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Frontier density pattern of dioxins

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Abstract

Ab initio molecular orbital calculations are performed to estimate the electron densities of the highest occupied molecular orbitals (HOMO) of 75 congeners of chlorinated dibenzo-*p*-dioxins and a nonchlorinated dibenzo-*p*-dioxin. Electron densities of HOMO on out of plane π orbitals of 12 carbons and two oxygens in the dioxin structure are used as variables in multivariate statistical analysis. Principal component analysis can classify 76 congeners of dioxins according to the principal component scores. All of the most toxic dioxins are involved in the group that has large negative values for both the first and the third principal component scores.

Keywords: Dioxins; Frontier density; Multivariate statistical analysis; Toxicity

1. Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) are members of a class of polyhalogenated hydrocarbons. Dioxins are known as the most dangerous compounds that human beings have ever synthesized. Among 75 congeners of chlorinated dibenzo-*p*-dioxins, 2,3,7,8-tetrachlorinated dibenzo-*p*-dioxin (2,3,7,8-T_4CDD) has strong toxicity. Dioxins induce both acute and chronic toxic effects. The LD_{50} of 2,3,7,8-T_4CDD is about 0.6 μg/kg for a guinea pig. Typical acute toxic effects are liver damage, chloracne, kidney abnormalities, etc. Carcinogenic and teratogenic effects are the most dangerous chronic toxicity of dioxins [1]. As dioxins are stable compounds, they accumulate mainly in fatty tissues of the human body. In some countries, the concentration of dioxins in mother's milk is not negligible. Recently, it has been confirmed that dioxin is a cancer hazard. Furthermore dioxin is suspected to be the endocrine disrupter [2]. Exposure to dioxin can cause severe reproductive and developmental problems (at levels 100 times lower than those associated with its cancer causing effects). Dioxin can also cause immune system damage and interfere with regulatory hormones.

The mechanism of toxic action of dioxin is based on analysis of the induction of microsomal aryl hydrocarbon hydroxylase (AHH) activity [3–5]. In view of structure activity relationship, dioxin congeners, which induce aryl hydrocarbon hydroxylase (AHH) activity, have two common properties: (1) planer structure; (2) halogen atoms occupy in at least three of the four lateral positions (positions 2, 3, 7, 8). And, for maximal potency, four halogen atoms in lateral positions [3,5].

In this study, we have investigated the relationship between structures and toxicity of dioxins by theoretical calculations. It is well known that the frontier electrons of the molecule are often responsible for the reactivity and molecular properties [6,7]. Recently the author has studied the relationship between structures and toxicity of dibenzofurans by their frontier density pattern [8]. In this work we focused on the electron density pattern of frontier orbitals, especially, HOMO of dioxins. Ab initio MO calculations are performed to obtain the electron densities of HOMO for 75 congeners of chlorinated dibenzo-*p*-dioxins and a nonchlorinated dibenzo-*p*-dioxin. These data are used to execute multivariate statistical analysis. Principal component analysis is used to extract the character of dioxins by their principal scores.

In this paper, a systematic number, which correlates with the substitution pattern of congeners, suggested by Ballschmiter et al. [9] is used to identify 76 congeners of dioxins.

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2. Methods

The dioxin structure with the labels for 12 carbons and two oxygens is shown in Fig. 1. Ab initio MO calculations for 75 congeners of chlorinated dibenzo-*p*-dioxins and a nonchlorinated dibenzo-*p*-dioxin are performed. The nonchlorinated dioxin is full optimized. This structure is used as dibenzo-*p*-dioxin skeleton. For chlorinated dioxins, chlorine atoms are adequately substituted to dibenzo-*p*-dioxin skeleton, where C–Cl distance and C–H distance are fixed to be 1.784 and 1.082 Å, respectively. GAUSSIAN 98 program systems [10] are used with the STO-3G basis sets. In order to obtain the frontier electron densities, the program is partly modified. A typical electron density distribution of HOMO of 1,2,6-trichlorinated dibenzo-*p*-dioxin (T_3CDD) is shown in Fig. 2. Largest densities of 0.349 and 0.329 are on oxygen atoms O_5 and O_{10}. 97.7% of HOMO densities of 1,2,6-T_3CDD are on 12 carbons and two oxygens. For all of the dioxin congeners, we have confirmed that the HOMO densities of dioxins are completely constructed from out of plane π electron densities of carbons, oxygens and chlorines. For 75 chlorinated dioxins, 95.6% of HOMO densities are on carbons and oxygens on average (max. is 99.8% and min. is 90.3%). From this we can see that the HOMO densities are large enough localized on carbons and oxygens. This will allow us to perform a multivariate statistical analysis by using only HOMO densities on carbons and oxygens as variables.

