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Electrostatic Potentials and CoMFA Analysis of Toxicity of Dioxins

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Abstract

The structures of dioxins were optimized by the ab initio molecular orbital method at the HF/6-31G* level with the Gaussian 98 program package, and then electrostatic potentials were mapped out to explore the pharmacophore image of the receptor complementary to that. The maps were additive on the constitutional atoms, showing similarly the negative values around the oxygen and chlorine atoms and the positive values around the carbon and hydrogen atoms; and with the similarity of the molecular shapes, the applicability of the Comparative Molecular Field Analysis (CoMFA) was anticipated. Total 18 compounds, to which the toxic equivalency factors (WHO-TEF) are given, were used for CoMFA. These compounds consist of three congeners: polychlorinated dibenzo-para-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar poly-chlorinated biphenyls (Coplanar PCBs). CoMFA was executed by using the receptor binding avidities (EC_{50}) as the activity. The receptor was rat hepatic cytosol aryl hydrocarbon receptor (AhR). The structures were superimposed by fitting pairs of atoms so as to minimize RMS of the distances. As the template molecule 1,2,3,7,8-PeCDD was selected. PCDFs were superimposed to PCDDs in planar position, but twisted PCBs went through from one side of PCDD to the other side. Electrostatic potential derived charges of CHelpG by ab initio HF/6-31G* calculations were given to the structures. Partial least squares gave a cross-validated correlation coefficient q^2 = 0.955 at the number of components 3. From the correlation, extrapolation to the higher value of bromine derivatives as 2,3,7,8-TBrDD and interpolation to the lower value as 1,3,7,8-TCDD were done.

Key Words: Electrostatic Potential, Comparative Molecular Field Analysis, CoMFA, Toxicity, ab initio Molecular Orbital, Dioxin, Aryl Hydrocarbon Receptor, AhR

Area of interest: Molecular Recognition

1. Introduction

The dioxins are considered to be endocrine disruptors, the so-called environmental hormones. The toxicities of these compounds as the induction of drug metabolizing enzyme P-450 type CYP1A1, are brought about by their binding to the aryl hydrocarbon receptor (AhR) as the initial step. Although AhR resembles the steroid hormone receptor ShR in its' mode of action, it is not a member of the ShR family, but another receptor-type transcription factor [1]. Its' endogenous ligand is not known. Its' binding to dioxins, the mode of action, and amino acid sequences [2] have been elucidated, but its' three dimensional structure has not yet been analyzed. By calculating the electrostatic potentials of dioxins, we have attempted from the ligand side to explore the pharmacophore image of the receptor complementary to that, and to execute the quantitative structure activity relationship by CoMFA [3].

2. Used Dioxins

Dioxins used for the calculation of the electrostatic potentials are shown in Figure 1. There are a total of 29 compounds of three congeners of dioxins: 7 polychlorinated dibenzo-para-dioxins (PCDDs), 10 polychlorinated dibenzofurans (PCDFs), 4 non-ortho coplanar poly-chlorinated biphenyls and 8 mono-ortho coplanar poly-chlorinated biphenyls (Coplanar PCBs) to which the toxic equivalency factors are given. (WHO-TEF [4], relative toxicity to that of the strongest 2,3,7,8-TCDD is taken as 1)

Among the 29 compounds, 18 compounds for which the receptor binding avidity EC_{50} of dioxins to the AhR of rat hepatic cytosol are given [5], were used for the calculation of CoMFA. Table 1 lists WHO-TEF and the receptor binding avidity (EC_{50}) of 18 compounds in Figure 1: polychlorinated dibenzo-*p*-dioxins (PCDDs) (1-3,7), polychlorinated dibenzofuranes (PCDFs) (8-12,14) and coplanar poly-chlorinatedbiphenyls (Coplanar PCBs) (19-20, 22-26, 28).

3. Optimization of Structure by *ab initio* Molecular Orbital

3.1 Optimization of Structure

The structures modeled by the Chem3D Pro [6] package using the MM2 force field were saved as MDL's Mol format, converted to Protein Data Bank's format, and then to the input coordinates of Gaussian's Z-matrix by means of the NewZMat utility.

The structures, optimized by the *ab initio* molecular orbital method at the HF/6-31G* level with the Gaussian 94 program operating on the supercomputer (Fujitsu vpp300) of The Japan Science and Technology Corporation were used [7].

The two benzene rings of PCDDs incline slightly toward each other to the line O-O connecting two oxygen atoms of the para dioxin ring, the inclination of OCDD is the largest at 14.88˚. As for TCDD, the calculation with the same basis set is reported [8] with their vibrational property. The

two benzene rings of PCBs twist considerably around the C-C bond connecting the two rings, 45.6˚, in non-ortho PCB and 68.7˚, in mono-ortho PCB. The result of PCBs is as seen in those of gas phase [9]. In crystals there is no twist between biphenyls but coplanar as they are so called [10].

