene at 100. This was done to account for the fact that the two time-windows between the traditionally used markers dibenzothiophene, benzonaphtho[2,1-*d*]thiophene and benzophenanthro[2,1-*d*]thiophene [10] are slightly different; one *I* unit in the first window corresponds to a different number of seconds than one *I* unit in the second window. The consequence is that data calculated from retention characteristics in the first time window cannot be used for compounds eluting in the second time window. The first and the third retention markers mentioned cover nearly the whole elution range of the PCDTs and were used for the indices in Tables 3, 4 and 5. To distinguish between the two retention index systems, the indices based on those two markers are indicated I_{PCDT}. The Cl_1- and Cl_2-DBTs provided parameters for each position in the molecule and for the intra-ring interactions (*ortho-*, *meta-* and *para-*effects). The principle inter-ring interactions of concern should arise when both the 4- and the 6-position are substituted. However, 4,6-Cl_2-DBT and its sulfone showed that such an effect does not exist and that the retention index of these compounds can be calculated from 4-chlorodibenzothiophene. This is in contrast to the PCDFs [9], where the effect has to be taken into consideration. Possibly it is a result of the smaller angle around sulfur in dibenzothiophene (C–S–C angle of 91.5° [20]) than around oxygen in dibenzo-

Table 2
Parameters for the calculation of retention indices (I_{calc}) of PCDTs and PCDTO$_2$s

	PCDT	PCDTO$_2$
a	15.36	8.44
b	16.16	9.96
c	16.17	10.54
d	14.77	16.41
e	0.96	1.26
f	−3.16	−3.21
g	−0.91	−0.86
Intercept	2.73	33.21

furan (C–O–C angle of 104.1° [21]) so that the two substitutents in the sulfur heterocycle show less steric interactions. Due to a lack of PCDTs substituted in both the 1- and the 9-position, we cannot give an estimate for this interaction which might be substantial for the same reason (closer approach in space due to the small angle around sulfur).

First a set of coefficients that describe the different contributions of the molecular features must be calculated. This was done through multiple linear regression using the I_{PCDT} values of 18 mono- to trichlorodibenzothiophenes. The equation used has the form:

$$I_{calc} = an_{1,9} + bn_{2,8} + cn_{3,7} + dn_{4,6} + en_{ortho}$$
$$+ fn_{meta} + gn_{para} + \text{intercept} \qquad (1)$$

The n values are whole numbers describing how many times a particular feature appears in the molecule, e.g., $n_{2,8}$ stands for the number of chlorine atoms in the 2- and 8-positions and n_{ortho} for the number of ortho-interactions present in the molecule. The parameters a–g resulting from the regression are listed in Table 2. The regression coefficient for the 18 PCDTs was $r = 0.9993$ and for the corresponding sulfones $r = 0.9992$. With these calculated parameters we proceed to predicting the retention indices for the higher-chlorinated dibenzothiophenes and their corresponding sulfones.

An example will be given to illustrate the process used for calculating the I_{calc} of a PCDT congener.
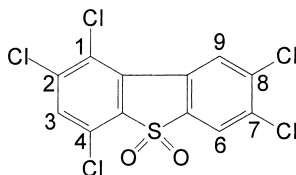
# of chlorine atoms in 1/9-position:	1	multiplied with	8.44	=	8.44
# of chlorine atoms in 2/8-position:	2	multiplied with	9.96	=	19.92
# of chlorine atoms in 3/7-position:	1	multiplied with	10.54	=	10.54
# of chlorine atoms in 4/6-position:	1	multiplied with	16.41	=	16.41
# of ortho-interactions:	2	multiplied with	1.26	=	2.52
# of meta-interactions:	1	multiplied with	- 3.21	=	- 3.21
# of para-interactions:	1	multiplied with	- 0.86	=	- 0.86
intercept (incl. value for "sulfone"):					33.21
				Σ	86.97
measured retention index:					84.64

Fig. 5. Calculation of the I_{calc} of 1,2,4,7,8-Cl$_5$-DBTO$_2$ with the parameters given in Table 2.

