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CORRELATION OF STRUCTURE WITH RETENTION INDEX FOR CHLORINATED DIBENZO-*p*-DIOXINS*

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SUMMARY

The method presented here allows calculation of the retention indices for the chlorinated dibenzo-*p*-dioxins under a specific set of experimental conditions, using a limited data set. This technique provides a simple way to obtain retention index data for those isomer standards not readily available. Thus, the technique can aid in structure assignment of specific congeners whether obtained from synthesis or found in environmental monitoring efforts. These calculations have proved highly successful in checking gas chromatographic retention data for internal consistency.

INTRODUCTION

Chlorinated dibenzo-*p*-dioxins (PCDDs) are compounds of great concern; high toxicity has been associated with many of the individual congeners in this series[1–3]. As a result, the U.S. Environmental Protection Agency (U.S. EPA) conducts monitoring of chlorinated dibenzodioxins and related compounds in the environment. This task is currently accomplished using the Resource Conservation and Recovery Act (RCRA) Method 8280 (hazardous waste matrices) and methods applied to the Contract Laboratory Program (CLP) using IFB Contract WA84-A002 (soil and sediment) and Method 613 (water matrix). To accomplish these efforts, analytical standards are desired for as many of the 75 dioxin congeners as possible. In addition, it would prove valuable if a simple method to provide structural assignment of a particular congener could be developed. Such a method would be useful for identifying synthetic products as well as unknown chlorinated dioxins found during environmental monitoring efforts.

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* This article has not been subjected to Agency policy review and therefore does not necessarily reflect the views of the Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

The gas chromatographic (GC) retention index (RI), relating the retention time of a compound to those of normal alkanes, has been used extensively to help characterize compounds within a closely related series. These calculations are based on parameters determined from a limited number of compounds within the series of interest, such as chlorinated biphenyls[4,5] and chlorinated dibenzofurans[6–8]. These compounds are considered relatively toxic, and few individual standards are readily available at reasonable cost. Therefore, the ability to calculate RI values for all members based upon asctual measurement of a few would be a distinct advantage.

This report presents a simple technique by which the RI of chlorinated dibenzo-*p*-dioxins can be calculated and the use of this information to resolve a variety of interesting structural problems.

EXPERIMENTAL

The retention indices of available chlorinated dioxin standards were determined on a Hewlett-Packard (Avondale, PA, U.S.A.) 5880A gas chromatograph (equipped with a flame ionization detector), splitless injector, and 60 m × 0.252 mm, I.D. (0.25 μm film thickness) DB-5 fused-silica capillary column (J & W Scientific, Rancho Cordova, CA, U.S.A.). The gas chromatograph was programmed from 170 to 340°C at 2°C/min with an initial hold of 1 min. The sample inlet valve for the splitless injector was activated at 0.01 min and deactivated at 0.65 min.

Helium was used as the carrier gas with a flow-rate at 340°C of 20.2 cm/s.

The normal C_{20}–C_{44} hydrocarbons were used (excluding C_{39}, C_{42} and C_{43}) to measure retention indices. The dioxin standards were obtained from the National Centers for Disease Control (CDC), Cambridge Isotope Labs. (Woburn, WA, U.S.A.), and Wright State University (Dayton, OH, U.S.A.).

The retention indices for the compounds were calculated using the linear retention index scale for linear temperature programmed GC runs as suggested by van Den Dool and Kratz[9].

DISCUSSION

In order to calculate the RIs for PCDDs we make the assumption that the RI for each specific isomers can be expressed as the sum of two halves of the molecule. Each dioxin isomer is divided as shown in Fig. 1.

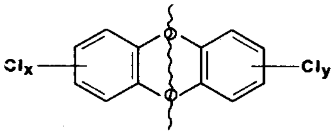

Fig. 1. Division of dioxin molecule to establish single ring indices.

This approach for the calculation of RIs was applied successfully to chlorinated biphenyls[4,5]. Because of the high degree of symmetry of the dioxin molecule, there are only ten possible "halves"; these halves and their respective half retention index values are shown in Fig. 2.

