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Journal of Chromatography A, 1025 (2004) 227–236
JOURNAL OF CHROMATOGRAPHY A
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Using new structurally related additive schemes in the precalculation of gas chromatographic retention indices of polychlorinated hydroxybiphenyls on HP-5 stationary phase

I.G. Zenkevich[a,*], M. Moeder[b], G. Koeller[b], S. Schrader[b]

[a] *Chemical Research Institute of St. Petersburg State University, Universitetsky pr., 26, St. Petersburg 198504, Russia*
[b] *Department of Analytical Chemistry, Center for Environmental Research Leipzig-Halle, Permoserstrasse 15, D-04318 Leipzig, Germany*

Received 3 July 2003; received in revised form 23 October 2003; accepted 27 October 2003

Abstract

A new additive scheme is proposed for the precalculation of gas chromatographic retention indices of complex organic compounds. The principal feature of this approach is the absence of previously calculated *I* increments for any structural fragments or functional groups in the molecule. Instead, arithmetical operations involving *I* values of simpler structural analogues of target compounds are used directly. *I* precalculation for polychlorinated hydroxybiphenyls (839 congeners) on the HP-5 stationary phase was chosen as one of the most important applications of the method under discussion. Such a large number of congeners cannot be obtained as reference samples and their gas chromatographic (GC)–mass spectrometric (MS) identification should therefore be based currently on precalculated *I* values.

Keywords: Retention indices; Retention prediction; Polychlorinated hydroxybiphenyls

1. Introduction

Using gas chromatographic (GC) retention parameters is sometimes the only way to identify compounds whose mass spectra are indistinguishable, such as in cases of isomeric or some isobaric compounds. For example, the quantification of technical mixtures of polychlorinated biphenyls (PCBs) entails assigning individual congeners due to their very different toxicological effects. Just PCBs have often been the subject of the development of suitable models to calculate GC retention indices (*I*) on the basis of structure–retention relationships. The most reliable assignment is based on the use of experimental data for reference substances. For instance, Ballschmiter and Zell [1] and Ballschmiter and co-workers [2] presented *I* values of PCBs that rest on an increment-assisted model derived from experimentally determined *I* data of a series of reference PCBs. A more sophisticated model was proposed by Robbat et al. [3] to describe the dependence of PCBs retention parameters on an SE-54 and DB-5 stationary phases on the structures of the analytes. The model included the influence of chlorine positions and structural features concerning interactions between chlorine atoms and the aromatic rings as well as between the skeletal structure of each PCB isomer. A similar model based on a linear relationship between GC retention times and molecular descriptors has been applied for polyhalogenated biphenyls [4–6]. The introduction of new stationary phases for PCB analyses usually needs the appropriate adaptation of *I* calculation models. Vetter and Luckas presented a system that uses twenty selected PCB congeners as a basis for the calculation of all other PCB retention times on GC stationary phases CP-Select, CP-Sil 8 CB and CP-Sil 19 CB compared to SE-54 [7,8]. The proposed model regarded the substitution pattern in terms of retention time increments, but provided acceptable data only in connection with a carefully selected and tested oven temperature program. Particularly in cases of more polar compound mixtures, the polarity of the stationary phase sharply influences GC separation as shown by Kurz and Ballschmiter [9] for the stereochemically challenging polychlorinated diphenyl ethers and tetrachloro-methyl-diphenylmethanes. Their investigation of the structure–retention relations were only focused on the

* Corresponding author. Tel.: +7-812-428-4045; fax: +7-812-428-6939.
E-mail address: igor@IZ6246.spb.edu (I.G. Zenkevich).

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doi:10.1016/j.chroma.2003.10.106

elution sequence of a selected number of congeners. Zenkevich [10] reviewed the utility and capability of *I* calculation for the GC identification of a number of chlorinated polycyclic aromatic compounds including PCBs and polychlorinated dibenzodioxins (PCDSs)/dibenzofurans (PCDFs). Modeling of physicochemical properties by semi-empirical computation has become a common approach in the field of quantitative structure–property relationship (QSPR) simulations [11,12]. Many models based on quantum and statistical mechanics have been created, although frequently they can only describe the gas chromatographic retention behavior of analytes on non- and semi-polar stationary phases.

Jalali-Heravi and Garkani-Nejad [13] used an extended set of molecular descriptors to calculate retention indices of benzene derivatives on the obsolete phase Apiezon MH. These descriptors encoded topological, geometric, electronic and physical properties of the halogenated and methylated benzenes and anisols. Despite the numerous molecular details implemented in the model, the agreement between calculated and experimental determined *I* values was not always satisfactory. Apart from incorrectly adapted temperature programs, the interactions of the actual molecular structure with the stationary phase are the main source of imponderability in the accurate precalculation of *I* values.

The "traditional" design of various additive schemes being used for the precalculation of GC *I* values often implies the summation of *I* values for any basic structure (I_0) and the set of increments for different structural fragments in the molecule (I_i), which is to be preliminary determined [11]:

$$I = I_0 + \sum k_i \, \Delta I_i \quad (1)$$

However, the precision of results when using this scheme of calculations is typically not very high. Aberrations appear owing to the need to process the increments ΔI_i themselves using appropriate reference compound sets. Generally, these ΔI_i increments can only be estimated with some errors and consequently their values need to be supplemented by corresponding standard deviations, $\Delta I_i \pm s_{\Delta I_i}$, describing the order of inaccuracy of the increment part. A successful adaptation of previously estimated increments on compounds with a different basic structure requires a suitable evaluation of their applicability, and since systematic errors appear the correction of results becomes necessary by introducing various additional parameters into the additive scheme. The essential consideration of these corrections makes numerous additive schemes very difficult and unattractive in practical use, as ascertained for instance in the system of atom, bond, and group refractions [14], numerous empirical rules for thermodynamics parameter precalculation [15], etc. Owing to the above-mentioned reasons there is an objective necessity to modify the "traditional" attitude to the additive schemes, at least for the precalculation of GC retention indices.