The parameters for each chlorine atom in dependence of its position in the molecule are added. Next the number of *ortho-*, *meta-* and *para*-interactions in the molecule is determined and the sum of their products with the appropriate parameters from Table 2 is added to the positional sum. In case of the PCDTs the intercept 2.73 and in case of the sulfones the intercept 33.21 is added. The calculation procedure is carried out in Fig. 5 with 1,2,4,7,8-Cl_5-DBT sulfone as an example. The calculated value 86.97 is in useful agreement with the observed retention index of 84.64; the difference in retention time is approx. 20 s.

The data for the PCDTs and the $PCDTO_2$s of known structure here are given in Table 3, where the measured and the predicted I_{calc} values are presented. The assignment of the Cl_7-DBTs and their sulfones will be discussed in detail elsewhere [22]. The calculated retention data for the 25 mono- to octachloro-$DBTO_2$s show a linear correlation with the measured I_{PCDT} with a correlation coefficient of $r = 0.9992$. The PCDTs also show an excellent linear correlation with a correlation coefficient of $r = 0.9994$. For the Cl_3-DBTs and their sulfones, for example, the difference between the measured and the predicted I_{calc} values is on the average less than 1.1%. This value, however, increases for the higher-chlorinated congeners to between 3.3 and 8.1% for Cl_4- and Cl_7-substituted congeners, respectively. The situation is more favorable for the $PCDTO_2$s where this value is lower than 2.8% for all chlorination degrees. Except for the highly substituted compounds, the agreement between the measured and the predicted retention indices is good enough to be of considerable use for our purposes. To improve the fit for congeners with five or more chlorine atoms, a correction is introduced (see below).

Is it now possible to use Eq. (1) and the parame-

Table 3
Measured (I_{PCDT}) and predicted (I_{calc}) retention indices of PCDTs and $PCDTO_2$s on DB-5ms based on dibenzothiophene and benzophenanthro[2,1-*d*]thiophene as retention markers

PCDT	I_{PCDT}	I_{calc}	$PCDTO_2$	I_{PCDT}	I_{calc}
1-	17.88	18.09	1-	41.23	41.65
2-	18.39	18.89	2-	42.69	43.17
3-	18.46	18.90	3-	43.50	43.75
4-	17.15	17.05	4-	49.41	49.62
1,3-	31.77	31.10	1,3-	49.56	48.56
1,4-	31.95	31.95	1,4-	57.19	57.20
1,6-	32.98	32.86	1,6-	58.22	58.06
2,3-	36.25	36.02	2,3-	55.24	54.97
2,4-	31.09	30.50	2,4-	56.85	56.37
2,6-	32.72	33.66	2,6-	59.70	59.58
2,8-	35.55	35.05	2,8-	53.74	53.13
3,6-	34.09	33.67	3,6-	60.40	60.16
4,6-	32.68	32.27	4,6-	66.23	66.03
1,3,6-	45.36	45.87	1,3,6-	65.28	65.39
1,3,7-	47.19	47.27	1,3,7-	59.27	59.52
2,3,8-	51.95	52.18	2,3,8-	64.66	65.93
2,4,6-	44.82	45.27	2,4,6-	72.41	72.78
2,4,8-	46.44	46.66	2,4,8-	65.97	66.33
2,3,6,8-	61.74	63.79	2,3,6,8-	76.72	78.13
1,2,4,7,8-	74.40	79.20	1,2,4,7,8-	84.64	86.97
1,2,3,4,6,7,8-	98.70	106.70	1,2,3,4,6,7,8-	110.50	111.28
1,2,3,4,6,7,9-	98.07	104.03	1,2,3,4,6,7,9-	108.37	107.64
1,2,3,4,6,8,9-	97.71	104.02	1,2,3,4,6,8,9-	106.56	107.06
1,2,3,4,7,8,9-	102.94	107.29	1,2,3,4,7,8,9-	105.19	103.31
Octa-	111.49	119.98	Octa-	119.50	116.91