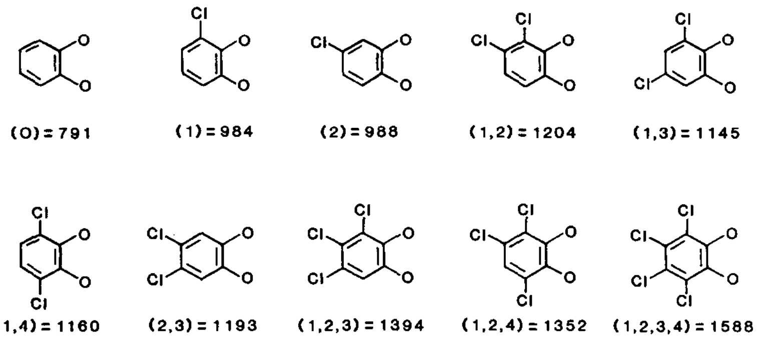

Fig. 2. Chlorinated dioxin single ring index values.

For calculation of the RI, we define the term "single-ring index" which is the numerical value each half shown in Fig. 2 contributes to the RI for the specific molecule in question. These values are also shown in Fig. 2. In addition to the single-ring index, this model requires a term for a ring-to-ring interaction when chlorine atoms occupy both the 1 and 9 positions or the 1, 4, 6 and 9 positions. The result is a simple mathematical model which describes the retention index as a function of molecular structure.

In order to determine the single-ring indices for each possible single-ring as shown in Fig. 2, and the value for the ring interaction, the retention indices for eleven carefully chosen PCDDs are required, and a simple system of eleven structure–RI relationships is developed. These relationships are shown in Fig. 3. It is immediately apparent that while 1,3,6,8- and 1,3,7,9-TCDD both have two 1,3-substituted single-rings, 1,3,7,9-TCDD elutes later due to some ring interaction effect, presumably between the 1,9 chlorines and oxygen. Experimentally this effect accounts for fourteen RI units (Fig. 3).

When two ring interactions are present (*i.e.*, all four 1, 4, 6, 9 positions occupied), a value of 21 RI units or 1.5 times the value for one ring interaction gave good correlation for available congener standards. RI = single-ring index A + single-ring index B + ring interaction effect. The eleven equations shown in Fig. 3 were chosen to permit calculation of a complete set of single ring indices from the imited set of standards which were available when this study was conducted. With the single-ring indices and terms for ring–ring interactions available it is possible to calculate the retention indices for the remaining PCDDs. The results of these calculations and experimentally determined retention indices are listed in Table I. Using eleven isomers to establish the model, 64 RIs were predicted. Fig. 4 is a plot of these calculated values against the experimental data for the 30 individual isomer standards used for testing the model. A linear correlation coefficient of 0.999 was obtained.

TESTS AND APPLICATIONS OF THE RETENTION INDICES

The TCDDs provided an initial test for this simple model. The U.S. EPA had obtained samples of each of the twenty-two TCDDs through cooperative Agreement No. CR908872-01-0 with Wright State University. These samples were intended for