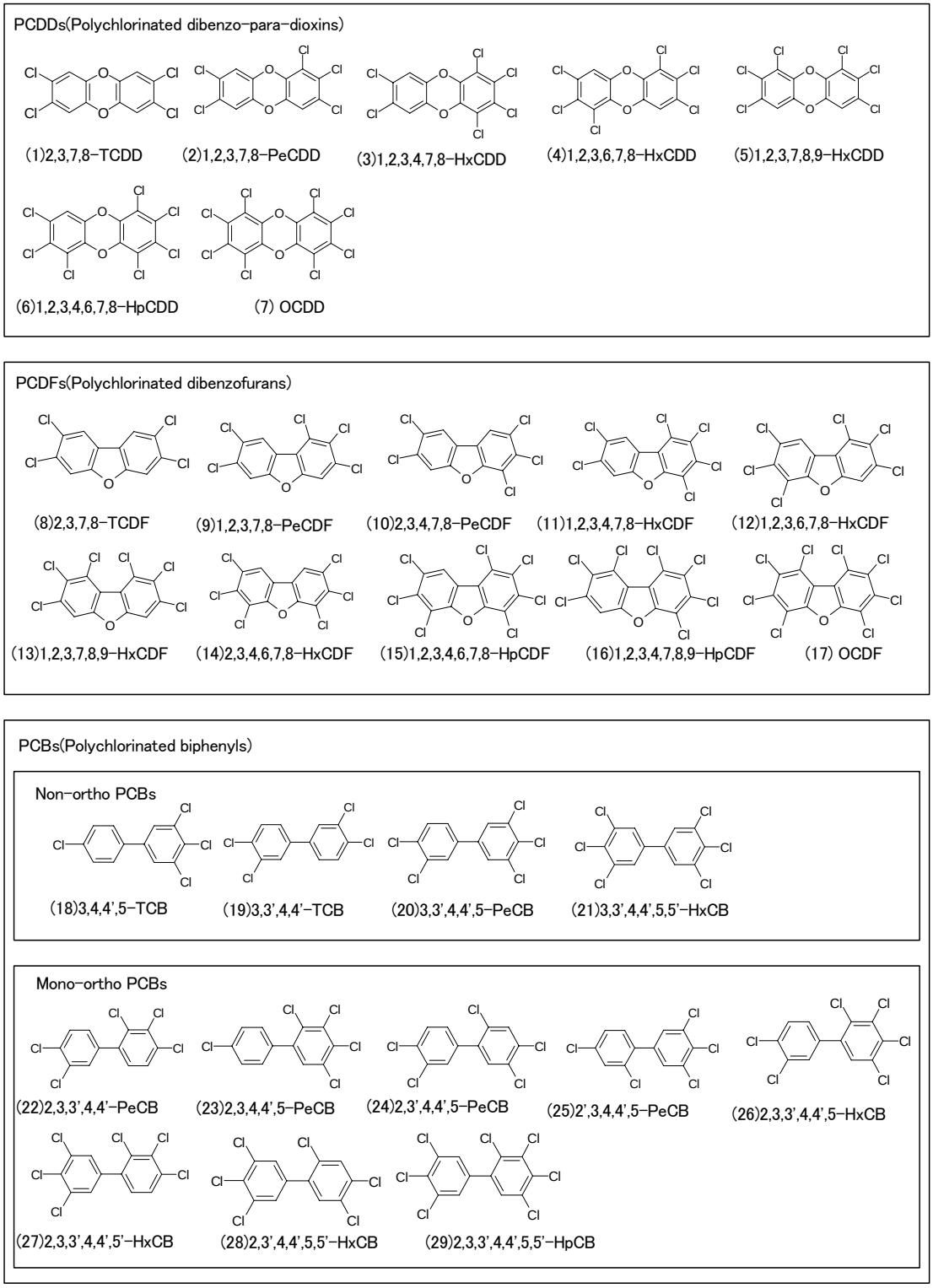
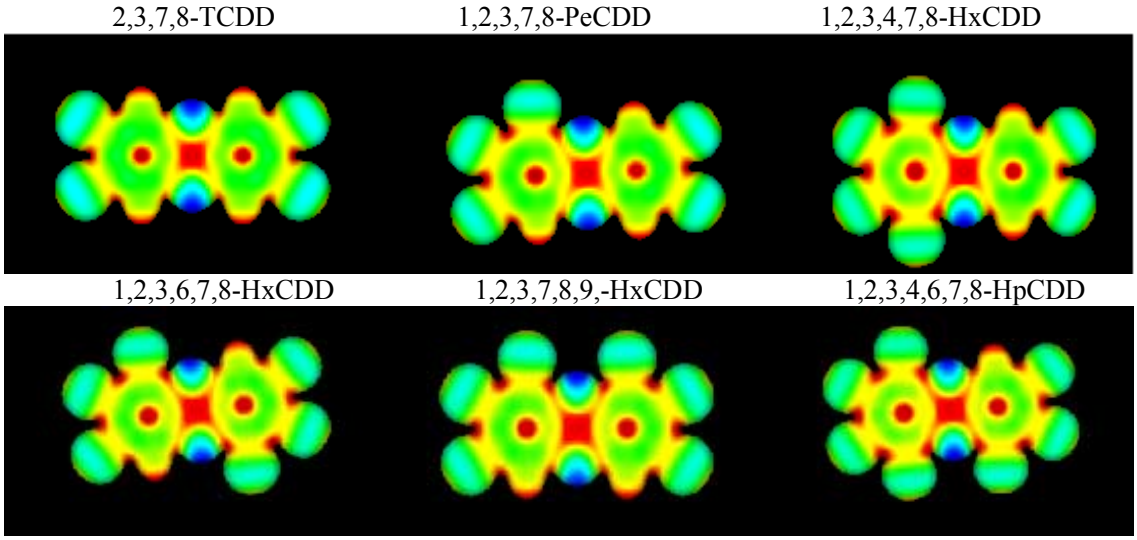