ters listed in Table 2 to calculate the retention indices of congeners with more than three chlorine atoms? To answer this question the I_{PCDT} of all synthesized PCDTs and all PCDTs found in a commercially available mixture, a chromatogram of which is shown in Fig. 6 were calculated and plotted against the number of chlorine atoms, see Fig. 7. It is obvious that the relationship between the chlorination degree and the retention index over all chlorination degrees is a non-linear function that has a linear branch for the mono- to tetrachlorodibenzothiophenes. The mean value of the retention indices for each of these four chlorination degrees was calculated and a linear correlation between these mean values (for a total of 31 compounds) and the number of chlorine atoms established. The equation found is $y = 14.792x + 3.2667$ with a regression coefficient of $r = 0.9998$. When all chlorination degrees (except octachlorodibenzothiophene, this congener elutes outside the temperature program) are considered, a cubic equation ($y = -0.387x^2 + 16.742x + 1.3405$, regression coefficient: $r = 0.9998$) describes the relationship between the number of

chlorine atoms in the molecule and the I_{PCDT} much better than a linear correlation. The straight line and the curve are shown in Fig. 7. The deviation of this curve from the straight line becomes more pronounced the larger the number of chlorine atoms in the congeners is. For Cl_5-DBTs ($n = 7$) the difference is 1.52, for the Cl_6-DBTs ($n = 7$) 4.11 and for the Cl_7-DBTs ($n = 4$) 7.46 (n is the number of isomers used for the calculation of the mean retention index). These values can be used to correct the predicted retention index I_{calc} to obtain the adjusted retention index I_{adj}.

For the chlorinated dibenzothiophene sulfones this effect is not observed. There is a linear relationship between the retention index I_{PCDT} and the chlorination degree over all congeners eluting in the temperature programmed area of the chromatogram, obeying the relationship $y = 10.724x + 34.389$ ($r = 0.9986$).

3.3. Identification of unknown PCDT congeners

With the help of the data above, we attempted to identify the congeners present in the commercially
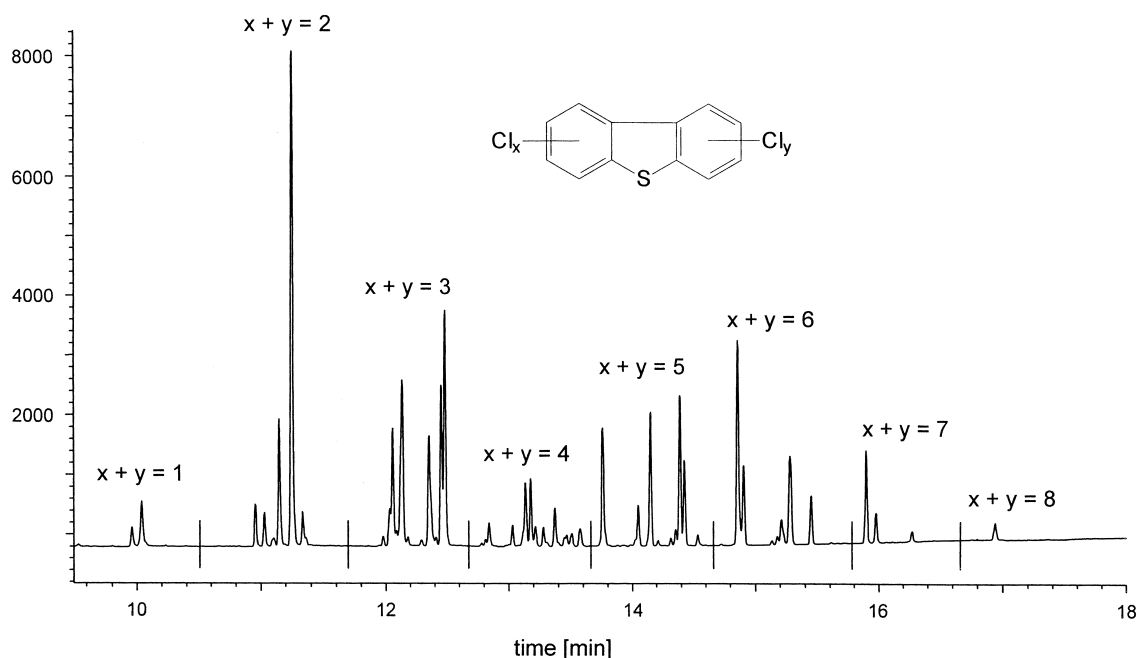

