Relationships between Biological Potency and Electronic States of Polychlorinated Dibenzofurans and Polychlorinated Biphenyls

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It was found that the differences between the frontier molecular orbital energies ($\epsilon_{\text{HOMO}} - \epsilon_{\text{LUMO}} = \Delta\epsilon$) in polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) have a relationship with the magnitude of the biological activity which is influenced by both the number and position of chlorine atom substituents on PCDFs and PCBs skeletons. Moreover, it was found that the $\Delta\epsilon$'s values of PCBs are classified into two types which coincide with the well-known classification of PCBs to types of 3-methylcholanthrene and phenobarbital according to their biological activities.

The relationship between $\Delta\epsilon$ and biological activity in these xenobiotics suggests that the congeners having small $\Delta\epsilon$ values such as 2,3,4,7,8-pentaCDF, 2,3,4,6,7-pentaCDF, 3,4,5,3',4'-pentaCB, and 3,4,5,3',4',5'-hexaCB form stable molecular complexes with an Ah-receptor, *e.g.* (2,3,4,7,8-PentaCDF-Ah-receptor), while the congeners having large $\Delta\epsilon$ values are strongly suggested to be unstable in a complex formation. Thus, this work presents an explanatory method to help understand the structure-activity relationship of the xenobiotics PCDFs and PCBs.

Keywords polychlorinated dibenzofuran; polychlorinated biphenyl; molecular orbital calculation; electronic state; toxicity; biological activity; structure-activity relationship

Polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) are artificial aromatic xenobiotics which share similar chemical structure and biological activity with polychlorinated dibenzo-*p*-dioxins (PCDDs) (Chart 1).

It is known that the widely distributed xenobiotics PCDFs and PCBs have potent toxicities and biological properties, and they induce a variety of hepatic microsomal enzymes such as cytochrome P-450 (448)-dependent monooxygenase, into activity, including aryl hydrocarbon hydroxylase (AHH),^{1a)} DT-diaphorase,^{1b,c)} and δ -aminolevulinic acid (δ -ALA) synthetase,^{1d)} *etc.* The biological activities of these compounds notably depend on both the number and the position of the chlorine substituents of the PCDD, PCDF, and PCB skeletons. Although a number of investigations have been carried out to seek the relationships between the position or the number of chlorine substituents and the inducing potency for enzymes or toxicity, the answer has not yet been elucidated. The results obtained so far suggested that the toxicity and enzyme-inducing capability produced by PCDDs, PCDFs, and PCBs may be connected to their interactions with a cytosolic Ah receptor.^{2,3)} Accordingly, structure-activity relationship (SAR) studies⁴⁾ were applied using molecular parameters such as lipophilicity (π), hydrogen-bonding capability (HB), electronegativity (σ), and sterimol (ΔB_5) to receptor binding affinity and enzyme-inducing ability.

In the previous work,⁵⁾ we demonstrated that the

difference in energy between the HOMO (ϵ_{HOMO}) and the LUMO (ϵ_{LUMO}) in PCDDs obtained by the Hückel molecular orbital method (HMO) showed a good linear correlation with the magnitude of toxicity and biological activity, such as acute toxicity, AHH activity, and δ -ALA synthetase activity. This result may point out that PCDDs having generally small $\Delta\epsilon$ show a large potency for enzyme induction, as well as powerful toxicity.

We applied a similar SAR technique for an explanation of the biological activity involved in PCDDs compared to PCDF and PCB congeners, and found that the magnitude of the biological activity is influenced by both the number and position of chlorine atom substituents on the PCDFs and PCBs skeletons. Also, we found that the $\Delta\epsilon$'s values of PCB congeners are classified into two types which coincide with the well-known classification of PCBs to 3-methylcholanthrene (MC)-type and phenobarbital (PB)-type according to their enzyme inducing abilities.

Theoretical Methods

HMO calculations of PCDFs and PCBs were carried out using the parameters in previous literature⁶⁾ to obtain ϵ_{HOMO} , ϵ_{LUMO} , and their differences ($\Delta\epsilon$). They are shown in Tables I and II.