Figure 1. Dioxins used for calculation of the electrostatic potentials

4. Electrostatic Potential Map

From the checkpoint file of Gaussian calculation, the electron density and the electrostatic potential were calculated at each grid point of medium size 80^3 of a cube by making use of the CubeGen utility of Gaussian 98W [11]. The electrostatic potential is an observable, which is different from that of the atomic charge from Mulliken's population analysis [12]. The electrostatic potential was mapped on the isosurface of the electron density near van der Waals radii by AVS Chemistry Viewer [13]. The maps in Figure 2 show that the electrostatic potential is negative of blue at chlorine and oxygen atoms, and positive of red at hydrogen and carbon atoms. The yellow regions show zero potential. It was said that the negative value of oxygen was especially large [14], but its' basis set is low, as STO-5G, and the map was a 2 dimensional drawing. The electrostatic potential maps show that they are characteristic of the molecular structures, additive on the constitutional atoms and the molecular point group. In the PCDD congener, the chlorine substitution at the 1,2,3 position strengthens the toxicity and at the 6,9 position weakens it. The PCDF and PCB congeners also show the same pattern regarding the substitution. It is considered, therefore, that the pharmacophore is complementary to the pattern of the superposition of 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD, which has the highest value of TEF. From the additive property of the electrostatic potential and the similarity of the molecular shapes, the applicability of CoMFA is anticipated. CoMFA postulates the linearity both with the steric and electrostatic fields.