Fig. 6. GC–FID chromatogram of the commercially available PCDT mixture on DB-5. Temperature program: 80°C held for 2 min, then programmed at 15°C min^{-1} to 295°C, held for 1 min.
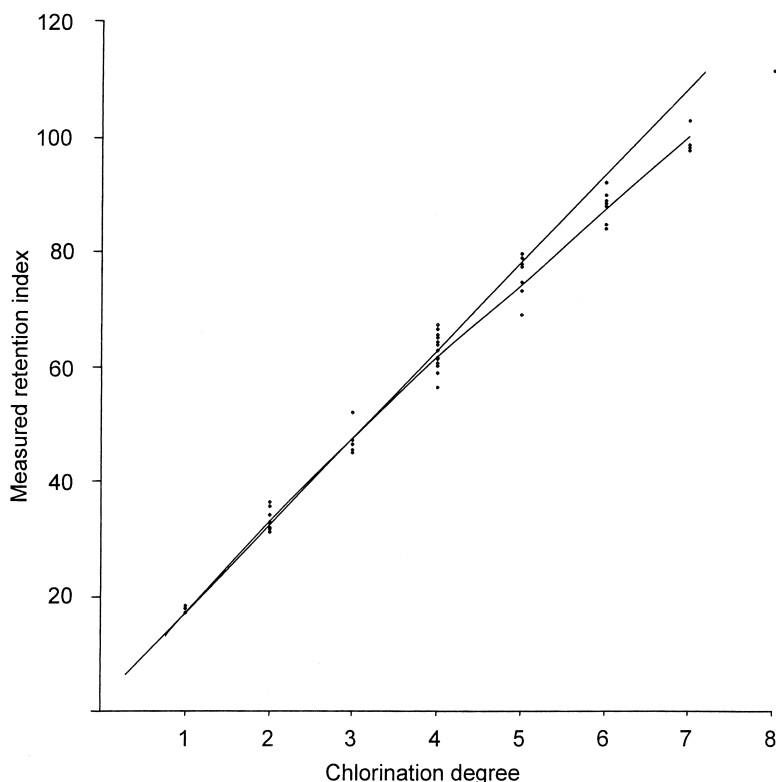
Fig. 7. Measured retention index vs. chlorination degree of all synthesized PCDTs and of the components of the commercially available PCDT mixture. Top line a first-order correlation, bottom line a second-order correlation.

available PCDT mixture (Fig. 6). First the Cl_5-DBTs will be discussed. The single ion chromatograms of them and their sulfones obtained after oxidation (see Experimental) show seven peaks (Fig. 8). In a first step the I_{calc} values of all the Cl_5-DBTs were calculated using the parameters listed above; the values (I_{calc}) are listed in Table 4. One isomer, 1,2,4,7,8-Cl_5-DBT was available from synthesis and showed coelution with one peak both as dibenzothiophene and as sulfone. This isomer is underlined in Table 4. Finally, the I_{calc} values of the pentachlorodibenzothiophenes were adjusted by subtracting the value of 1.52, the average deviation of the mean I from the first-order correlation (see above), to get the I_{adj} values. These data are listed in Table 4. It should be noted that this correction in principle involves no more than the addition of an

extra parameter to Eq. (1) of the form $1.52n_5 + 4.11n_6 + 7.46n_7$ where $n_5 = -1$ for all Cl_5-DBTs and 0 for all other congeners and similarly for n_6 and n_7. Thus this extra term is as much based on a best correlation with the experimentally determined data as are any of the other terms in Eq. (1).

In a second step we assumed that all isomers in the mixture should be substituted in the 2- and the 8-positions, the reason being that the PCDTs were synthesized by direct chlorination of dibenzothiophene and these positions are the most reactive toward nucleophilic substitution. This is corroborated by the finding that among the Cl_3-DBTs, 2,4,8-Cl_3-DBT is one of the most abundant isomers in the PCDT mixture. The 10 isomers fulfilling this criterion are highlighted in bold type in Table 4.