The alignment of the dioxin molecule will affect the results of multivariate statistical analysis. For dibenzofurans we have suggested the suitable method to exclude the arbitrariness from the alignment rule [8]. For dioxins we will use the similar method as follows. If we adopt the different alignment rule, 1,2,6-T_3CDD is the same with 1,6,7-T_3CDD, 3,4,9-T_3CDD and 4,8,9-T_3CDD. As shown in Fig. 2, considering the symmetry, only eight variables (X_1, X_2, X_3, X_4, X_a, X_b, X_5, X_{10}) are needed to describe the HOMO densities of 1,2,6-T_3CDD. Eight variables have four different data sets to identify the HOMO densities of 1,2,6-T_3CDD. That is, the first data set correspond to the HOMO densities on C_1, C_2, C_3, C_4, C_a, C_b, O_5, O_{10}, the second one is on C_4, C_3, C_2, C_1, C_b, C_a, O_{10}, O_5, the third one is C_9, C_8, C_7, C_6, C_d, C_c, O_5, O_{10} and the fourth one is C_6, C_7, C_8, C_9, C_c, C_d, O_{10}, O_5. Concrete values of the four data sets of 1,2,6-T_3CDD (#15-1, #15-2, #15-3 and #15-4) are also shown in Fig. 2. These four

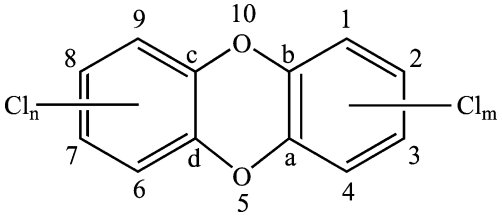
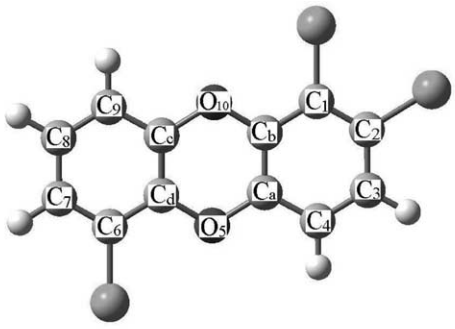
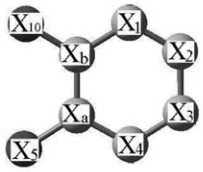


Fig. 1. Structure and labeling of a dibenzo-*p*-dioxin molecule. *m*, *n* = 1–4 for polychlorinated dibenzo-*p*-dioxins. *m*, *n* = 0 for nonchlorinated dibenzo-*p*-dioxin.



	C_1	C_2	C_3	C_4	C_a	C_b	O_5
HOMO densities	0.011	0.124	0.112	0.025	0.171	0.160	0.349
	C_c	C_d	C_6	C_7	C_8	C_9	O_{10}
HOMO densities	0.184	0.189	0.017	0.135	0.132	0.015	0.329



Variable	X_1	X_2	X_3	X_4	X_a	X_b	X_5	X_{10}
	C_1	C_2	C_3	C_4	C_a	C_b	O_5	O_{10}
#15-1	0.011	0.124	0.112	0.025	0.171	0.160	0.349	0.329
	C_4	C_3	C_2	C_1	C_b	C_a	O_{10}	O_5
#15-2	0.025	0.112	0.124	0.011	0.160	0.171	0.329	0.349
	C_9	C_8	C_7	C_6	C_d	C_c	O_5	O_{10}
#15-3	0.015	0.132	0.135	0.017	0.189	0.184	0.349	0.329
	C_6	C_7	C_8	C_9	C_c	C_d	O_{10}	O_5
#15-4	0.017	0.135	0.132	0.015	0.184	0.189	0.329	0.349

Fig. 2. HOMO densities of 1,2,6-trichlorinated dibenzo-*p*-dioxin (T_3CDD) (#15) on carbon and oxygen atoms. Variables and data sets for multivariate statistical analysis are also shown.

data sets are equally treated as independent data sets in multivariate statistical analysis. In this study we use the principal component analysis (PCA) which is one of the useful methods of multivariate statistical analysis [11]. As each of 304 samples (76 congeners × 4 data sets) have eight variables, PCA is performed for the data group of 304 samples × 8 variables. The correlation matrix of the data group is diagonalized in PCA. The eigenvectors are called principal components. The principal components, which have large eigenvalues, are used to characterize the data group. In this study, principal component is linear

Table 1
Toxic equivalency factors (TEFs) of dioxin congeners

No.	Structure	TEF[a]
48	2,3,7,8-T_4CDD	1
54	1,2,3,7,8-P_5CDD	1
66	1,2,3,4,7,8-H_6CDD	0.1
67	1,2,3,6,7,8-H_6CDD	0.1
70	1,2,3,7,8,9-H_6CDD	0.1
73	1,2,3,4,6,7,8-H_7CDD	0.01
75	1,2,3,4,6,7,8,9-OCDD	0.0001

[a] From Ref. [12].