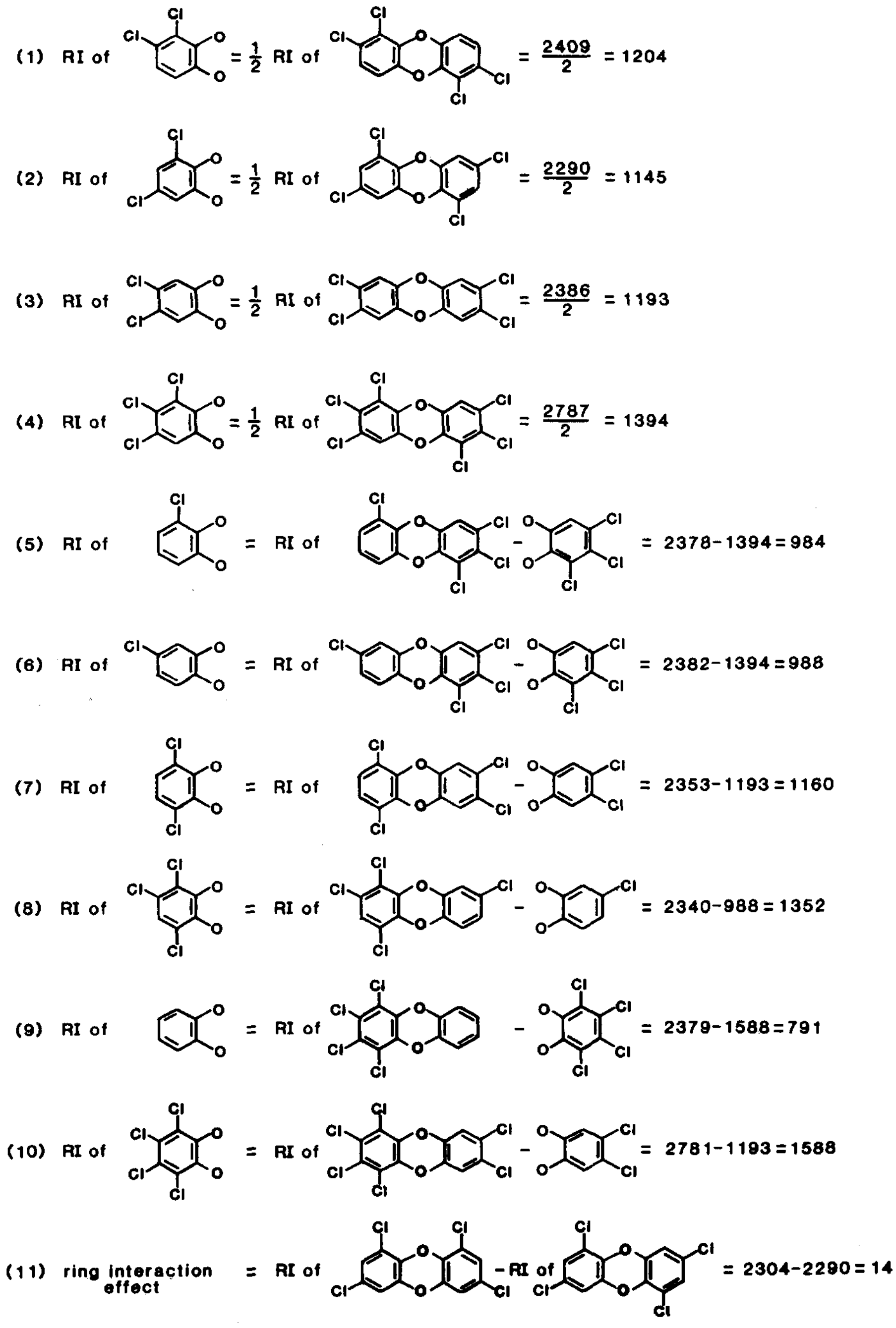

Fig. 3. Calculation procedures for single ring indices.

TABLE I

CONGENERS OF PCDDs LISTED BY INCREASING RI (CALCULATED)

Isomer	*RI*			*Isomer*	*RI*		
	Calculated	*Measured*	*Δ*		*Calculated*	*Measured*	*Δ*
1,3	1936			1,2,6,9	2378	2378	0
1,4	1951			1,2,3,4	2379	2379★	0
1,6	1968			1,2,3,7	2382	2382★	0
1,7	1972			1,2,3,8	2382	2382	0
1,8	1972			2,3,7,8	2386	2386★	0
2,7	1976	1985	−9	1,2,3,9	2392	2392	0
2,8	1976	1985	−9	1,2,7,8	2397	2400★★	−3
1,9	1982			1,2,6,7	2408	2408★	0
2,3	1984	1993	−9	1,2,8,9	2422	2428	−6
1,2	1995			1,2,4,7,9	2511	2501	+10
1,3,6	2129			1,2,4,6,8	2511	2501	+10
1,3,7	2133			1,2,4,6,9	2533		
1,3,8	2133			1,2,3,6,8	2539		
1,3,9	2143			1,2,4,7,8	2545		
1,2,4	2143	2152	−9	1,2,3,7,9	2553		
1,4,8	2148			1,2,3,6,9	2568		
1,4,9	2158			1,2,4,6,7	2570		
1,7,8	2177			1,2,4,8,9	2570		
2,3,7	2181			1,2,3,4,7	2576	2573	+3
1,2,3	2185			1,2,3,4,6	2586		
1,2,6	2188			1,2,3,7,8	2587	2587	0
1,2,7	2192			1,2,3,6,7	2598	2604	−6
1,2,8	2192			1,2,3,8,9	2612	2623	−11
1,2,9	2202			1,2,4,6,8,9	2725	2713	+12
1,3,6,8	2290	2290★	0	1,2,4,6,7,9	2725	2713	+12
1,3,7,9	2304	2304★	0	1,2,3,4,6,8	2747	2742	+5
1,3,6,9	2319	2315	+4	1,2,3,6,8,9	2760		
1,3,7,8	2338	2340	−2	1,2,3,6,7,9	2760		
1,2,4,7	2340	2340	0	1,2,3,4,6,9	2769		
1,2,4,8	2340	2340★	0	1,2,3,4,7,8	2781	2781★	0
1,4,6,9	2341	2341	0	1,2,3,6,7,8	2788	2788★	0
1,2,6,8	2349	2349★★	0	1,2,3,7,8,9	2802		
1,2,4,6	2350	2346	+4	1,2,3,4,6,7	2806	2812	−6
1,2,4,9	2350	2346	+4	1,2,3,4,6,7,9	2961	2949	+12
1,4,7,8	2353	2353★	0	1,2,3,4,6,7,8	2996	2994	+2
1,2,7,9	2363	2364★★	−1	1,2,3,4,6,7,8,9	3197	3196	+1
1,2,3,6	2378	2378★	0				