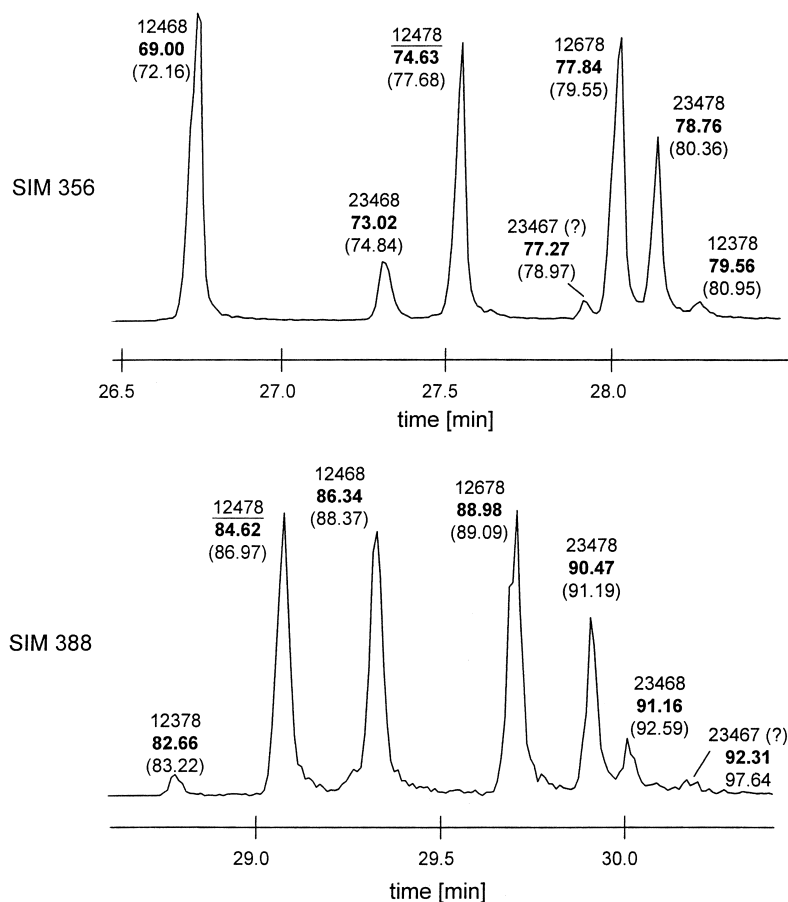In a third step we note that only those isomers can

Fig. 8. Single ion monitoring (SIM) chromatograms of pentachlorodibenzothiophenes (top) and their corresponding sulfones (bottom) on DB-5ms. The peak labels indicate: first line the suggested substitution position, the second line the measured I_{PCDT}, the third line the corrected predicted I_{adj} for the parent compounds and the calculated I_{calc} for the sulfones, respectively. Temperature program: 80°C held for 2 min, then programmed at 8°C min^{-1} to 320°C, held for 5 min.

be present in the mixture that have retention data in agreement with the predicted values for both the parent *and* for the corresponding sulfones. This is true only for the six isomers marked with a star. The difference between the observed and the predicted retention indices (I_{adj} and I_{calc}, respectively) of these isomers ranges from 1.8 to 4.6% for the parent compounds and from 0.1 to 2.8% for their corresponding sulfones. These six isomers also make chemical sense from the point of view of their bearing chlorine atoms in the 2-, 4- and 8-positions. Further support for the identification is rendered by the observation that peaks in the two chromatograms

for each DBT and its sulfone are of similar relative height. The suggested identifications are indicated in Fig. 8.

The seventh Cl_5-DBT present in detectable amounts in this mixture might be 2,3,4,6,7-Cl_5-DBT. The disagreement between the observed and the predicted I_{adj} is 2.2% for the parent compound but for the sulfone it is 5.6%. We therefore do not consider this identification to be final. In Fig. 8 the substitution pattern, the measured retention indices (I_{PCDT}) and the predicted retention indices I_{adj} and I_{calc} of the seven PCDTs and their corresponding sulfones are presented.