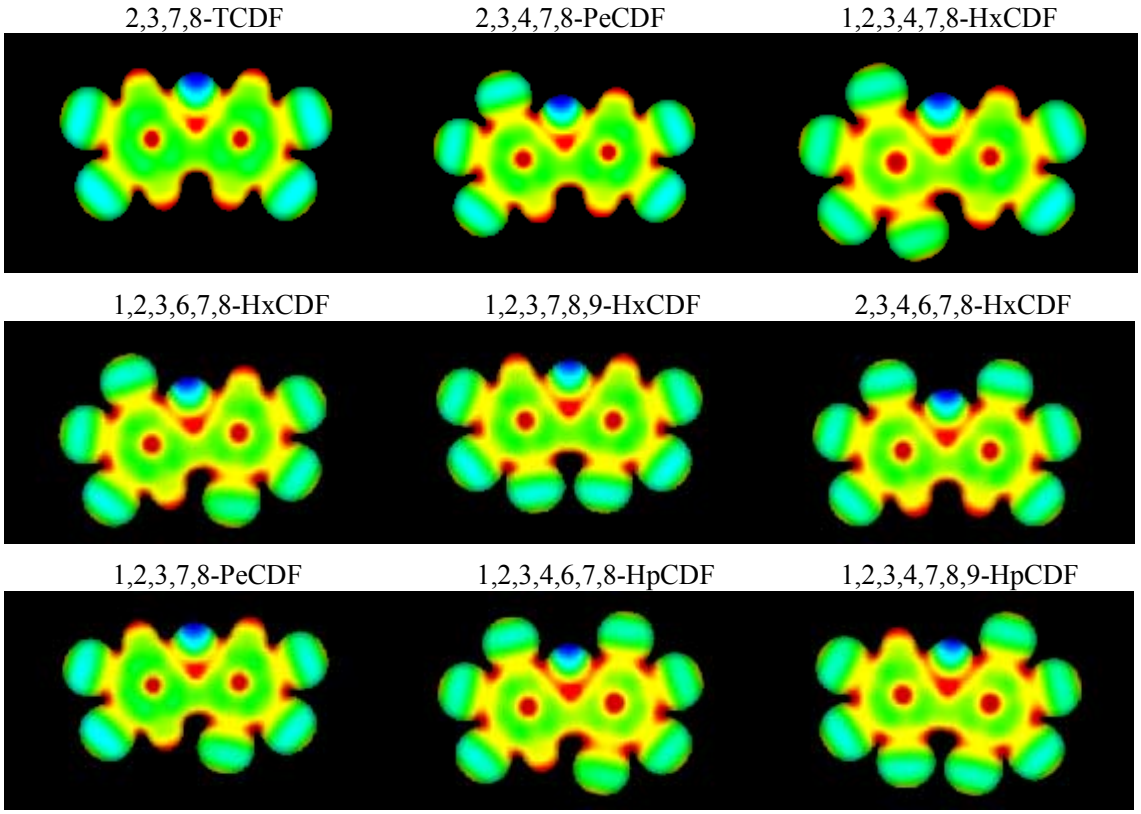
(a) PCDD



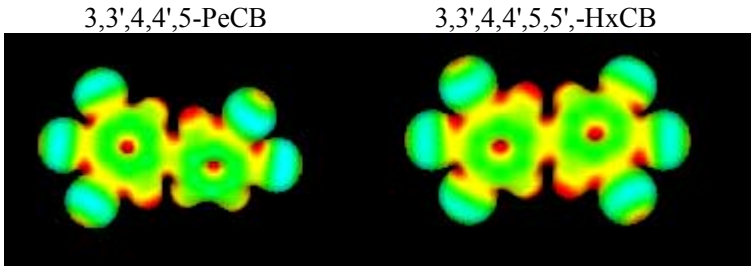
blue -0.04, yellow 0, red 0.16 a.u.

Figure 2. Electrostatic Potential Map of Dioxins; (a) PCDD

(b) PCDF



(c) PCB



blue -0.04, yellow 0, red 0.16 a.u.

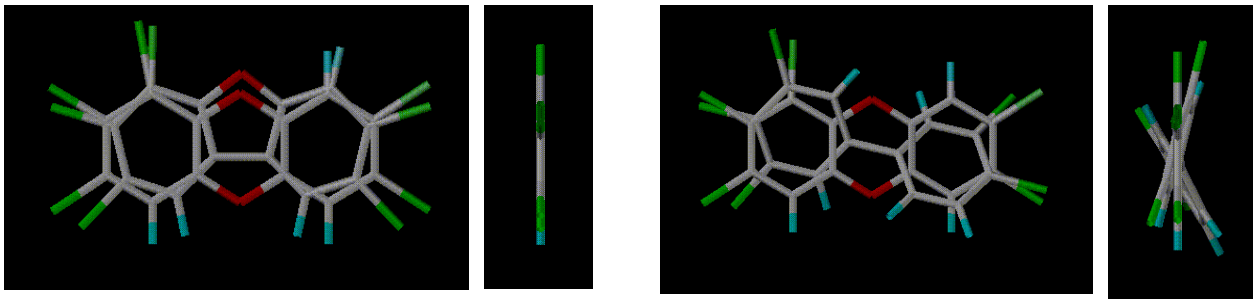
Figure 2. Electrostatic Potential Map of Dioxins; (b) PCDF, (c) PCB

5. Comparative Molecular Field Analysis (CoMFA)

5.1 Superimposition of Structures-

As the template structure, not 2,3,7,8-TCDD of the higher symmetry C_{2v} but 1,2,3,7,8-PeCDD of unsymmetrical C_1, which has the same highest TEF, was selected to avoid the arbitrariness of the

superimposing directions. The other PCDDs are superimposed on the template, dibenzodioxin ring as the common substructure. Fitting 2,3,4,7,8-PeCDF (2) and 3,3',4,4',5-PeCB (7), which has the highest TEF, and the receptor binding avidity in each congeners to the template of 1,2,3,7,8-PeCDD (6), including the substituted chlorines by 'atom fit', then each congener is superimposed by the 'align data base' option, with a dibenzofuran ring as the common substructure for PCDFs and a biphenyl ring for PCBs. As shown in Figure 3a, PCDFs were superimposed on PCDDs in planar orientation, and the oxygen atom of the furan ring is on that of the dioxy ring, as is that of Safe [5] [15] and McKinney [16] [17]. The other case of PeCDD and PCB, is different from that of Safe in which PeCDD and PCB are also in a plane, but twisted PCBs went through from one side of PCDD to the other as shown in Figure 3b. In the case of McKinney, PCB was superimposed on PCDD's force field, yet the resulted planes were still relaxed. Of the 29 compounds, the 18 compounds used for the calculation of CoMFA are shown in Figure 4 with their orientations of upward and downward, left and right.



a) 1,2,3,7,8-PeCDD(6) and 2,3,4,7,8-PeCDF(2) b) 1,2,3,7,8-PeCDD(6) and 3,3',4,4',5-PeCB(7)

Figure 3. Superposition of Structures

5.2 Electric Charge

As the electric charge on atoms, rather than the atomic charge by population analysis, we used the electrostatic potential derived charge CHelpG obtained by the *ab initio* molecular orbital method of HF/6-31G* level with Gaussian 98.