★ Used to develop the model.

★★ See text for basis of assignment.

use as environmental monitoring standards and therefore structural assignment verifications were needed.

Syntheses of these TCDD standards were accomplished by the self or crossed condensations of appropriate potassium chlorophenates. Such syntheses have been reported by Buser and Rappe[10,11], Nestrick and co-workers[12,13] and Taylor *et al.*[14]. Structure determinations of the products, however, and matching of chromatograph-

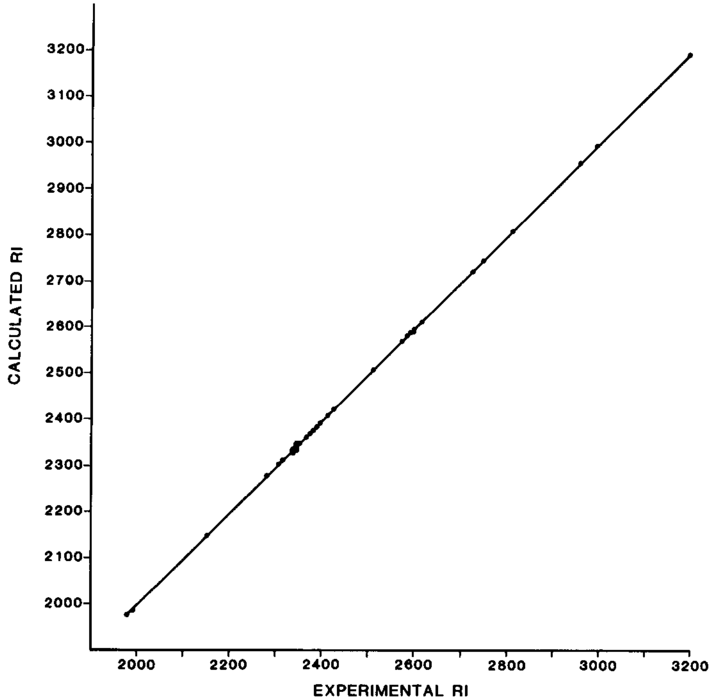

Fig. 4. Calculated RI *vs.* experimental RI of dichlorodibenzodioxin through octachlorodibenzodioxin.

ic relative retention times [gas and high-performance liquid chromatographic (HPLC)] with specific isomers were complicated due to a competing reaction pathway known as the Smiles rearrangement. The intermediate postulated for this rearrangement positions one ring perpendicular to the other; final products result from a second 90° rotation, in either direction (Fig. 5). For example when 2,3,6-trichlorophenol is allowed to self-condense the products may be envisioned as arising from a "normal" condensation (1,4,6,9; 1,2,6,9; 1,2,6,7) or from the spirocyclic intermediate (1,2,8,9; 1,2,6,9)[12].

The suspected high toxicities of these compounds made large scale isolation and structure determination by conventional techniques a matter of concern, so indirect methods were applied to determine the substitution patterns of specific TCDDs. Relative retention times for all 22 TCDDs on non-polar and polar GC columns[10–14] and on normal and reversed-phase high-performance liquid chromatographic (HPLC) columns[12–14] have been reported and used in developing structure

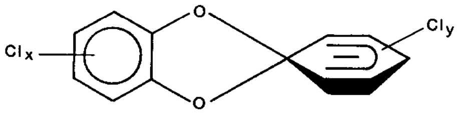

Fig. 5. Smiles rearrangement intermediate.

assignments. Published results were in agreement on the assignments for 19 of the 22 TCDDs, based upon synthesis, relative retention times and photolytic degradation and pattern recognition techniques. The proposed assignments by different research groups for 1,2,7,8-, 1,2,7,9- and 1,2,6,8-TCDDs were not in agreement (see Table II). This was assumed to be due to the relationship of the 1,2,7,9- and 1,2,6,8-TCDDs by the Smiles rearrangement (Fig. 5), and complications resulting from impure phenols often used in the synthesis of 1,2,7,8-TCDD.