Table 2
Correlation matrix of eight variables in principal component analysis

	X_1	X_2	X_3	X_4	X_a	X_b	X_5	X_{10}
X_1	1.000							
X_2	−0.293	1.000						
X_3	0.023	0.923	1.000					
X_4	−0.818	0.023	−0.293	1.000				
X_a	−0.391	0.965	0.891	0.149	1.000			
X_b	0.149	0.891	0.965	−0.391	0.812	1.000		
X_5	−0.516	0.183	−0.086	0.749	0.264	−0.073	1.000	
X_{10}	0.749	−0.086	0.183	−0.516	−0.073	0.264	−0.231	1.000

combination of eight variables X_1–X_{10} that are related to HOMO densities of dioxins. The coefficients of each principal component are used to interpret the character of the principal component. The score of the principal components of each congener of dioxins are also calculated. Each dioxin has four scores for each principal component. We sum these four scores to obtain total scores. Each dioxin has one total score for each principal component that is used in further discussions.

3. Results and discussion

3.1. Toxicity of dioxins

Toxic equivalency factors (TEFs) are useful concept to estimate the toxicity of dioxins. The TEF concept is the most plausible and feasible approach for risk assessment of halogenated aromatic hydrocarbons with dioxin-like properties. TEF is unit to evaluate the intensity of toxicity of dioxins based on the toxicity of 2,3,7,8-T_4CDD that is taken as 1 unit. World Health Organization (WHO) [12] reported the TEFs of dioxins for mammals, fish and birds as shown in Table 1. Due to insufficient environmental and toxicological data, TEFs are only established for the 2,3,7,8-substituted PCDDs and PCDFs and the non- and momo-*ortho* PCBs.

3.2. Principal component analysis

Table 2 shows the correlation matrix among eight variables. Seven pairs show large correlations (>0.8). That is, X_1 and X_4 (−0.818), X_2 and X_3 (0.923), X_2 and X_a (0.965), X_2 and X_b (0.891), X_3 and X_a (0.891), X_3 and X_b (0.965), X_a and X_b (0.812). We can see that variables X_2, X_3, X_a and X_b correlate positively with each other. This means that a dioxin which has a large HOMO density on C_2 (C_8) atom tends to have large HOMO densities on C_3 (C_7), C_a (C_d) and C_b (C_c), vice versa. Table 3 shows the eigenvalues and coefficients of the three principal components, which have eigenvalues are larger than 0.5. From now, we use these three principal components (PC1, PC2 and PC3) as main principal components to analyze the data. For PC1,

Table 3
Coefficients of the principal component transformation

	PC1	PC2	PC3
Eigenvalue	3.771	3.027	0.854
X_1	−0.068	0.537	0.240
X_2	0.504	−0.100	−0.053
X_3	0.504	0.100	−0.053
X_4	−0.068	−0.537	0.240
X_a	0.490	−0.155	0.031
X_b	0.490	0.155	0.031
X_5	0.042	−0.422	0.662
X_{10}	0.042	0.422	0.662