This work proposes an *I* prediction procedure that completely avoids the stage of preliminary precalculation of any increments and replaces it by the direct using of *I* databases, namely the data for simpler structural analogues of compounds being characterized. In order to demonstrate the performance of the novel approach, the group of polychlorinated hydroxybiphenyls (OH-PCBs, hydroxylated PCBs) was chosen as the subject of interest.

PCBs belong to a group of pollutants that is still widely spread in the environment despite being banned in the USA in 1978 and in Germany in 1983. Their environmental stability and toxicity, including endocrine disrupting properties, are a known potential risk impairing the health and reproductive capability of living organisms [16]. The sustainable removal of this kind of compounds is therefore vital. Apart from incineration and deposition, biodegradation techniques are playing an increasing role in the remediation of PCB-contaminated material. As previously demonstrated [17], the preliminary steps in PCB degradation are both hydroxylation and dechlorination reactions. The exact structural identification of the individual metabolites and products is essential for risk assessment and the interpretation of degradation pathways. In the case of PCB transformation the principal analytical problem is the assignment of a large number of possible congeners. The group of hydroxylated PCBs includes 839 compounds [18], nearly four times more than the 209 existing PCB congeners. Consequently, identifying individual OH-PCB congeners is a great challenge. The mass spectra of isomers within a specific group of homologues hardly differ from each other, and so GC retention data seem to be the only suitable parameters for assigning individual congeners. But as few references materials are available, the experimental determination of the entire set of *I* values is an unrealistic venture. However, the precalculation of these *I* values seems to be a promising approach.

Previously, the results of this work have been presented only as abstract [19], but have already been cited in a recent review on the high-resolution GC of PCBs [20].

2. Materials and methods

2.1. Microscale synthesis of chlorinated hydroxybiphenyls

Septum sealed glass vials of 1 ml volume were used as reaction vessels. Two milligrams of individual 2-, 3- or 4-hydroxybiphenyl (Merck, Darmstadt, Germany), respectively, were dissolved in 50 μl *n*-heptane (Merck) and treated with a droplet of 38% HCl and $KMnO_4$ in aqueous solution. The solution was magnetically stirred for 10 min. The organic layer was separated and mixed with a standard reference mixture (Fluka, Sigma–Aldrich, Seelze, Germany) containing *n*-alkanes with carbon numbers from 8 to 40. One microliter of this solution was injected into the GC–mass spectrometric (MS) system. All solvents and reagents were used without additional purification.

2.2. GC–MS analysis

GC–MS analysis was carried out with a HP5980 series II gas chromatograph coupled to a mass-selective detector. A HP-5 MS (polydimethyl siloxane with 5% phenyl groups) capillary column of 30 m, 0.25 mm i.d. and a film thickness of 0.25 μm (Agilent Technolgies, Waldbronn, Germary) was used with the following temperature program: initial temperature 50 °C—3 min, ramp 3 °C/min, final isotherm 280 °C—20 min. The injector and ion source temperature were set to 280 and 180 °C, respectively. Helium was used as carrier gas at a constant linear velocity of 25 cm/s. Mass spectra were detected in the mass range between 50 and 500 Da with a scan rate of 1 spectrum/s.

An important basis for the precalculation of unknown I values is the accuracy of the experimental GC retention data involved. Applying the experimental equipment resulted in an average relative standard deviation of 0.1% for the retention times for the set of n-alkanes and target analytes (n = 4, average absolute reproducibility of I values were about ±1–2 IU).

2.3. LC–NMR analysis

Before the products of the hydroxybiphenyl chlorination were used for retention index determination, the assignment of congeners was carried out by LC–NMR. The LC instrument (Institute of Chemical Technology, Prague, Czech Republic) consisted of an gradient mixer (Model 1155, Bischoff, Leonberg, Germany), an HPLC pump equipped with a micropump head (Model 2250, Bischoff) and a UV detector (Lambda 1000, Bischoff) operated at 254 nm. The NMR spectra were recorded on a Bruker AMX 600 spectrometer at 600.13 MHz with a $^{1}H-^{13}C$ inverse LC probe head (4 mm i.d., of measuring cell with an active volume of 120 μl) (Bruker-Daltronik, Bremen, Germany).

The LC separation was performed on a Vydac RP-18 column of 150 mm × 4.6 mm i.d. and 5 μm particle size (Vydac, Hesperia, CA, USA) with a mobile phase of A: acetonitrile and B: $^{2}H_2O$ (both supplied by Merck) at a flow of 1 ml/min. The gradient started at a mixture of A–B (55:45). After 35 min A has been increased to 75% and returned after 40 min to the initial eluent composition. Stopped flow ^{1}H NMR and stopped flow TOCSY spectra were used to exactly assign the substitution positions of chlorine atoms introduced in the hydroxybiphenyls [20].

2.4. Calculation of GC retention indices and sources of reference data

I values of target analytes in linear temperature programming regime have been calculated from their retention times using the linear–logarithmic relationship [$f(t_R) = t_R + q \log t_R'$] [21,22] that is the generalization of previously proposed logarithmic Kovats I system [$f(t_R) = \log t_R'$] [23] and linear retention indices introduced by Van den Dool and Kratz [$f(t_R) = t_R$] [24]:

$$I_x = \frac{I_n \text{ (before the ratio)} + \text{(ratio)}(I_{n+1} - I_n) \times [f(t_{R,x}) \times f(t_{R,n})]}{[f(t_{R,n+1}) - f(t_R)]}$$

where $t_{R,x}$, $t_{R,n}$ and $t_{R,n+1}$—net retention times of analyte and reference n-alkanes ($t_{R,n} < t_{R,x} < t_{R,n+1}$) with postulated $I_n = 100n_C$ values, $t_R' = t_R - t_0$, where t_0 is hold-up time (if $t_R >> t_0$, parameter t_0 can be neglected under calculations owing to its small influence on the results); auxiliary parameter q should be evaluated from retention times of three consecutively eluting n-alkanes and for any parts of chromatograms it provides the precise linear interpolation I values versus $f(t_R) = t_R + q \log t_R'$ [25]:

$$q = \frac{t_{R,n-1} + t_{R,n+1} - 2t_R}{2 \log t_{R,n}' - \log t_{R,n-1}' - \log t_{R,n+1}'}$$

Statistically processed averaged I values of model organic compounds on standard non-polar polydimethyl siloxanes used in the testing of the calculation method were taken from the collection of the corresponding author and followed on all available published data since the 1980s.