TABLE II

TCDD STRUCTURE ASSIGNMENTS

Label	*RRT* (DB-5)*	*RRT* (SP-2330)*	*Old, alternate assignment***	*Current assignment***
1,2,7,8 (1,2,7,9)	0.97	0.97	1,2,7,8 (first)	1,2,6,8 (first)
1,2,6,8 (1,2,7,8)	0.98	1.03	1,2,6,8 (middle)	1,2,7,9 (middle)
1,2,7,9 (1,2,6,8)	1.01	1.07	1,2,7,9 (last)	1,2,7,8 (last)

* Relative to 2,3,7,8-TCDD.
** Order of GC elution in parentheses.

A plot (Fig. 6) of the calculated retention indices *versus* the observed retention indices for the nineteen TCDDs whose structures were in agreement resulted in a straight line with a correlation coefficient of 0.998. In view of this excellent correlation we were able to check the structure assignments for the isomers in question by testing each research group's set of data for internal consistency (Figs. 6 and 7). Plots of each group's assignment *versus* the calculated RI values for the TCDDs revealed that each data set was internally consistent (*i.e.*, the plot was linear) if one choice of structure assignments was used, but linearity was not obtained on any of the data sets if other isomer assignments were used (Table II).

After this data analysis was performed, an authentic standard of one isomer in question (1,2,7,8-TCDD) was purchased (Cambridge Isotope Labs.). This standard was synthesized by a method which only yielded that one isomer[15]. This authentic 1,2,7,8-TCDD was found to have the same retention index as that predicted for the assignment which resulted in a linear data set. Conversations regarding the alternate isomer assignments (Table II) with a colleague in the field[16] (who generously checked the U.S. EPA standards and an authentic 1,2,7,8-TCDD standard) led to agreement with our assignments for the three TCDDs in question. Hence, for the first time the principal investigators reached agreement upon a single set of assignments for the TCDDs.

The synthesis, structure assignments, and chromatographic relative retention times (RRT) of penta- through octachlorodibenzo-*p*-dioxins have been reported by several groups[17–21]. The published experimental conditions for GC relative retention time include multiple GC phases using both isothermal and temperature programmed

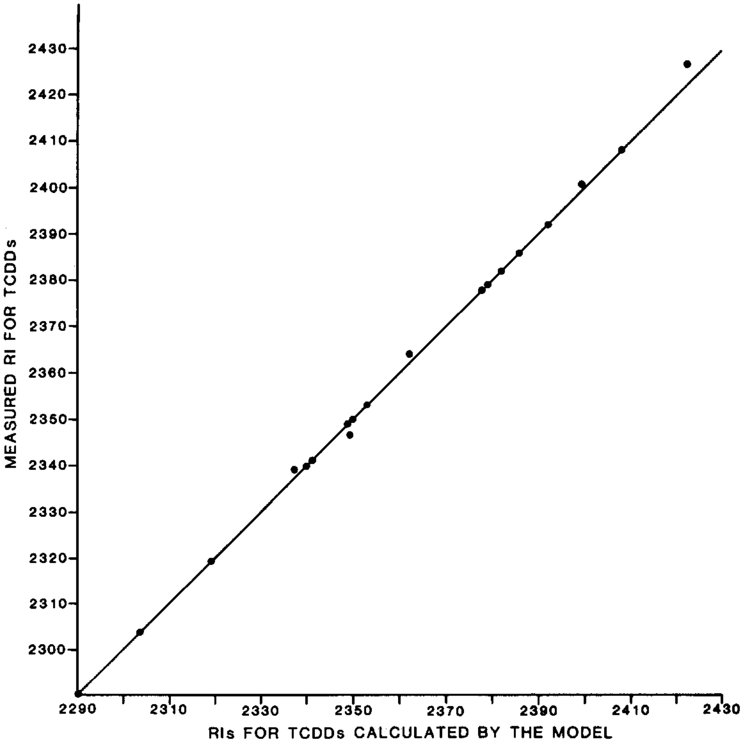

Fig. 6. Calculated RI *vs.* experimental RI for TCDDs.