5.3 CoMFA

CoMFA was executed by using the receptor binding avidities (EC_{50}) as the activity. The receptor used was the rat hepatic cytosol aryl hydrocarbon receptor (AhR). Partial least squares analysis gave a cross-validated correlation coefficient $q^2 = 0.955$ with the number of components 3. The regression line and the observed and predicted value are shown in Figure 5 and Table 1. The lower part of the line corresponds approximately to the PCB congeners, and the upper part to the PCDF and PCDD congeners. Contributions of the steric and electrostatic fields are nearly equal, that is, 51 versus 49 %. It is considered that the higher q^2 obtained was caused by the correct selection of unsymmetrical 1,2,3,7,8-PeCDD rather than symmetrical 2,3,7,8-TCDD as the template structure for superimposition for one thing, and the precise calculation by *ab initio* MO for another. The binding site of the receptor seems to be unsymmetrical. The weakest activity of the PCB congeners seems to be due to the mono-ortho substituted chlorine. The results have been anticipated from the electrostatic potential maps.

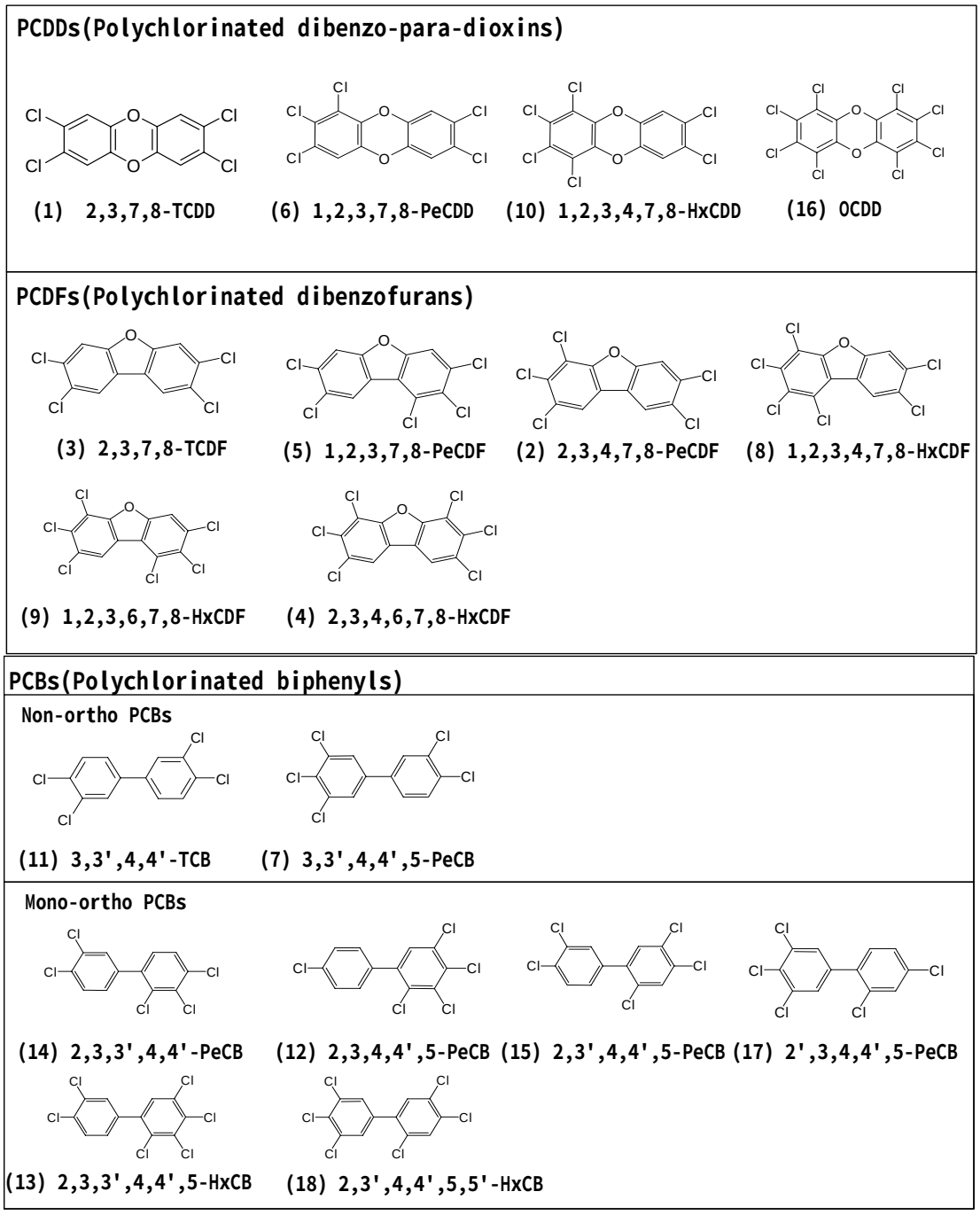


Figure 4. Structures of Dioxins used for CoMFA;

with orientations of upward and downward, and left and right, the numbers of the compounds also correspond to the order of receptor binding avidity