The measurements of inter-ring dihedral angles (θ) of PCBs have been established by photoelectron spectroscopy (PES).^{7b)} HMO calculations of the congeners were achieved by the well-known following approximation considering these angles:

$$\beta' = \beta \cos \theta$$

where β is related to the simple Hückel resonance integral.

The biological assays, DT-diaphorase activity,^{1b,c)} cytochrome P-450 (448) activity,^{1b,c)} the accumulation in rat liver,^{1b)} benzpyrene 3-hydroxylation activity,^{1b)} and the change in weight of rat liver,⁸⁾ are cited from the literature and are compared to the calculated values.

Results and Discussion

PCBs are polychlorinated aromatic compounds having a non-planar structure owing to the presence of rotational conformers between phenyl-phenyl rings in contrast with PCDFs and PCDDs. A striking characteristic of PCBs' biological activity is its isomer dependence; namely, it was reported that chlorine substitutions at both *para* and, at

Chart 1. Structure of Polychlorinated Dibenzofurans (a) and Polychlorinated Biphenyls (b)

TABLE I. Calculated Hückel MO Energy Levels and Point Groups for PCB Congeners

Compound	Point group	Irreducible representation	HMO energy levels ^{a)}		
			ϵ_{homo}	ϵ_{lumo}	$\Delta\epsilon$
3,4,5,3',4',5'-	D ₂	3a + 3b ₁ + 6b ₂ + 6b ₃	0.6197	-0.8218	1.4415
2,4,6,2',4',6'-	D ₂	3a + 3b ₁ + 6b ₂ + 6b ₃	0.7294	-0.9599	1.6893
2,4,5,2',4',5'-	C ₂	9a + 9b	0.6428	-0.9246	1.5674
2,3,4,2',3',4'-	C ₂	9a + 9b	0.7122	-0.9372	1.6494
4,4'-	D ₂	2a + 2b ₁ + 5b ₂ + 5b ₃	0.6745	-0.8163	1.4908
3,5,3',5'-	D ₂	2a + 2b ₁ + 5b ₂ + 5b ₃	0.7500	-0.8023	1.5523
3,4,3',4'-	C ₂	8a + 8b	0.6395	-0.8189	1.4584
3,4,2',4'-	C ₁	16a	0.6825	-0.8834	1.5659
3,4,5,3',4'-	C ₁	17a	0.6291	-0.8204	1.4495
2,3,6,2',3',6'-	C ₂	9a + 9b	0.6891	-0.9461	1.6352
2,3,4,3',4',5'-	C ₁	18a	0.6666	-0.8870	1.5536
2,3,4,3',4'-	C ₁	17a	0.6782	-0.8853	1.5635
2,5,2',5'-	C ₂	8a + 8b	0.6971	-0.8926	1.5898
Non	D ₂	2a + 2b ₁ + 4b ₂ + 4b ₃	0.7715	-0.7714	1.5429

a) In β unit.

TABLE II. Calculated Hückel MO Energy Levels and Point Groups for PCDF Congeners

Compound	Point group	Irreducible representation	HMO energy levels ^{a)}		
			ϵ_{homo}	ϵ_{lumo}	$\Delta\epsilon$
2,3,4,7,8-	C ₁	18a''	0.5846	-0.7417	1.3263
2,3,7,8-	C _{2v}	8a ₂ + 9b ₁	0.6022	-0.7401	1.3423
1,2,3,7,8-	C ₁	18a''	0.5781	-0.7470	1.3251
1,2,3,4,7,8-	C ₁	19a''	0.5467	-0.7481	1.2948
1,2,7,8-	C ₁	17a''	0.6128	-0.7359	1.3487
2,3,4,6,7-	C ₁	18a''	0.5771	-0.7424	1.3195
1,2,4,6,8-	C ₁	18a''	0.6134	-0.7284	1.3418
2,4,8-	C ₁	16a''	0.6988	-0.7197	1.4185
2,8-	C _{2v}	7a ₂ + 8b ₁	0.7248	-0.7178	1.4426
1,3,6,8-	C ₁	17a''	0.6204	-0.7367	1.3571
1,2,3,4,6,7-	C ₁	19a''	0.5385	-0.7521	1.2906
1,2,3,6,7,8-	C ₁	19a''	0.5651	-0.7486	1.3137
1,2,4,6,7,8-	C ₁	19a''	0.5757	-0.7391	1.3148
1,2,4,7,9-	C ₁	18a''	0.5796	-0.7371	1.3167
1,2,6,7,8-	C ₁	18a''	0.5987	-0.7375	1.3362
2,3,6,7-	C ₁	17a''	0.6009	-0.7359	1.3368

a) In β unit.