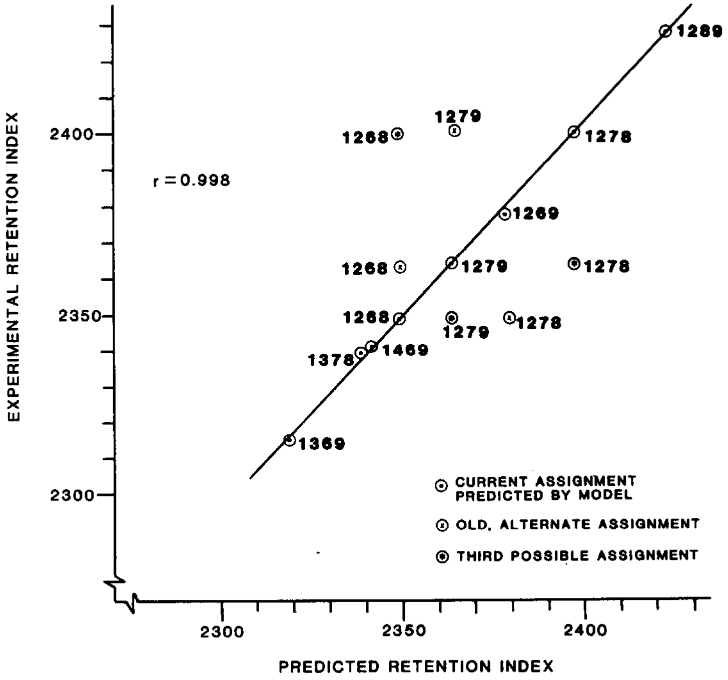

Fig. 7. (2,2)Substituted TCDDs. 13 Isomers: 5 used for model and 8 predictions.

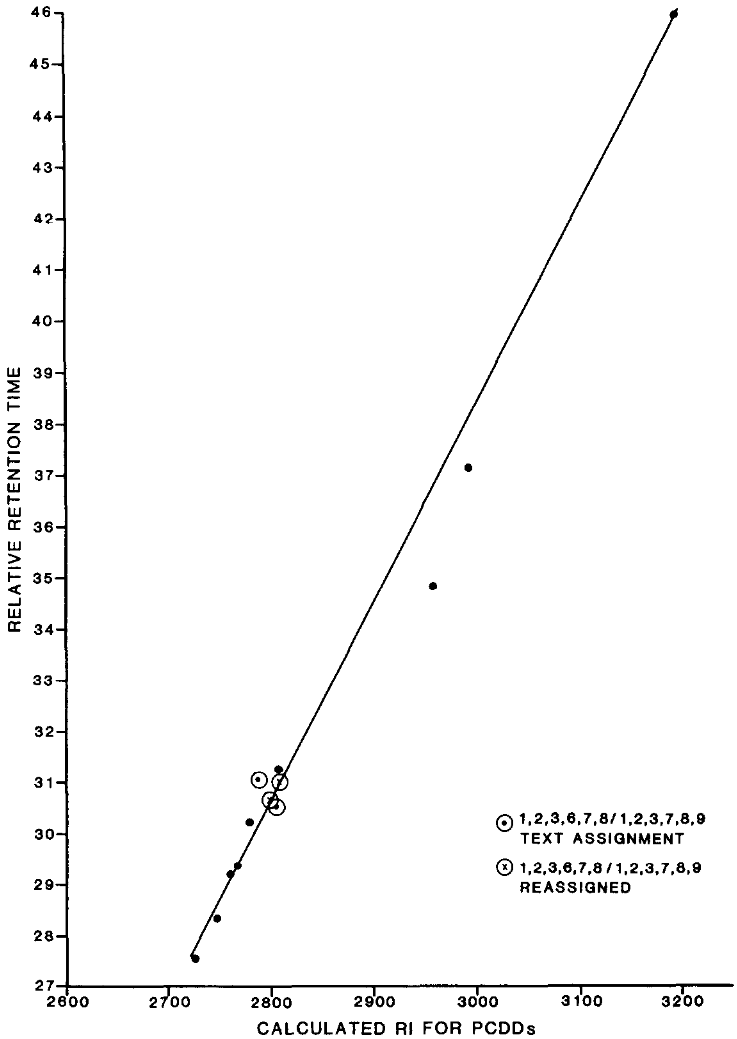

Fig. 8. RRT *vs.* calculated RI for PCDDs. From ref. 17.