Table 4

Pentachlorodibenzothiophenes and sulfones: calculated (I_{calc}), corrected (I_{adj}) with a correction factor of 1.52 for the PCDTs and measured (I_{PCDT}) retention indices[a]

PCDT	I_{calc}	I_{adj}	I_{PCDT}	PCDTO$_2$	I_{calc}	I_{PCDT}
1,2,4,6,8-*	73.68	72.16	69.00	1,2,3,7,9-	77.23	
1,3,4,6,8-	73.69	72.17		1,2,4,7,9-	80.98	
1,2,4,7,9-	74.28	72.76		**1,2,3,8,9-**	81.12	
1,3,4,7,9-	74.29	72.77		1,3,4,7,9-	81.56	
1,2,4,6,9-	75.13	73.61		**1,2,3,7,8-***	83.22	82.66
1,3,4,6,9-	75.14	73.62		1,2,3,4,9-	83.50	
1,2,3,4,6-	75.61	74.09		**1,2,3,6,8-**	84.62	
1,2,3,4,9-	76.20	74.68		**1,2,4,8,9-**	84.87	
2,3,4,6,8-*	76.36	74.84	73.02	**1,2,3,4,8-**	85.02	
1,2,3,6,8-	76.95	75.43		1,3,6,7,8-	85.20	
1,3,6,7,8-	76.96	75.44		1,2,3,6,9-	85.45	
1,2,3,4,8-	77.00	75.48		1,2,6,7,9-	85.45	
1,2,3,4,7-	77.01	75.49		1,2,3,4,7-	85.60	
1,2,3,7,9-	77.55	76.03		**1,2,4,7,8-***	86.97	84.62
1,2,4,6,7-	77.81	76.29		<u>1,3,4,7,8-</u>	87.55	
1,4,6,7,8-	77.81	76.29		**1,2,4,6,8-***	88.37	86.34
1,3,4,6,7-	77.82	76.30		1,3,4,6,8-	88.95	
1,2,4,8,9-	78.39	76.87		**1,2,6,7,8-***	89.09	88.98
1,2,3,6,9-	78.40	76.88		1,2,4,6,9-	89.20	
1,2,6,7,9-	78.40	76.88		1,2,3,6,7-	89.67	
1,2,4,7,8-*	79.20	77.68	74.63	1,3,4,6,9-	89.78	
<u>1,3,4,7,8-</u>	79.21	77.69		**2,3,4,7,8-***	91.19	90.47
2,3,4,6,7-	80.49	78.97	77.27	1,2,3,4,6-	91.47	
1,2,6,7,8-*	81.07	79.55	77.84	**2,3,4,6,8-***	92.59	91.16
1,2,3,6,7-	81.08	79.56		1,2,4,6,7-	93.42	
1,2,3,8,9-	81.66	80.14		1,4,6,7,8-	93.42	
2,3,4,7,8-*	81.88	80.36	78.76	1,3,4,6,7-	94.00	
1,2,3,7,8-*	82.47	80.95	79.56	2,3,4,6,7-	97.64	92.31

[a] All based on dibenzothiophene and benzophenanthro[2,1-d]thiophene. For the meaning of the bold type and the stars, see text.

The identification of PCDTs in complex mixtures demonstrates another advantage of the oxidation step of the PCDTs to their sulfones. With this simple reaction the identification of PCDTs becomes more reliable since two independent data sets are available for each compound and both of them have to agree with calculated values in order to lead to a firm identification.

This identification procedure has been carried out in essentially the same way for the Cl$_4$-DBTs in the commercial mixture (see Table 5). 2,3,6,8-Cl$_4$-DBT was available from synthesis and its presence in the PCDT mixture was confirmed by coelution. It is underlined in Table 5. The relative difference between the measured and the calculated retention index is less than 4.5%. Fig. 9 shows the single ion

chromatograms of the Cl$_4$-DBTs and their sulfones. The substitution pattern, the measured and the calculated retention indices are given. The most abundant isomer is suggested to be 1,2,4,8-Cl$_4$-DBT. As for the pentachloro congeners, all identified isomers have at least one chlorine atom in the 2- and/or 4-position. The assignment of the Cl$_4$-DBT is chemically sensible but it should be conclusively proved with more reference compounds.