least, two *meta* positions resulted in maximum activity, whereas chlorine substitutions at *ortho* positions resulted in decisively decreased activity.^{1b,8,9)} The inducing patterns for hepatic microsomal cytochrome P-450 (448) of PCBs are classified into phenobarbital (PB)-type induction and 3-methylcholanthrene (MC)-type induction. Although 3,4,5,3',4'-pentaCB and 3,4,5,3',4',5'-hexaCB are known as the most potent MC-type inducers of PCBs, 2,5,2',5'-tetraCB and 2,4,6,2',4',6'-hexaCB, *etc.* exhibited less activity than the aforementioned congeners.

The $\Delta\epsilon$'s obtained so far by HMO calculation incorporating a dihedral angle (θ)⁷⁾ for PCB congeners were compared with the inducing patterns of MC-types and PB-types, as shown in Table III. It is understood that MC-type PCBs, which have an approximately coplanar structure reactive with a hepatic cytosol receptor, have smaller $\Delta\epsilon$'s than those of PB-type PCBs.

For example, Table III shows that the $\Delta\epsilon$ of 3,4,5,3',4',5'-hexaCB and 3,4,3',4'-tetraCB, which are known

TABLE III. The Relationship between $\Delta\epsilon$ and Two Distinct Groups of the PB- and the MC-Types for PCB Congeners

Compound	Type of inducer	$\Delta\epsilon$ (β)	Compound	Type of inducer	$\Delta\epsilon$ (β)
2,4,6,2',4',6'-	PB	1.6893	3,4,3',4'-	MC	1.4584
2,4,5,2',4',5'-	PB	1.5674	3,4,5,3',4'-	MC	1.4495
2,3,4,2',3',4'-	PB	1.6494	3,4,5,3',4',5'-	MC	1.4415
2,3,6,2',3',6'-	PB	1.6352	2,3,4,3',4'-	Intermediate	1.5634
3,4,2',4'-	PB	1.5659			
2,5,2',5'-	PB	1.5898			
4,4'-	PB	1.4908			

as a prototype of MC-type PCBs, is smaller than that of 2,4,6,2',4',6'-hexaCB, 2,3,4,2',3',4'-hexaCB, and 2,5,2',5'-tetraCB, regarding the induction potencies of PB-type PCBs.

It has been reported that the induction potencies and toxicities of several individual PCBs depend on the amounts of cytochrome P-450 (448),^{1b)} activity of DT-diaphorase^{1b)} and BP hydroxylase,^{1b)} and the amount of lipid accumulation in rat liver,^{1b)} *etc.* Figures 1 and 2 show the relationship between the $\Delta\epsilon$'s of PCBs and the logarithmic values of relative activity relative to the amounts of cytochrome P-450, the activity of BP hydroxylase, and the activity of DT-diaphorase.

As can be seen from the figures, the reductive order of $\Delta\epsilon$ is parallel to increases in the induction potency, 2,5,2',5'-tetraCB < 2,4,3',4'-tetraCB < 3,4,3',4'-tetraCB < 2,3,4,3',4'-pentaCB < 3,4,5,3',4'-pentaCB < 3,4,5,3',4',5'-hexaCB. Therefore, $\Delta\epsilon$'s can explain the decrease or increase of inductive activity depending on the substitution pattern of the chlorine atom on the PCB skeleton. It is also known that the DT-diaphorase activity of 2,3,4,3',4'-pentaCB decreases to approximately 1/5 compared with that of 3,4,5,3',4'-pentaCB.^{1b)} Such a difference can be understood as changes in their electronic structure due to the difference in chlorine substitution position. This difference can be observed in the difference of $\Delta\epsilon$ in which the $\Delta\epsilon$ of 2,3,4,3',4'-pentaCB is about 0.11 β larger than that of 3,4,5,3',4'-pentaCB. From these results, it is recognized that $\Delta\epsilon$ may show a difference in potency for biological effects which is based on the position as well as the number of chlorine substitution.