conditions. Certain isomers shift elution ordering significantly when GC column polarity is increased from non-polar to very polar, but retention order is apparently constant with non-polar and slightly polar phases. Fortuitously, analyses of these more highly chlorinated congeners of dibenzo-*p*-dioxin are almost invariably performed with relatively non-polar chromatographic phases, since polar phases cause their retention times to increase greatly. While caution must be exercised when comparing results from different chromatographic phases and different temperature programs, we have plotted the calculated retention indices (obtained from a non-polar DB-5 column) against the relative retention times reported by various workers for these compounds. If this analysis is restricted to relatively non-polar columns it pro-

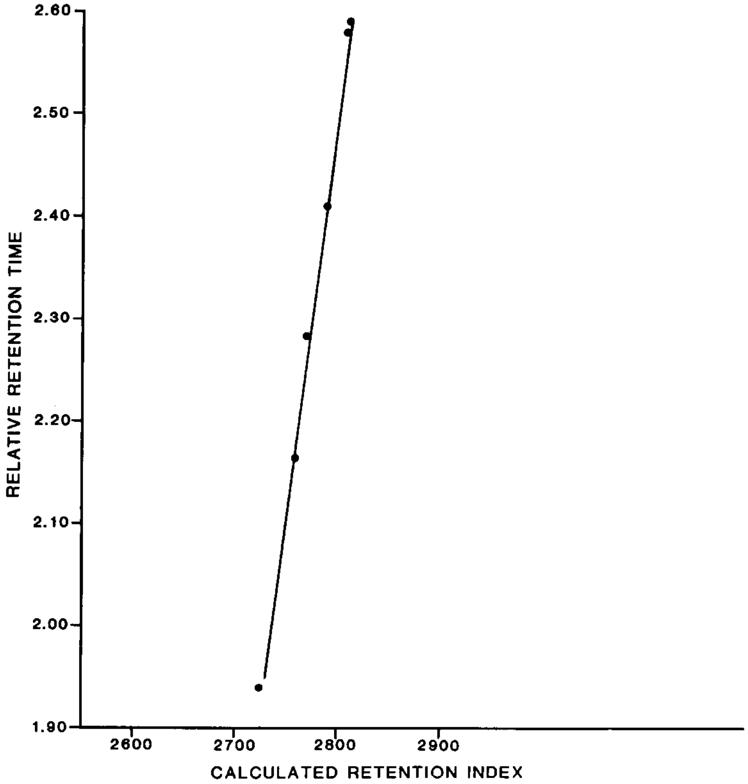

Fig. 9. RRT *vs.* calculated RI for PCDDs. From ref. 19.

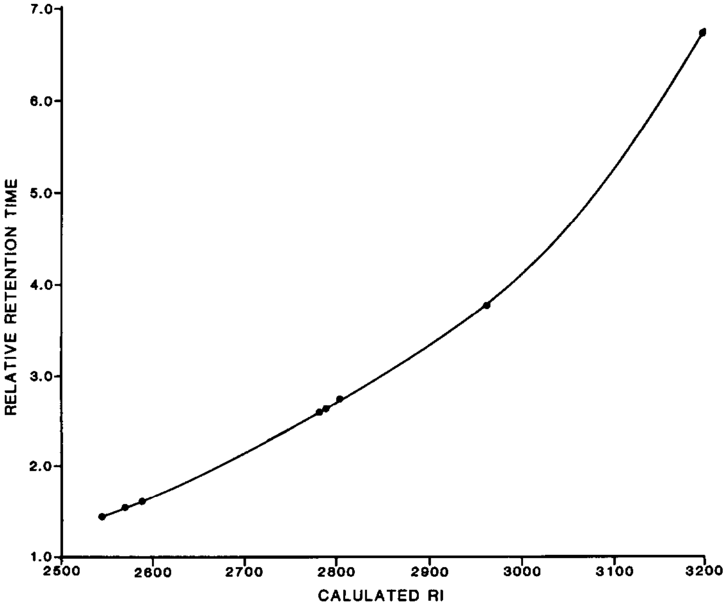

Fig. 10. RRT *vs.* calculated RI for PCDDs. From ref. 20.

vides a method by which peak assignments may be tested for internal consistency. We show such plots in Figs. 8–11.