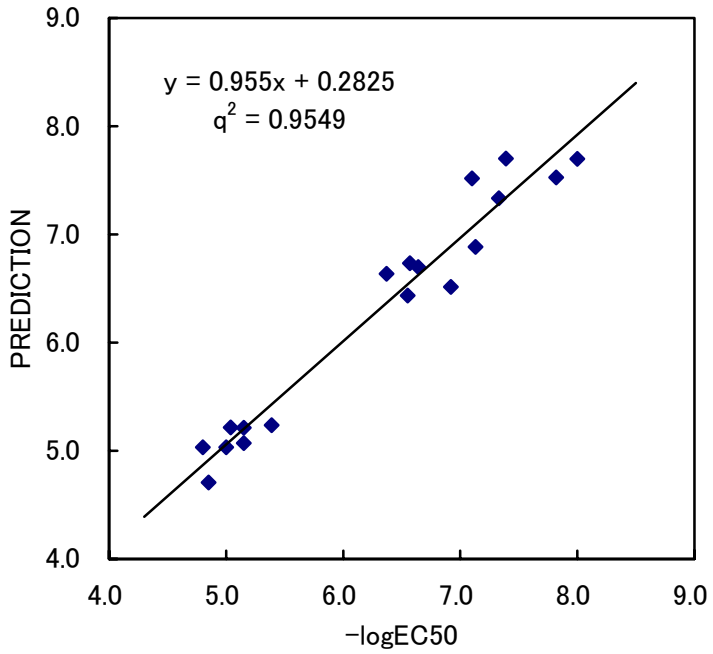


Figure 5. CoMFA of Toxicity of Dioxins； Plots of Observed－Predicted Value

5.4 Isosurface Map

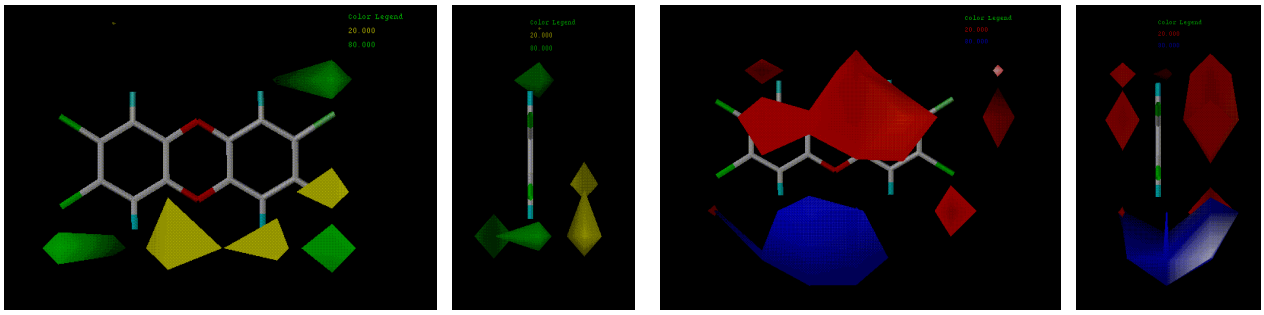
Isosurface maps of the steric and electrostatic fields are shown in Figure 6. Larger values of steric field at the lower left part and those of the electrostatic field at the lower part in the front view correspond to larger activities.

Table 1. CoMFA of Toxicity of Dioxins； Observed Activity-Predicted Value

NO	Dioxins	TEF[a)]	EC_{50}[b)]	$-\log EC_{50}$	PREDICT[c)]
1	2,3,7,8−TCDD	1.00	$1.0X10^{-8}$	8.00	7.699
2	2,3,4,7,8−PeCDF	0.50	1.5	7.82	7.528
3	2,3,7,8−TCDF	0.10	4.1	7.39	7.701
4	2,3,4,6,7,8−HxCDF	0.10	4.7	7.33	7.335
5	1,2,3,7,8−PeCDF	0.05	7.45	7.13	6.886
6	1,2,3,7,8−PeCDD	1.00	7.9	7.10	7.518
7	3,3',4,4',5−PeCB	0.10	$1.2\ X10^{-7}$	6.92	6.515
8	1,2,3,4,7,8−HxCDF	0.10	2.3	6.64	6.698
9	1,2,3,6,7,8−HxCDF	0.10	2.7	6.57	6.735
10	1,2,3,4,7,8−HxCDD	0.10	2.8	6.55	6.435
11	3,3',4,4'−TCB	0.0001	4.3	6.37	6.635
12	2,3,4,4',5−PeCB	0.0005	$4.1\ X10^{-6}$	5.39	5.237
13	2,3,3',4,4',5−HxCB	0.0005	7.1	5.15	5.071
14	2,3,3',4,4'−PeCB	0.0001	7.1	5.15	5.214
15	2,3',4,4',5−PeCB	0.0001	9.1	5.04	5.215
16	OCDD	0.0001	>1 .0	5.00	5.032
17	2',3,4,4',5−PeCB	0.0001	1.4	4.85	4.706
18	2,3',4,4',5,5'−HxCB	0.00001	1.6	4.80	5.033