3. Discussion

Any additive schemes of GC I precalculation based on Eq. (1) need I data for basic structural analogues (I_0) and the set of increments ΔI_i for various molecular fragments X_i. An evaluation of these increments implies the subtraction of I values for non-substituted compounds $B_j - H$ from corresponding substituted compounds $B_j - X_i$ at different B_j:

$$\Delta I_i(H \rightarrow X_i) = I(B_j - X_i) - I(B_j - H) \quad (2)$$

This subtraction can be followed by averaging of ΔI_i data for concrete compounds, i.e. the generalization of the parameter $\langle \Delta I_i \rangle$ on the whole structural transformations.

The main disadvantage of this "traditional" approach is that information is lost on the initial structures B_j, preventing evaluation of the restrictions of this additive scheme in its applications. Theoretically every ΔI_i value is affected by many sources of variation which should be listed together with them, although in most cases this additional information is omitted owing to objective restrictions. For instance, in the benzene ring any increments ΔI_i are different for the same substituents in *meta*-positions from groups in the molecule, *ortho*-positions (so-called *ortho*-effect) and *para*-positions (an optimal case for π-conjugation, which leads to the a non-additive increase in molecular polarizability and, hence, the non-additivity of retention parameters). Within aliphatic series of compounds ΔI_i values usually vary for the same substituents at primary, secondary and tertiary carbon atoms [26]. In all cases, any extra steric hindrance in the molecules can change these parameters unpredictably.

To increase the precision of additive schemes, the abovementioned structural uncertainties need to be avoided when they are used. Hence, the principal proposition is to exclude

the stage of preliminary calculation of any increments ΔI_i and replace it by direct operations with structures of organic compounds and, in parallel, with their I values.

This general thesis can be illustrated by the following schemes in simplest line notation. An evaluation of I values for structures R–X and A–Z (–A)–B requires the selection of a few precursors and ways of transforming their structures:

$$R–H + R'–X - R'–H = R–X$$

$$A–Z–A + A–Z–B - Z–A = A–Z(–A)–B$$

We can also duplicate these calculation schemes starting from other sets of precursors:

$$R–CH_3 + R''–X - R''–CH_3 = R–X,$$

or

$$R–Y + Z–X - Z–Y = R–X$$

and

$$A–Z(–A)–C + A–Z–B - A–Z–C = A–Z(–A)–B$$

Since there are no preferences for one particular mode of calculation compared to the others, all partial sub-results should be averaged. Finally, the I value of any compounds can be evaluated directly from the I values of their simpler structural analogues as a result of data summation and subtraction. It is important to note, that after the arithmetical treatment of data for different chemical structures, each structure fragment in the resulting molecule need only be mentioned once.

The possibility of applying the above-mentioned arithmetical operations (not only summation, but also subtraction) to GC retention indices follows from the first proposition by Kovats [23]. Accordingly, the additivity of I values means the evaluation of these parameters for compounds of type A–B from the data for compounds A–A and B–B, namely $I(A–B) \approx [I(A–A) + I(B–B)]/2$. The last equality can be transformed into a mathematically equivalent form, $I(A–A) \approx 2I(A–B) - I(B–B)$. Further generalization of the same additive scheme results in the above-mentioned equations.

All the general recommendations presented above are illustrated below with some very simple examples. The estimated products of the radical chlorination of isopropylbenzene (cumene) [$PhCH(CH_3)_2$, I 914 ± 6] are two chlorinated derivatives, namely (2-chloro-1-methyl)-ethylbenzene $PhCH(CH_3)CH_2Cl$ (a) and (1-chloro-1-methyl)ethylbenzene, $PhC(CH_3)_2Cl$ (b). No retention or mass spectral data for these isomers were available about these isomers before the experiment. Two peaks of products with I 1070 ± 1 and 1152 ± 1 are registered on the chromatograms (capillary column with OV-101). Hence, their identification needs the precalculation of I values in any suitable way. The preferable approach is the method under discussion. Isomer (a) has a primary chlorine atom. It is necessary to choose a structural analogue also with primary chlorine as a precursor, namely (2-chloroethyl)benzene. The transformation of structures in this case will be as follows:

$$PhCH_2CH_2Cl + PhCH(CH_3)_2 - PhCH_2CH_3 \rightarrow PhCH(CH_3)CH_2Cl \quad (a)$$

$$1090 \pm 1 + 914 \pm 6 - 854 \pm 9 = 1150 \pm 11$$

Hence, the second product in the reaction mixture is the expected primary chloride.

Isomer (b) contains tertiary chlorine atom, and so it is preferable to choose a precursor with tertiary chlorine substituent and non-aromatic nature, such as *tert*-butyl chloride:

$$PhCH(CH_3)_2 + (CH_3)_3C\text{-}Cl - CH(CH_3)_2 \rightarrow PhC(CH_3)_2Cl \quad (b)$$

$$914 \pm 6 + 540 \pm 6 - 376 \pm 2 = 1078 \pm 9$$

This result suggests, that the first eluting product in the reaction mixture analyzed is the expected tertiary chloride.

The second example, the precalculation of the I value of 2′,4′-dimethylacetophenone, illustrates the application of this approach within the series of aromatic compounds, when the relative positions of substituents in the ring need to be borne in mind.

The principal structural feature of this molecule is *ortho*-location of the methyl and acetyl groups. The best way to reflect this feature in the additive scheme is to choose as the starting analogue the compound which already has these two fragments in the same position, for instance 2′-methylacetophenone. Both modes 1 and 2 of precalculation give comparable results (1229 ± 13 and 1223 ± 14 IU), which can be averaged to 1226. Standard deviation of this averaged value can be estimated as $[(s_1^2 + s_2^2)/2]^{0.5} \approx 14$. If we start from unsubstituted acetophenone, we should compensate for the absence of this fragment if possible by using I data for other compounds with functional groups in *ortho*-positions, namely 2,4-dimethylbenzaldehyde (mode 3). Nevertheless, the resulting I value in this case is higher than in first two ones (1239 ± 16) (see Fig. 1).