Naturally it was of interest to see if this system be used to establish the presence of 2,3,7,8-tetrachloro-dibenzothiophene in the mixture, since this substitution pattern is central to toxicological studies. According to Table 5, 2,3,7,8-Cl$_4$-DBT should have an I_{calc} of 69.31, i.e., the compound elutes after all other isomers, and the sulfone an I_{calc} of 76.73. The last

Table 5

Tetrachlorodibenzothiophenes and sulfones: calculated (I_{calc}) and measured (I_{PCDT}) retention indices based on dibenzothiophene and benzophenanthro[2,1-*d*]thiophene[a]

PCDT	I_{calc}	I_{PCDT}	PCDTO$_2$	I_{calc}	I_{PCDT}
2,4,6,8-	58.27	56.42	1,3,7,9-	64.75	–
1,3,6,8-	58.87	–	1,2,7,9-	68.64	–
1,3,7,9-	59.47	–	1,2,3,9-	69.90	–
1,4,6,8-	59.72	–	1,3,7,8-	70.74	–
1,3,6,9-	60.32	–	**1,2,3,8-**	71.42	73.28
1,2,4,6-	60.68	–	1,2,3,7-	72.00	–
1,3,4,6-	60.69	–	1,3,6,8-	72.14	–
1,2,3,4-	60.84	–	**1,2,8,9-**	72.53	–
1,4,6,9-	61.17	–	1,3,6,9-	72.97	–
1,2,4,9-	61.27	–	1,2,4,9-	73.65	–
1,3,4,9-	61.28	–	1,3,4,9-	74.23	–
1,2,4,8-	62.07	60.67	**1,2,7,8-**	74.63	–
1,2,4,7-	62.08	–	1,2,3,4-	75.06	–
1,3,4,8-	62.08	–	**1,2,4,8-**	75.17	75.35
1,3,4,7-	62.09	–	1,2,4,7-	75.75	–
2,4,6,7-	62.40	–	1,3,4,8-	75.75	–
1,2,6,8-	62.98	61.24	**1,2,6,8-**	76.03	75.81
1,3,6,7-	63.00	–	1,3,4,7-	76.33	–
2,3,4,6-	63.36	–	**2,3,7,8-**	76.73	–
1,2,7,9-	63.58	–	1,2,6,9-	76.86	–
2,3,6,8-	63.79	61.70	1,3,6,7-	77.19	–
1,4,6,7-	63.85	–	1,2,3,6-	77.87	–
1,2,3,6-	63.95	–	1,6,7,8-	77.87	–
1,6,7,8-	63.95	–	**2,3,6,8-**	78.13	76.26
1,3,7,8-	64.39	–	1,4,7,8-	78.96	–
1,2,6,9-	64.43	–	**2,3,4,8-**	79.39	–
1,2,3,9-	64.54	–	**2,4,6,8-**	79.53	78.10
2,3,4,8-	64.75	–	2,3,4,7-	79.97	–
2,3,4,7-	64.76	–	1,4,6,8-	80.36	–
1,4,7,8-	65.24	–	1,2,6,7-	81.08	80.62
1,2,3,8-	65.34	63.88	1,4,6,9-	81.19	–
1,2,3,7-	65.35	–	1,2,4,6-	81.62	–
3,4,6,7-	66.53	–	1,3,4,6-	82.20	–
1,2,6,7-	67.11	64.22	2,3,6,7-	83.18	81.20
1,2,8,9-	67.69	–	2,4,6,7-	84.58	–
2,3,6,7-	67.92	65.60	1,4,6,7-	85.41	–
1,2,7,8-	68.50	–	2,3,4,6-	85.84	–
2,3,7,8-	69.31	–	3,4,6,7-	89.63	–

[a] For the meaning of the bold type, see text.

small peak expected from 2,3,7,8-Cl$_4$-DBTO$_2$ so that no information on the presence or absence of this sulfone can be gained. Therefore we must conclude that a failure to identify this congener in this mixture from the predicted retention properties is rather due to the sample than to limitations of the predictive method we have established.