a) Toxicity Equivalent Factor b) EC_{50} (M)=Rat Hepatic Cytosol Receptor Binding Avidity c) Predicted Value

a) Steric Field　　b) Elctrostatic Field



Front View　Side View　Front View　Side View

green；larger volume, yellow；smaller volume→larger activity(binding avidity)
blue；positive charge, red；negative charge→larger activity(binding avidity)

Figure 6.　Isosurface Map

5.5 Prediction of Toxicity

From the results of CoMFA obtained above, the prediction of the receptor binding avidities were tested on the compounds that have no TEF, so are not included in CoMFA. The compounds tested are in Figure 7 with their orientations. Extrapolation was done to the higher value of bromine derivatives as 2,3,7,8-TBrDD and interpolation to the lower value as 1,3,7,8-TCDD. The compounds to be tested were fitted to the template compound and the ligand binding avidity was estimated. The results of the prediction are shown in Table 2. The prediction of the lower value was

Table 2.　Prediction of Receptor Binding Avidity：Comparison with Observed Value

NO	COMPOUND USED FOR PREDICTION	TEMPLATE COMPOUND	Fit Atom RMS value	−logEC50 RBA	PREDICTED VALUE
Extra polation					
21	2,3,7,8−TBrDD	2,3,7,8−TCDD	0.005	8.82	7.64
22	2,3−diBr−7,8−diCDD; up	2,3,7,8−TCDD	0.006	9.35	7.62
23	2,3−diBr−7,8−diCDD; down				7.38
Interpolation					
24	1,3,7,8−TCDD; up, left	2,3,7,8−TCDD	0.011	6.10	7.44
25	1,3,7,8−TCDD; up, right		0.013		6.70
26	1,3,7,8−TCDD; down, left		0.013		6.61
27	1,3,7,8−TCDD; down, right		0.011		6.64
28	1,2,4,7,8−PeCDD; up, left	1,2,3,7,8−PeCDD	0.011	5.96	6.40
29	1,2,4,7,8−PeCDD; up, right		0.032		6.39
30	1,2,4,7,8−PeCDD; down, left		0.032		6.41
31	1,2,4,7,8−PeCDD; down, right		0.030		6.23
32	2,6,7−triCDF; up	2,3,4,6,7,8−HxCDF	0.018	6.34	7.28
33	2,6,7−triCDF; down	1,2,3,4,7,8−HxCDF	0.017		7.20

fairly good but those of the bromine derivatives were lower than the observed value.