If we completely neglect the *ortho*-position of the methyl and acetyl groups, the reliability of the results decreases sharply. An attempt to estimate the I of 2′,4′-dimethylacetophenone from the data for non-substituted acetophenone, *m*-xylene and benzene seems ineffective because the resulting value 1249 ± 14 (see Fig. 2) far exceeds the experimentally determined I of 2′,4′,-dimethylacetophenone of 1223 ± 8 on standard non-polar phases, which is not acceptable for practical use. On the other hand, the calculated I of 1249 ± 14 points instead to configurations of dimethylacetophenones without any *ortho*-substituents. Since we used I data for *m*-xylene, the last evaluation characterizes I of 3′,5′-dimethylacetophenone.

The statements mentioned above were applied to the precalculation of I values of members of a newly investigated

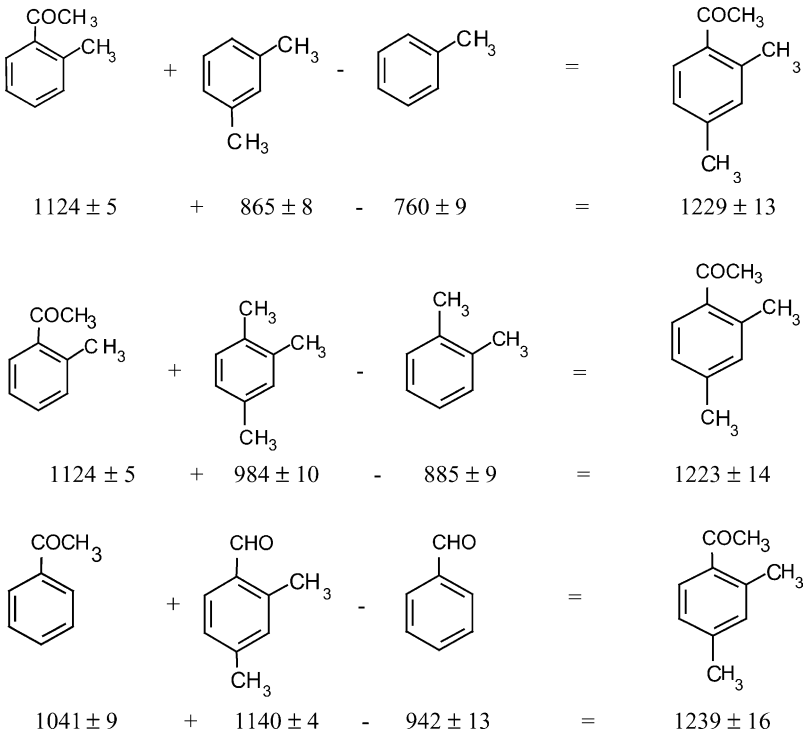

Fig. 1. Example of *I* precalculation for 2,4-dimethyl acetophenone on standard non-polar stationary phases.

class of ecotoxicants-polychlorinated hydroxybiphenyls. The number of 839 congeners within this group exceeds those for PCBs with a total of 209 congeners. As a result, the synthesis of all the individual references and the experimental determination of their *I* values would be a very time-consuming process, and so precalculation of *I* values seems to be an attractive alternative to facilitate identification of individual OH-PCB congeners.

The application of the new modification of additive schemes for *I* values precalculation values needs some initial information, preferably of an experimental nature, concerning the *I* data for structural analogues. For this work, this means that for OH-PCBs it is necessary to possess the data on non-chlorinated hydroxybiphenyls as well as on the *I* values of all 209 PCB congeners on HP-5 stationary phase. Because the most frequently published retention data for PCBs are retention times measured on DB-5 or SE-54 stationary phases, net or relative retention times had to be recalculated or the *I* values of PCBs found in the literature [27–29] had to be translated into the comparable scale. The retention indices for some PCBs were used as the reference data for this recalculation instead of *n*-alkanes. A list of some experimental data is presented in Table 1.

In order to evaluate the precalculated *I* data for OH-PCBs some congeners were synthesized from non-chlorinated hydroxybiphenyls on a microscale. The identification of the congeners produced was carried out by mass spectra as well as LC–NMR experiments. The experimentally measured *I* values of selected OH-PCBs are presented in Table 2. All these values have been used as “starting points” for precalculation of *I* values for other OH-PCBs in accordance with algorithm proposed.

This set of information is enough for the calculation of *I* values of all possible OH-PCBs, both the simplest members of series, and polychlorinated compounds. For example, the *I* precalculation ways for 2-chloro-3-hydroxy-, 3,4′-dichloro-2-hydroxy- and for 3,6-dichloro-2-hydroxybiphenyl are shown in detail as Fig. 3. The last of these examples is the congener found in the solution after enzymatic degradation of PCB 9 (see below) [30].

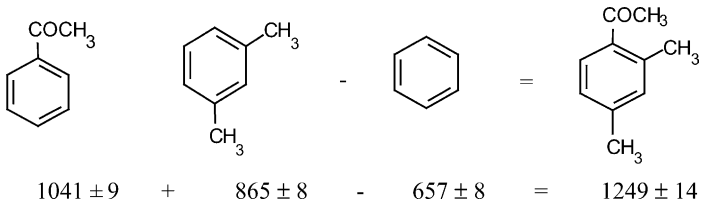

Fig. 2. Precalculation example which includes unsuited increments.