On the other hand, the distribution of these congeners is also strongly isomer-dependent in its accumulation in certain animal tissue. Figures 3 and 4 show that $\Delta\epsilon$ expresses correlation diagrams with the liver lipid content in rats and the liver concentration in chicks.

It was recognized that there are linear correlations between $\Delta\epsilon$'s, toxicity in rat liver, and liver concentrations of PCBs in chicks.

PCDFs, similarly to PCDDs and PCBs, are well-known as a potent toxic compound and a potent inducer of hepatic microsomal enzyme systems. The toxicity and inducing ability of these congeners are also due to the position as well as the number of chlorine substituents on the PCDF skeleton. Yoshimura *et al.*^{1c)} assayed the DT-diaphorase, cytochrome P-450 (448) inducing ability and the accumulation of PCDFs in rat liver. The AHH inducing ability and cytosolic receptor binding affinities have been reported by Bandiera *et al.*,¹⁰⁾ Safe *et al.*,¹¹⁾ McKinney *et al.*,¹²⁾ and

Fig. 1. The Correlation Diagram between $\Delta\epsilon$ and $\log$ (relative BP hydroxylation activity) and $\log$ (relative amount of cytochrome P-450 (448)) in Rats
1, 3,4,5,3',4',5'-hexaCB; 2, 3,4,5,3',4'-pentaCB; 3, 2,3,4,3',4'-pentaCB; 4, 3,4,3',4'-tetraCB; 5, 2,4,3',4'-tetraCB; 6, 2,5,2',5'-tetraCB.

Fig. 2. The Correlation Diagram between $\Delta\epsilon$ and $\log$ (relative DT-diaphorase activity) in Rats

1, 3,4,5,3',4',5'-hexaCB; 2, 3,4,5,3',4'-pentaCB; 3, 2,3,4,3',4'-pentaCB; 4, 3,4,3',4'-tetraCB; 5, 2,4,3',4'-tetraCB; 6, 2,5,2',5'-tetraCB.

Poland *et al.*^{1a,13)} Considering the results obtained so far, the potency of the activity in PCDFs increases in the following order of chlorine substitution pattern: 2,3,4,7,8- > 1,2,3,7,8- > 2,3,7,8- > 1,2,3,4,6,7- > 2,3,4,6,7- > 1,3,6,8- > 2,8-.

The correlation diagrams shown in Figs. 5—7 shows that there are rough linear correlations between $\Delta\epsilon$'s and the inducing ability of DT-diaphorase,^{1c)} cytochrome P-450 (448),^{1c)} and the accumulation in rat liver,^{1c)} respectively. The plots suggest a comparatively good correlation between $\Delta\epsilon$ and the biological activity as $\log_{10}$ (DT-diaphorase activity), $\log_{10}$ (cytochrome P-450 (448) activity), and $\log_{10}$ (rat liver concentration).

Although 2,3,7,8-tetraCDF has C_{2v} geometrical symmetry concerning chlorine position in the skeleton, the related compounds do not always show the greatest

Fig. 3. The Correlation Diagram between $\Delta\epsilon$ and $\log$ (liver concentration) in Chicks

1, 3,4,5,3',4',5'-hexaCB; 2, 2,4,5,2',4',5'-hexaCB; 3, 2,3,4,2',3',4'-hexaCB; 4, 2,4,6,2',4',6'-hexaCB; 5, 2,3,6,2',3',6'-hexaCB.

biological activity. Namely, those PCDFs having three or more chlorine atoms in lateral ring positions, such as 2,3,7-triCDF, 2,3,8-triCDF, and 2,3,7,8-tetraCDF, are minimally exhibited MC-type inductions, and it was found that the $\Delta\epsilon$ values of chlorine substituted congeners at the C-4, C-6, and C-4, 6 positions were smaller than that in the original. For instance, the $\Delta\epsilon$ values of 2,3,4,7,8-pentaCDF (1.3263) and 2,3,4,6,7-pentaCDF (1.3195) were both smaller, by 0.016 and 0.047 (in terms of β), when compared with that of 2,3,7,8-tetraCDF (1.3423). The $\Delta\epsilon$ values of 1,3,6,8-tetraCDF (1.3571) and 2,8-diCDF (1.4426), however, are larger by 0.015 and 0.100 than that of 2,3,7,8-tetraCDF. Furthermore, though a decreasing order of induction potencies was 1,2,6,7,8-pentaCDF > 2,3,6,7-tetraCDF > 1,2,7,8-tetraCDF, the order was nearly parallel to the increasing order of $\Delta\epsilon$.