4. Conclusions

An extensive study of the retention behavior of polychlorinated dibenzothiophenes on non-polar gas chromatographic columns has been carried out. These investigations were possible because a useful set of PCDTs with a known substitution pattern was available. The influence of chlorine atoms in the different positions as well as effects depending on the substitution pattern have been quantified using a multiple linear regression. The 4-position in the dibenzothiophene molecule influences the retention of the PCDTs to a considerable degree, a fact which is true also for the sulfones but then with an opposite influence. Intra-ring interactions make a noticeable contribution to the retention as well; if two chlorine atoms are in an *ortho*-position to each other the *I* increases, if they are in *meta*- or *para*-position the *I* decreases. Based on this knowledge a first attempt to predict *I* values has been done and it is shown to be a helpful tool for the identification of PCDTs if reference compounds are not available. Several congeners were identified in a mixture through the retention data of themselves and their sulfones, illustrating how information on the retention behavior can be deduced without a need to synthesize every single congener.

eluting peak with an unambiguous isotopic pattern of a Cl$_4$-DBT is the one labeled with the measured retention index 67.21 in Fig. 9. The very minor peak labeled 69.62 showed the presence of the correct isotopic masses but with untypical ratios so that it cannot be conclusively said to be a Cl$_4$-DBT. In the retention area where the sulfone is expected to elute, there is a cluster of major peaks that swamp the
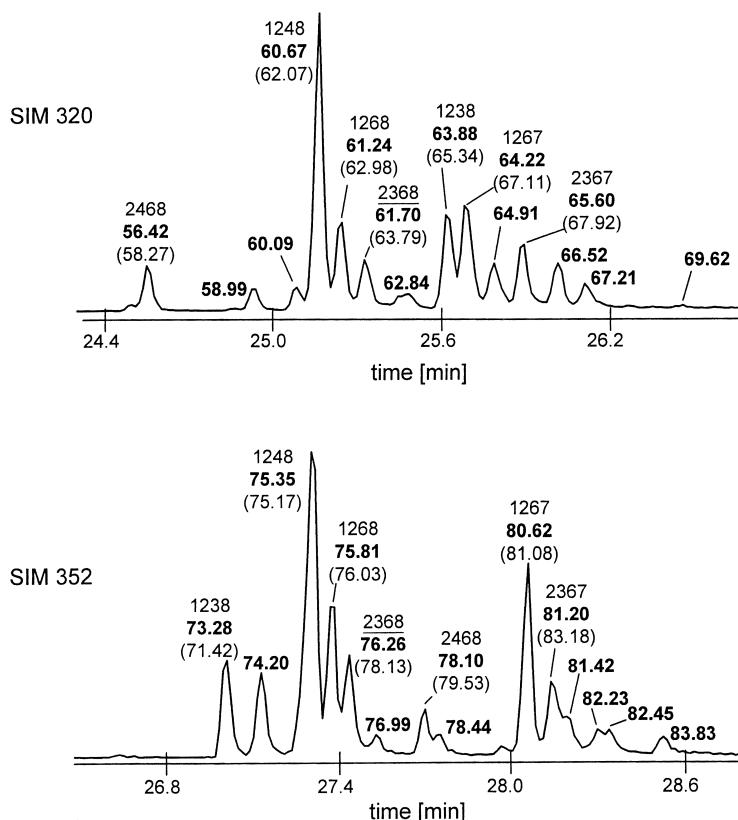
Fig. 9. SIM chromatograms of tetrachlorodibenzothiophenes (top) and their corresponding sulfones (bottom) on DB-5ms. The peak labels indicate: top line the suggested substitution positions, numbers in bold type the measured I_{PCDT}, numbers in parentheses the calculated I_{calc}. Temperature program: 80°C held for 2 min, then programmed at 8°C min^{-1} to 320°C, held for 5 min.

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