Table 1
Experimental retention indices (I with standard deviations, s_I, on HP-5 stationary phase) of structural analogues, synthetic precursors and some related compounds for target polychlorinated hydroxybiphenyls: PCBs, hydroxybiphenyls, chlorinated benzenes and phenols; results of recalculation of retention times of selected reference biphenyls into their retention indices

Compound	M_r	I(HP-5)	s_I
Polychlorinated biphenyls (Ballschmitter No.)			
2,2′-Cl_2 (4)	222	1606	4
2,5-Cl_2 (9)	222	1672	4
2,4,4′-Cl_3 (28)	256	1881	1
2,2′,5,5′-Cl_4 (52)	290	1935	2
2,2′,4,5,5′-Cl_5 (101)	324	2130	1
2,2′,3,4,4′,5′-Cl_6 (138)	358	2311	1
2,2′,4,4′,5,5′-Cl_6 (153)	358	2373	2
2,2′,3,4,4′,5,5′-Cl_7 (180)	392	2526	1
Hydroxybiphenyls			
Unsubstituted biphenyl	154	1379	3
2-OH	170	1506	2
3-OH	170	1704	2
4-OH	170	1723	2
4,4′-$(OH)_2$	186	2046	1
2,2′-$(OH)_2$	186	1662	1
Chlorinated benzenes			
Mono-Cl	112	844	2
1,4-Cl_2	146	1013	1
1,3-Cl_2	146	1005	2
1,2-Cl_2	146	1003	3
1,2,3-Cl_3	180	1204	2
1,2,3,5-Cl_4	214	1317	2
1,2,4,5-Cl_4	214	1321	1
1,2,3,4-Cl_4	214	1383	2
Cl_5	248	1509	1
Chlorinated phenols			
Unsubstituted phenol	94	992	1
2-Cl	128	990	1
2,4-Cl_2	162	1165	2
3-Me,4-Cl	142	1297	1
2,4,6-Cl_3	196	1348	1
Cl_5	264	1748	2
I values recalculated from retention times of PCBs ($n_{Cl} \leq 3$) (Ballschmitter No.)			
2-Cl (1)	188	1482	6
3-Cl (2)	188	1560	4
4-Cl (3)	188	1564	5
2,3-Cl_2 (5)	222	1695	9
2,3′-Cl_2 (6)	222	1676	9
2,4-Cl_2 (7)	222	1653	2
2,4′-Cl_2 (8)	222	1704	9
2,6-Cl_2 (10)	222	1591	7
3,3′-Cl_2 (11)	222	1746	9
3,4-Cl_2 (12)	222	1756	9
3,4′-Cl_2 (13)	222	1760	9
3,5-Cl_2 (14)	222	1748	9
4,4′-Cl_2 (15)	222	1776	3
2,2′,3-Cl_3 (16)	256	1789	10
2,2′,4-Cl_3 (17)	256	1761	3
2,2′,5-Cl_3 (18)	256	1772	11
2,2′,6-Cl_3 (19)	256	1708	12
2,3,3′-Cl_3 (20)	256	1880	6
2,3,4-Cl_3 (21)	256	1877	6
2,3,4′-Cl_3 (22)	256	1896	9
2,3,5-Cl_3 (23)	256	1820	12
2,3,6-Cl_3 (24)	256	1784	12
2,3′,4-Cl_3 (25)	256	1855	9
2,3′,5-Cl_3 (26)	256	1846	8
2,3′,6-Cl_3 (27)	256	1786	10
2,4,5-Cl_3 (29)	256	1846	10
2,4,6-Cl_3 (30)	256	1737	3
2,4′,5-Cl_3 (31)	256	1864	4
2,4′,6-Cl_3 (32)	256	1797	8
2′,3,4-Cl_3 (33)	256	1873	7
2′,3,5-Cl_3 (34)	256	1814	8
3,3′,4-Cl_3 (35)	256	1967	3
3,3′,5-Cl_3 (36)	256	1918	5
3,4,4′-Cl_3 (37)	256	1980	5
3,4,5-Cl_3 (38)	256	1942	2
3,4′,5-Cl_3 (39)	256	1931	2

Table 2
Experimental I data for some OH-PCBs synthesized by chlorination of hydroxybiphenyls and unambiguously identified in reaction mixtures on HP-5 MS stationary phase

No.	Hydroxy-PCBs	$I \pm s_I$
1	2-Cl, 3-OH	1636 ± 2
2	2-Cl, 5-OH	1728 ± 3
3	3-Cl, 2-OH	1691 ± 1
4	3-Cl, 4-OH	1742 ± 2
5	3-Cl, 6-OH	1712 ± 1
6	4-Cl, 3′-OH	1852 ± 1
7	2,4-Cl_2, 3-OH	1764 ± 2
8	2,4-Cl_2, 5-OH	1841 ± 3
9	2,4′-Cl_2, 3-OH	1870 ± 1
10	2,4′-Cl_2, 5-OH	1940 ± 1
11	2,5-Cl_2, 6-OH	1802 ± 2
12	2,6-Cl_2, 3-OH	1764 ± 2
13	3,4′-Cl_2, 4-OH	1950 ± 2
14	3,5-Cl_2, 2-OH	1871 ± 1
15	3,5-Cl_2, 4-OH	1846 ± 3
16	2,4,6-Cl_3, 3-OH	1971 ± 2
17	3,4′,5-Cl_3, 2-OH	2091 ± 1

Analogously, by the same manner we can precalculate several individual I values of the last compound tally well with each other, which give after averaging the I of 3,6-dichloro-2-hydroxybiphenyl (1802 ± 6 IU). Comparison with the corresponding experimentally determined I value of 1802 ± 2 shows an excellent agreement. Other examples of good coincidence of precalculated and experimental I values are 3,4-dichloro-4′-hydroxy- and 2,3′,4-trichloro-4′-hydroxybiphenyls (Table 3).