Since the electronic structures of PCDFs and PCBs depend on the substitutional pattern of the chlorine atom, $\Delta\epsilon$ may explain the differences in the effect of the chlorine

Fig. 4. The Correlation Diagram between $\Delta\epsilon$ and log (relative weight of liver lipids) in Rats

1, 3,4,5,3',4',5'-hexaCB; 2, 3,4,5,3',4'-pentaCB; 3, 3,4,3',4'-tetraCB; 4, 2,3,4,3',4'-pentaCB; 5, 2,4,3',4'-tetraCB; 6, 2,5,2',5'-tetraCB.

Fig. 5. Plot of $\Delta\epsilon$ and Logarithm Value of Relative Cytochrome P-450 Activity in Rats

1, 1,2,3,4,6,7-hexaCDF; 2, 2,3,4,6,7-pentaCDF; 3, 2,3,4,7,8-pentaCDF; 4, 2,3,7,8-tetraCDF; 5, 2,3,6,7-tetraCDF; 6, 1,2,6,7,8-pentaCDF; 7, 1,2,7,8-tetraCDF; 8, 1,3,6,7-tetraCDF; 9, 1,2,4,6,8-pentaCDF; 10, 1,3,6,8-tetraCDF; 11, 2,8-diCDF.

substituted pattern on biological activities. The xenobiotic effects such as PCDDs, PCDFs, and PCBs exert on cellular biology express that the dioxin-receptor protein complex moves into the nucleus, where it turns on the activity of a specific set of genes called the Ah locus.^{2,3,13)} The potencies of toxicity and enzyme inducing ability vary according to the stabilization of the cytosolic receptor-PCDFs (or PCBs) complex. The dioxin-receptor protein complex is an example of a reversible molecular recognition process. In our model, it is demonstrated that the $\Delta\epsilon$'s of these xenobiotics are in inverse proportion to the stabilization energy of the interaction between these xenobiotics and the Ah receptor. Therefore, the stabilization of the xenobiot-

Fig. 6. Plot of $\Delta\epsilon$ and Logarithm Value of Relative DT-Diaphorase Activity in Rats

1, 1,2,3,4,6,7-hexaCDF; 2, 2,3,4,6,7-pentaCDF; 3, 2,3,4,7,8-pentaCDF; 4, 2,3,7,8-tetraCDF; 5, 1,2,6,7,8-pentaCDF; 6, 2,3,6,7-tetraCDF; 7, 1,2,7,8-tetraCDF; 8, 1,3,6,7-tetraCDF; 9, 1,2,4,6,8-pentaCDF; 10, 1,3,6,8-tetraCDF; 11, 2,8-diCDF.

Fig. 7. Plot of $\Delta\epsilon$ and Logarithm Value of Relative Concentration in Rat Liver

1, 1,2,3,4,6,7-hexaCDF; 2, 2,3,4,6,7-pentaCDF; 3, 2,3,4,7,8-pentaCDF; 4, 2,3,7,8-tetraCDF; 5, 1,2,6,7,8-pentaCDF; 6, 2,3,6,7-tetraCDF; 7, 1,2,7,8-tetraCDF; 8, 1,2,4,6,8-pentaCDF; 9, 1,3,6,7-tetraCDF; 10, 1,3,6,8-tetraCDF; 11, 2,8-diCDF.

ics-Ah receptor complex (e.g., [PCDFs-Ah] and [PCBs-Ah]) increases as the $\Delta\epsilon$ of the xenobiotics decreases. From these results, it is considered that any PCDF (or PCB) with a small $\Delta\epsilon$ acquires large stabilization energy. This suggests that the equilibrium moves to the right-hand side with a small $\Delta\epsilon$ to show strong biological activity (Chart 2). Consequently, the induction potencies may be closely related to the concentration of the congeners which move into the genes.