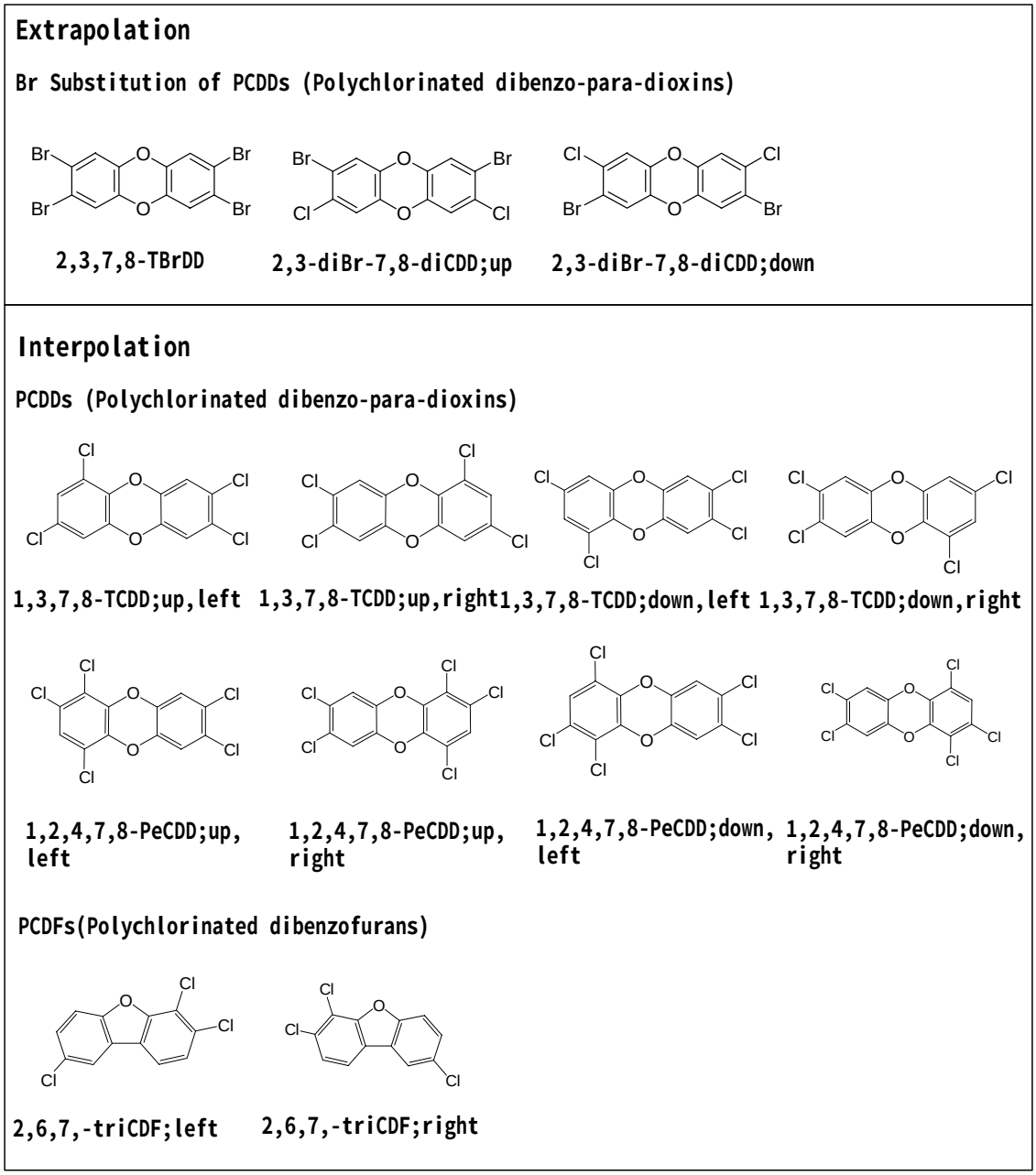


Figure 7. Structure of Compounds used for Prediction of Receptor Binding Avidity : with orientations of upwards and downwards, and left and right

6. Conclusion

As anticipated from the results of the precise calculations of the electrostatic potentials on the three congeners of dioxins by the *ab initio* molecular orbital method, the Comparative Molecular Field Analysis using the receptor binding avidities (EC_{50}, *M)* of the rat hepatic cytosol aryl

hydrocarbon receptor (AhR) as a measure of toxicity, gave the highest cross-validated correlation coefficient $q^2 = 0.955$. The three-dimensional mode of action of dioxins to the aryl hydrocarbon receptor would be a subject for further research.

Acknowledgment

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ダイオキシン類の静電ポテンシャルと毒性の CoMFA

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要旨

ダイオキシン類が結合する芳香族炭化水素レセプター(AhR)に関してはその作用、アミノ酸配列の解明等が進んでいるが、X線結晶解析等の3次元構造の解析はまだ成されていない。そこで毒性等価指数(WHO-TEF)が与えられているダイオキシン類のPCDD系列4個,PCDF系列6個,PCB系列8個計18個の化合物について*ab initio* 分子軌道法ソフトGaussian 98でHF/6-31G*レベルで構造最適化を行うと共にその静電ポテンシャル図を求めた。静電ポテンシャル図はいずれも酸素と塩素の辺りが負で、炭素と水素の辺りは正で構成原子に関して加成的であってCoMFAの成立が予期された。それで毒性値としてAhR結合能 (RBA)、ラット肝サイトソルのEC_{50}, Mの-logEC_{50}で立体場及び静電場による比較分子場解析CoMFAを行った。原子電荷としては静電ポテンシャル誘導電荷のCHelpGを使用した。1,2,3,7,8-PeCDDを基準としてそれに他の化合物を置換塩素まで含めた各原子対で重ねる。PCDFはPCDDと平面的に重なるが、PCBはビフェニル環が互いに捩れているのでPCDDの片側から他方へ貫通する。すべてを重ね合わせてから成分数3で部分最小二乗法PLSを行い最終モデルの交差確認相関係数 q^2=0.955 と高い値を得た。立体場と静電場の寄与の割合は51%対49%でほぼ等しい。この相関から外挿と内挿による予測を行った。結合能の高い2,3,7,8-TBrDD、2,3-diBr-7,8-diCDD等の臭素誘導体の外挿では実測値よりも低いが、1,3,7,8-TCDD、1,2,4,7,8-PeCDD等の内挿では逆に高いが実測値に大体近い。

キーワード: 静電ポテンシャル, 比較分子場解析, CoMFA, 毒性、ab initio 分子軌道法, ダイオキシン, 芳香族炭化水素受容体, AhR

領域区分：分子認識