Table 3
Precalculated and experimental I values for 3,4-dichloro-4′-hydroxy- and 2,3′,4-trichloro-4′-hydroxybiphenyls

OH-PCB	I value	
	Experimental	Precalculated
3,4-Cl_2, 4′-OH	1950 ± 2	1945 ± 10
2,3′,4-Cl_3, 4′-OH	2057 ± 2	2054 ± 14

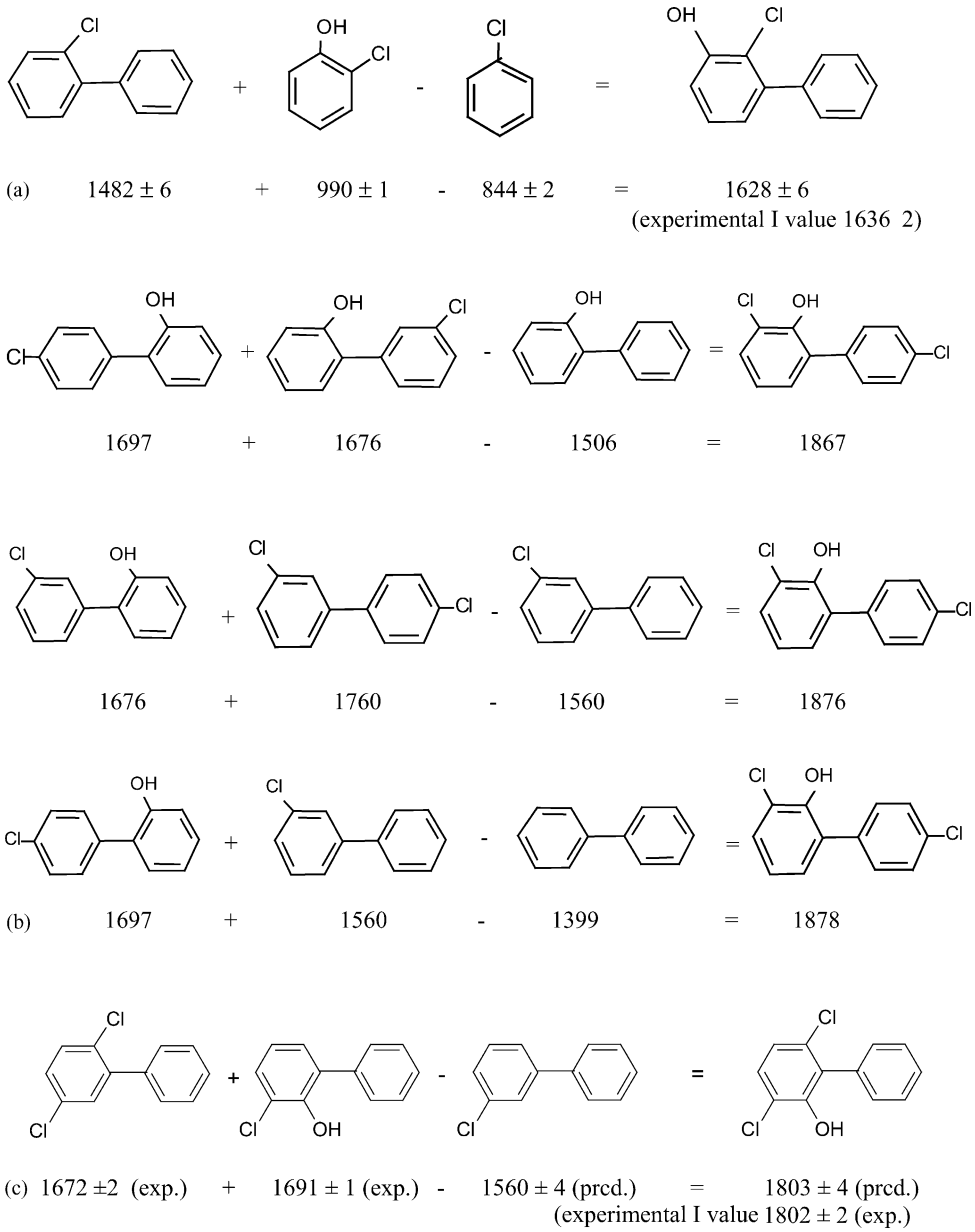

Fig. 3. (a) *I* precalculation for 2-chloro-3-hydroxybiphenyl. (b) *I* precalculation for 3,4′-dichloro-2-hydroxybiphenyl (experimental *I* value is unavailable) in three different ways. (c) *I* precalculation for 2,5-dichloro-6-hydroxybiphyenyl, one of the OH-PCB congener; that has been found in the solution of PCB 9 after its enzymatic degradation.

The precalculated *I* values of OH-PCBs were applied to assign the structural configuration of metabolites found in PCB degradation experiments [31]. For example, the mechanism of the enzymatically initiated degradation of 2,5-dichlorobiphenyl (PCB 9) is indicated by a selective hydroxylation. The two products appearing in the chromatogram of a GC–MS analysis (Fig. 4) are characterized by similar mass spectra. Thus, a congener specific identification is only possible by using their GC retention parameters.

The experimentally determined *I* values of 1800 ± 2 for component at 16.851 min and 1836 ± 3 for the product at 18.648 min retention time corresponds very well with *I* data precalculated for the congeners 3,6-dichloro-2-hydroxybiphenyl ($I_{precalcd} = 1802 \pm 8$) and 2,5-dichloro-3-hydroxybiphenyl with $I_{precalcd}$ of 1834 ± 15. The formation of these metabolites seems to be a logical consequence of a hydroxyl radical attack on the 2,5-dichlorobiphenyl (PCB 9). Another degradation product, a monochloro hydroxybiphenyl was identified as 3-chloro-2-hydroxybiphenyl with an experimental *I* of 1689 and a precalculated *I* of 1691 (see No. 3, Table 2). The postulated loss of a chlorine atom was confirmed by the identification of the congener on the basis of the full set of *I* values precalculated for all possible monochloro hydroxybiphenyls.

Table 4
United set of predicted and experimentally measured retention indices of polychlorinated hydroxybiphenyls (first part of complete data for congeners with $n_{Cl} \leq 3$)