Fig. 8 is a plot of the calculated retention indices against relative retention times obtained by Buser[17], on an OV-61 glass capillary column using a temperature program of 180 to 240°C at 2°C/min. This analysis reveals that either the retention times for the 1,2,3,6,7,8 and 1,2,3,7,8,9 isomers (related by the Smiles rearrangement) are reversed from the predicted order, or the structure assignments should be reversed. In 1980 Cantrell demonstrated that the structure assignments should be reversed[18].

Fig. 9 is a plot of the calculated retention indices *versus* relative retention times obtained by Lamparski and Nestrick[19] on a 0.6% OV-17 silicone + 0.40% Poly S-179 on 80–100 mesh column, run isothermally at 280°C. Note that the structure assignments are consistent with predicted retention times, and in the instances where coelution is predicted, the compounds coelute (1,2,4,6,7,9 and 1,2,4,6,8,9) or nearly so (1,2,3,6,7,9 and 1,2,3,6,8,9; ΔRRT = 0.006 min). Note also that this technique predicts that the 1,2,3,6,7,8 structure should be assigned to the peak eluting at 2.410

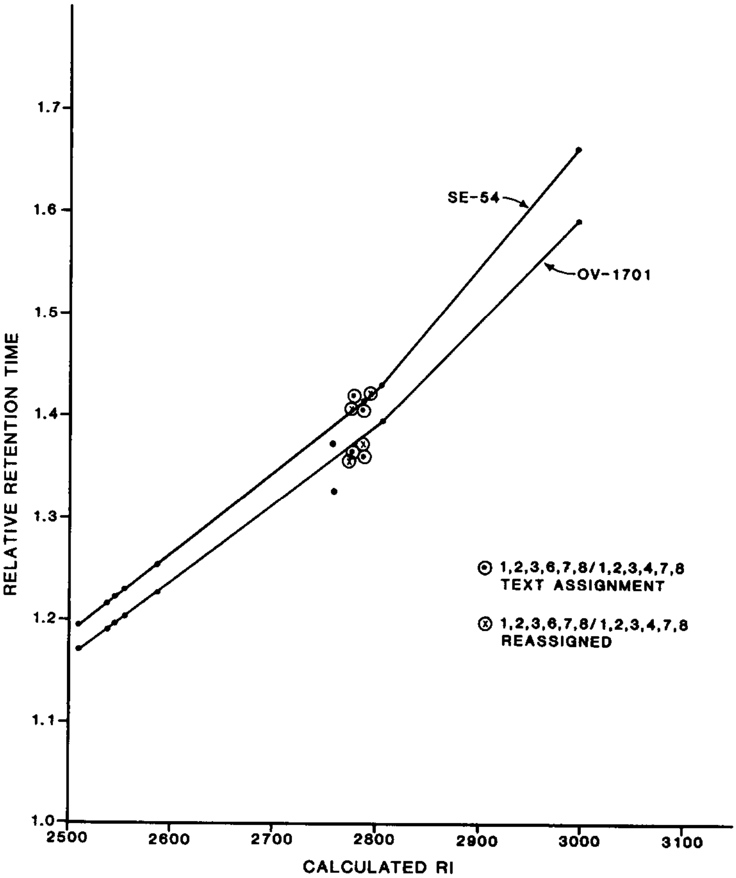

Fig. 11. RRT *vs.* calculated RI for PCDDs. From ref. 21.

min in their study. At that time they were not able to distinguish between 1,2,3,6,7,8 and 1,2,3,7,8,9 substituted isomers.

Fig. 10 is a plot of the calculated retention indices *versus* the relative retention times obtained by Korfmacher and Mitchum[20] on a 50-m fused-silica column (226°C, isothermal). While non-linear, the plot is a smooth continuous function. Since the RIs presented here were developed for temperature programmed GC, a non-linear function may be a reasonable result when isothermal GC data are plotted against these RI values.

Fig. 11 is a plot of our calculated retention indices against the relative retention times obtained by Humppi and Heinola[21] on both SE-54 and OV-1701. We note that two pairs of isomers which are related by the Smiles rearrangement are predicted to coelute; coelution was observed for both pairs (1,2,4,6,8/1,2,4,7,9 and 1,2,3,6,7,9/1,2,3,6,8,9) on both columns. We also note that the retention times for two of the compounds are reversed from those predicted by this model (1,2,3,6,7,8 and 1,2,3,4,7,8).

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