Finally, let us consider why $\Delta\epsilon$ explains biological activity

Chart 2. The Illustration for Relationship between $\Delta\epsilon$ and the Equilibrium of PCDF-Ah Receptor Binding Complex

so well. The $\Delta\epsilon$ value approximately corresponds to the least electron excitation energy from ϵ_{homo} to ϵ_{lumo} . Namely, the polarization interaction energy (Π)¹⁴⁾ between the highest occupied MO ϕ_{homo} and the lowest unoccupied MO ϕ_{lumo} on one reacting substrates is given by:

$$\Pi = 2 \times (H_{\text{homo, lumo}})^2 / (\epsilon_{\text{homo}} - \epsilon_{\text{lumo}}) = 2 \times (H_{\text{homo, lumo}})^2 / \Delta\epsilon$$

where the term is defined as follows:

$$H_{\text{homo, lumo}} = \int \phi_{\text{homo}} \hat{H} \phi_{\text{lumo}} dr$$

In these Eqs., the symbol $\hat{H}$ stands for the total Hamiltonian of the system.

Therefore, it is considered that any PCDF (or PCB) with a small $\Delta\epsilon$ acquires large stabilization energy, and binds strongly with the binding site of an Ah receptor.

References

- 1) a) A. Poland, E. Glover, and A. S. Kende, *J. Biol. Chem.*, **251**, 4936 (1976); b) H. Yoshimura, S. Yoshihara, N. Ozawa, and M. Miki, *Ann. N.Y. Acad. Sci.*, **320**, 179 (1979); c) S. Yoshihara, K. Nagata, H. Yoshimura, H. Kuroki, and Y. Masuda, *Toxicol. Appl. Pharmacol.*, **59**, 589 (1981); d) A. Poland and E. Glover, *Mol. Pharmacol.*, **9**, 736 (1973).
- 2) R. L. Rawls, *Chem. & Eng. News*, **1983**, No. 6, 38.
- 3) A. Poland, J. Knutson, and E. Glover, *Clin. Physiol. Biochem.*, **3**, 147 (1983).
- 4) a) J. D. McKinney and P. Singh, *Chem.-Biol. Interact.*, **33**, 271 (1981); b) S. H. Safe, *Ann. Rev. Pharmacol. Toxicol.*, **26**, 371 (1986).
- 5) S. Kobayashi, A. Saito, Y. Ishii, T. Tanaka, and S. Tobinaga, *Chem. Pharm. Bull.*, **39**, 2100 (1991).
- 6) "Reactivity Indices for Biomolecules," ed. by C. Chin and P. Song, Texas Tech. Press, Lubbock, Texas, 1981, p. 175.
- 7) a) J. D. McKinney, K. E. Gottschalk, and L. Pedersen, *J. Mol. Struct.*, **104**, 445 (1983); b) J. J. Dynes, F. L. Baudais, and R. K. Boyd, *Can. J. Chem.*, **63**, 1292 (1985).
- 8) J. A. Goldstein, J. D. McKinney, G. W. Lucier, P. Hickman, H. Bergman, and J. A. Moore, *Toxicol. Appl. Pharmacol.*, **36**, 81 (1976).
- 9) T. Sawyer and S. Safe, *Toxicol. Lett.*, **13**, 87 (1982).
- 10) S. Bandiera, T. Sawyer, M. Romkes, B. Zmudzka, L. Safe, G. Mason, B. Keys, and S. Safe, *Toxicology*, **32**, 131 (1984).
- 11) G. Mason, K. Farrell, B. Keys, J. Piskorska-Pliszczynska, L. Safe, and S. Safe, *Toxicology*, **41**, 21 (1986).
- 12) a) J. D. McKinney, T. Darden, M. A. Lyrly, and L. G. Pedersen, *Quant. Struct.-Act. Relat.*, **4**, 166 (1985); b) G. Long, J. McKinney, and L. Pedersen, *ibid.*, **6**, 1 (1987).
- 13) A. Poland and J. C. Knutson, *Ann. Rev. Pharmacol. Toxicol.*, **22**, 517 (1982).
- 14) K. Fukui and H. Fujimoto, *Bull. Chem. Soc. Jpn.*, **41**, 1989 (1968).