Hydroxy-PCBs (in parentheses the Ballschmiter No. of the corresponding PCB is indicated)	M_r	I(HP-5)	s_I
2-Cl (1) 3-OH	204	1628 (1636)[a]	6
2-Cl (1) 4-OH	204	1748	6
2-Cl (1) 5-OH	204	1728[b]	3
2-Cl (1) 6-OH	204	1625	16
2-Cl (1) 2′-OH	204	1674	21
2-Cl (1) 3′-OH	204	1818	10
2-Cl (1) 4′-OH	204	1817	11
3-Cl (2) 2-OH	204	1691[b]	1[c]
3-Cl (2) 4-OH	204	1742[b]	2
3-Cl (2) 5-OH	204	1914	7
3-Cl (2) 6-OH	204	1712[b]	1
3-Cl (2) 2′-OH	204	1676	18
3-Cl (2) 3′-OH	204	1849	12
3-Cl (2) 4′-OH	204	1861	5
4-Cl (3) 2-OH	204	1697	6
4-Cl (3) 3-OH	204	1715	14
4-Cl (3) 2′-OH	204	1680	18
4-Cl (3) 3′-OH	204	1852[b]	1
4-Cl (3) 4′-OH	204	1872	10
2,2′-Cl_2 (4) 3-OH	238	1749	7
2,2′-Cl_2 (4) 4-OH	238	1870	8
2,2′-Cl_2 (4) 5-OH	238	1868	13
2,2′-Cl_2 (4) 6-OH	238	1730	13
2,3-Cl_2 (5) 4-OH	238	1868	17
2,3-Cl_2 (5) 5-OH	238	1886	5
2,3-Cl_2 (5) 6-OH	238	1843	5
2,3-Cl_2 (5) 2′-OH	238	1879	17
2,3-Cl_2 (5) 3′-OH	238	1996	24
2,3-Cl_2 (5) 4′-OH	238	2003	15
2,3′-Cl_2 (6) 3-OH	238	1837	12
2,3′-Cl_2 (6) 4-OH	238	1942	11
2,3′-Cl_2 (6) 5-OH	238	1924	15
2,3′-Cl_2 (6) 6-OH	238	1798	18
2,3′-Cl_2 (6) 2′-OH	238	1819	13
2,3′-Cl_2 (6) 4′-OH	238	1853	7
2,3′-Cl_2 (6) 5′-OH	238	2006	10
2,3′-Cl_2 (6) 6′-OH	238	1829	18
2,4-Cl_2 (7) 3-OH	238	1764[b]	2
2,4-Cl_2 (7) 5-OH	238	1841[b]	3
2,4-Cl_2 (7) 6-OH	238	1823	18
2,4-Cl_2 (7) 2′-OH	238	1842	5
2,4-Cl_2 (7) 3′-OH	238	1984	8
2,4-Cl_2 (7) 4′-OH	238	1998	5
2,4′-Cl_2 (8) 3-OH	238	1870[b]	1
2,4′-Cl_2 (8) 4-OH	238	1972	19
2,4′-Cl_2 (8) 5-OH	238	1940[b]	1
2,4′-Cl_2 (8) 6-OH	238	1784	13
2,4′-Cl_2 (8) 2′-OH	238	1866	12
2,4′-Cl_2 (8) 3′-OH	238	1824	8
2,5-Cl_2 (9) 3-OH	238	1834	15
2,5-Cl_2 (9) 4-OH	238	1767	10
2,5-Cl_2 (9) 6-OH	238	1803 (1802)[a]	2
2,5-Cl_2 (9) 2′-OH	238	1849	8
2,5-Cl_2 (9) 3′-OH	238	1946	5
2,5-Cl_2 (9) 4′-OH	238	1957	8
2,6-Cl_2 (10) 3-OH	238	1764[b]	2
2,6-Cl_2 (10) 4-OH	238	1784	16
2,6-Cl_2 (10) 2′-OH	238	1789	?
2,6-Cl_2 (10) 3′-OH	238	1927	9
2,6-Cl_2 (10) 4′-OH	238	1922	12
3,3′-Cl_2 (11) 2-OH	238	1974	4
3,3′-Cl_2 (11) 4-OH	238	1929	7
3,3′-Cl_2 (11) 5-OH	238	2033	17
3,3′-Cl_2 (11) 6-OH	238	1874	17
3,4-Cl_2 (12) 2-OH	238	1892	11
3,4-Cl_2 (12) 5-OH	238	1921	6
3,4-Cl_2 (12) 6-OH	238	1900	10
3,4-Cl_2 (12) 2′-OH	238	1883	15
3,4-Cl_2 (12) 3′-OH	238	2054	13
3,4-Cl_2 (12) 4′-OH	238	2064	12
3,4′-Cl_2 (13) 2-OH	238	1874 (1886)[a]	8
3,4′-Cl_2 (13) 4-OH	238	1945 (1950)[a]	2
3,4′-Cl_2 (13) 5-OH	238	2044	15
3,4′-Cl_2 (13) 6-OH	238	1890	15
3,4′-Cl_2 (13) 2′-OH	238	1875	12
3,4′-Cl_2 (13) 3′-OH	238	1939	18
3,5-Cl_2 (14) 2-OH	238	1871[b]	1
3,5-Cl_2 (14) 4-OH	238	1846[b]	3
3,5-Cl_2 (14) 2′-OH	238	1846	10
3,5-Cl_2 (14) 3′-OH	238	2035	11
3,5-Cl_2 (14) 4′-OH	238	2046	13
4,4′-Cl_2 (15) 2-OH	238	1887	17
4,4′-Cl_2 (15) 3-OH	238	1949	17
2,2′,3-Cl_3 (16) 4-OH	272	1978	11
2,2′,3-Cl_3 (16) 5-OH	272	1992	10
2,2′,3-Cl_3 (16) 6-OH	272	1952	11
2,2′,3-Cl_3 (16) 3′-OH	272	1956	6
2,2′,3-Cl_3 (16) 4′-OH	272	2082	9
2,2′,3-Cl_3 (16) 5′-OH	272	2072	13
2,2′,3-Cl_3 (16) 6′-OH	272	1935	8
2,2′,4-Cl_3 (17) 3-OH	272	1874	5
2,2′,4-Cl_3 (17) 5-OH	272	1941	10
2,2′,4-Cl_3 (17) 6-OH	272	1916	12
2,2′,4-Cl_3 (17) 3′-OH	272	1922	5
2,2′,4-Cl_3 (17) 4′-OH	272	2068	21
2,2′,4-Cl_3 (17) 5′-OH	272	2037	7
2,2′,4-Cl_3 (17) 6′-OH	272	1907	12
2,2′,5-Cl_3 (18) 3-OH	272	1934	8
2,2′,5-Cl_3 (18) 4-OH	272	1972	14
2,2′,5-Cl_3 (18) 6-OH	272	1912	11
2,2′,5-Cl_3 (18) 3′-OH	272	1934	9
2,2′,5-Cl_3 (18) 4′-OH	272	2052	4
2,2′,5-Cl_3 (18) 5′-OH	272	2047	7
2,2′,5-Cl_3 (18) 6′-OH	272	1917	6
2,2′,6-Cl_3 (19) 3-OH	272	1876	6
2,2′,6-Cl_3 (19) 4-OH	272	2079	8
2,2′,6-Cl_3 (19) 5-OH	272	1856	5
2,2′,6-Cl_3 (19) 3′-OH	272	1860	6
2,2′,6-Cl_3 (19) 4′-OH	272	1981	8
2,2′,6-Cl_3 (19) 5′-OH	272	1980	7
2,2′,6-Cl_3 (19) 6′-OH	272	1840	10
2,3,3′-Cl_3 (20) 4-OH	272	2048	4
2,3,3′-Cl_3 (20) 5-OH	272	2068	7
2,3,3′-Cl_3 (20) 6-OH	272	2027	6
2,3,3′-Cl_3 (20) 2′-OH	272	2016	11
2,3,3′-Cl_3 (20) 4′-OH	272	2065	4
2,3,3′-Cl_3 (20) 5′-OH	272	2161	8
2,3,3′-Cl_3 (20) 6′-OH	272	2011	9
2,3,4′-Cl_3 (22) 4-OH	272	2060	8
2,3,4′-Cl_3 (22) 5-OH	272	2071	17
2,3′,4-Cl_3 (25) 4′-OH	272	2054 (2057)[a]	14
2,4,6-Cl_3 (30) 3-OH	272	1971[b]	2
3,4′,5-Cl_3 (39) 2-OH	272	2091[b]	1