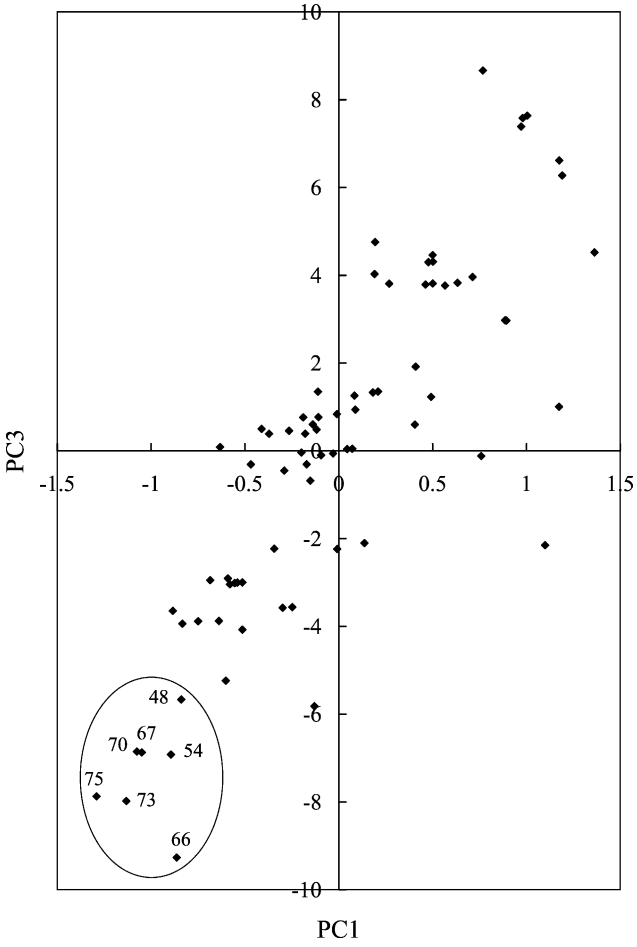


Fig. 3. Principal component analysis on HOMO densities. Projection of the scores on the plane of the first principal component (PC1) vs. the third principal component (PC3). Several dioxins are identified by the numbering in Table 1.

Table 4
Top 10 of the dioxin congeners which have the lowest values of total scores of the first and the third principal components

Rank no.	Congener no.	Total scores of PC1 (score #1)	Congener no.	Total scores of PC3 (score #3)
1	75	−1.290	66	−9.266
2	73	−1.132	73	−7.979
3	70	−1.076	75	−7.871
4	67	−1.049	54	−6.919
5	54	−0.894	67	−6.871
6	74	−0.885	70	−6.849
7	66	−0.863	50	−5.818
8	48	−0.839	48	−5.665
9	68	−0.834	60	−5.237
10	69	−0.749	63	−4.076

Table 5
Top 10 of the dioxin congeners which have the smallest values of sum of HOMO densities on eight carbons (C_2, C_3, C_7, C_8, C_a, C_b, C_c and C_d) and two oxygens (O_5 and O_{10})

Rank no.	Congener no.	Sum of HOMO densities on eight carbons	Congener no.	Sum of HOMO densities on two oxygens
1	75	1.1802	66	0.6155
2	73	1.1911	73	0.6232
3	70	1.1981	75	0.6251
4	67	1.1989	54	0.6255
5	74	1.2061	67	0.6272
6	66	1.2066	70	0.6273
7	54	1.2102	50	0.6301
8	68	1.2116	48	0.6301
9	69	1.2130	60	0.6337
10	48	1.2176	29	0.6398

variables X_2, X_3, X_a and X_b have large coefficients of 0.504, 0.504, 0.490 and 0.490, respectively. For PC2, variables X_1, X_4, X_5 and X_{10} have large coefficients of 0.537, −0.537, −0.422 and 0.422, respectively. For PC3, variables X_5 and X_{10} have large coefficients of 0.662.

3.3. Principal component scores

To extract the character of each congener of dioxins we have calculated principal component scores of three main principal components for 76 dioxin congeners. As the score of each sample for the principal component is inner product between the coefficients of the principal component and the standardized data set of each sample, generally speaking large coefficients in each principal component contribute much in calculating the score. In this study each dioxin congener has four scores for each principal component. We sum these four scores to obtain total scores for each dioxin. Plots of scores of the first principal component vs. those of the third are shown in Fig. 3 with the positions of the most toxic dioxins (#48, #54, #66, #67, #70, #73 and #75). In this graph, all of the most toxic dioxins have large negative values for both PC1 and PC3 axes. In Table 4, top 10 dioxins of the lowest total scores of the first and the third principal components are shown. For both score #1 and score #3, all of the most toxic 7 dioxins (#66, #73, #75, #54, #67, #70 and #48) rank within the top eight.

As the coefficients of PC1 have large values for the variables X_2, X_3, X_a and X_b, low values of score #1 will be ascribed to relatively small values of HOMO densities on carbons (C_2 (C_8), C_3 (C_7), C_a (C_d) and C_b (C_c)). Similarly, as the coefficients of PC3 have large values for the variables X_5 and X_{10}, low values of score #3 will be ascribed to relative small values of HOMO densities on oxygens (O_5 and O_{10}). So we have calculated the sum of the HOMO density values on eight carbons (C_2, C_3, C_7, C_8, C_a, C_b, C_c and C_d) and two oxygens (O_5 and O_{10}), respectively. The top 10 dioxins that have the smallest sum of HOMO densities on eight carbons and on two oxygens are shown in Table 5. All of the toxic dioxins are included in the top 10 for both the smallest density values on eight carbons and on two oxygens.

4. Conclusions

Seventy-six congeners of dioxins are classified by the principal component analysis using HOMO densities as variables. All of the most toxic dioxins are included in the group that has large negative score values for both the first and the third principal components. The first principal component related to HOMO densities on eight carbons and the third principal component related to HOMO density on oxygens in dioxin structure. It was found that the toxic dioxins have relatively small total electron densities of HOMO on carbons and oxygens. Quantum chemometrical approach suggested in this work will be one of the useful theoretical methods to study toxicity of dioxin-like compounds.

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