[a] Selected comparisons of precalculated and experimentally measured (in parentheses) I values.
[b] Experimentally measured I values; there are no precalculated data.
[c] For experimentally determined I values here and later corresponding standard deviations are presented.

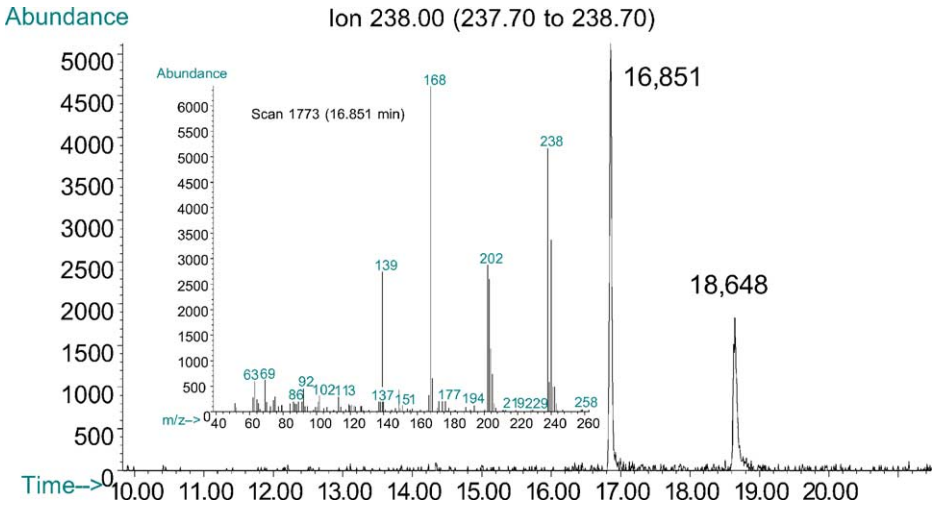

Fig. 4. The ion trace chromatogram of *m/z* 238 shows the record of hydroxylated dichlorobiphenyls after enzymatic degradation of 2,5-dichlorobiphenyl (PCB 9). The congeners produced are assigned as 3,6-dichloro-3-hydroxybiphenyl (16.851 min, mass spectrum included) and 2,5-dichloro-3-hydroxy-biphenyl (18.648 min) [25]. Time scale in min.

The first part the complete data set of predicted and experimentally measured retention indices for polychlorinated hydroxybiphenyls with $n_{Cl} \leq 3$ of is shown in Table 4. All compounds are listed in the order of ascending Ballschmiter numbers of initial PCBs (from 1 to 39) and differ by position of OH within each sub-group. This table also includes all experimentally measured *I* values mentioned in Table 2 when the theoretical estimations of them are not fulfilled. Of course, an evaluated standard deviations of precalculated *I* values exceed the same s_I parameters for experimentally measured data that may increase the uncertainty of GC identification of target congeners. In these cases of overlapping *I* windows the relative elution order or extra chemical information (for instance, preferable alternative positions of hydroxylation of PCB molecules) should be taken into account.

Starting from the data presented in this table, *I* values of OH-PCBs with $n_{Cl} > 3$ can be estimated by manner, which seems equivalent to the mathematical iteration procedure.

4. Conclusion

Identifying all the possible 839 congeners of chlorinated hydroxybiphenyls cannot be based on their mass spectra alone and requires the use of retention indices. The *I* values for OH-PCBs cannot be experimentally determined owing to the difficulty of synthesizing so many congeners. The best way to solve this problem today is to use the precalculated *I* values.

A special version of the additive scheme is proposed. It does not include any preliminarily determined increments of retention indices and is based on the direct operations involving structures of simpler analogues of target compounds and their retention indices. The choice of various sets of these analogues for any compound permits us to characterize precalculated *I* values by their standard deviations, which is very important for analytes with a large number of isomers.

This enables the exhaustive *I* database for all possible hydroxy-PCBs to be processed. The first part of this database for congeners with no more than number three chlorine atoms is presented in the paper.

Acknowledgements

The authors would like to thank Dr. A. Preiss of the Fraunhofer Institute for Toxicology and Aerosol Research at Hannover for performing a series of HPLC–NMR experiments to assign the structural configuration of the OH-PCBs synthesized. Furthermore, we are most grateful to Ludmilla Chroma of the Institute of Chemical Technology at Prague, Department of Biochemistry and Microbiology for providing some individual PCB congeners.

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