Toxicity of Dioxins: Role of an Absolute Hardness–Absolute Electronegativity Diagram (η–χ Diagram) as a New Measure in Risk Assessment

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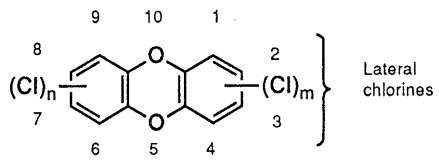
The differences in biological activities among polychlorinated dibenzo-*p*-dioxins (dioxins) are strongly dependent on the substitution pattern of chlorine at various positions on the parent dibenzo-*p*-dioxin molecule. The absolute hardness, η, of dioxins shows a good correlation with the potency of biological activity and the chlorine substitution pattern. The result means that the soft dioxins have a small HOMO–LUMO gap, and are more toxic than the hard dioxins. Therefore, the values of absolute hardness, η, of dioxins can be used to predict their toxic potency (dioxin hardness). Moreover, we show that the absolute hardness–absolute electronegativity (η–χ) diagrams, as an activity coordinate, play an important role as a new measure in the assessment of the toxicity and potency of the biological activity of dioxins.

Key words absolute hardness electronegativity diagram; toxicity; dioxin-water complex; risk assessment; hardness concept

Polychlorinated dibenzo-*p*-dioxins (dioxins or PCDDs) can be classified as xenobiotics and enviromental pollutants. These compounds are composed of a tricyclic system substituted with chlorine at various positions. There are 75 possible derivatives of PCDDs. These congeners elicit toxic phenomena, including the induction of various enzymes, a decrease in body weight, acnegenic edema, liver disorder, immunosuppression, *etc.*[1–3] In particular, 2,3,7,8-tetrachlorinated dibenzo-*p*-dioxin(2,3,-7,8-tetra-CDD) is the most toxic of the dioxins.

The differences in physiological activities of PCDDs are dependent on their chlorine substitution pattern on the parent dibenzo-*p*-dioxin (DD). Poland, *et al.* concluded that: a) at least three positions among C-2, C-3, C-7, and C-8 must contain chlorine substituents, b) at least one hydrogen atom must be on the PCDD skeleton, and c) biological activity as a result of the halogen substituent decreases in the order Br > Cl > F.[4] Three approaches, hydrophobic interaction,[5] electrostatic potential analysis,[6] and charge transfer interaction as electron acceptor (or electron donor),[7] have been used to investigate the structure–activity relationships for PCDDs. However, it is not yet understood why the differences in potency regarding the toxicity and biological activity of dioxins are affected by the number and position of chlorine substitutions.

Previously,[8] we found that the energy difference ($\Delta\varepsilon$) between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) of dioxins shows a good linear correlation with the potency of toxicity and biological activity. We show here that the absolute hardness (η) of dioxins also correlates well with the potency of biological activity and their chlorine substitution pattern. The absolute hardness of dioxins is an expression of whether their chemical properties are hard or soft, according to the principle of hard and soft acids and bases (HSAB).[9] We found that soft dioxins are more toxic than hard dioxins. These results led us to introduce a new method, plotting of an absolute hardness–electronegativity diagram (η–χ diagram), as a measure of predicting the relative toxic potencies of dioxins.



The toxicity and induction responses have been proposed to involve initial binding of the dioxins to the aryl hydrocarbon receptor (AhR) in cytosol which is encoded by the aryl hydrocarbon (Ah) gene. Understanding the molecular interpretation of the relationship between binding affinities with the Ah receptor of dioxins in the binding site and chlorination pattern, the quantity of electron transfer (ΔN) from the amino acid residue to dioxin can be calculated.

Furthermore, the dioxin–H_2O binding model should be meaningful for predicting the potency of toxicity. In this model, we found that the conformation of dioxin–H_2O complexes with water of 1,9-di-, 1,4,6,9-tetra-, and 1,2,3,4,6,7,8,9-octa-CDDs, *etc.* is in a bended form. However, the conformation of the complexes of laterally chlorinated dioxins such as 2,3,7-tri-, 2,3,7,8-tetra-, and 1,2,3,7,8-penta-CDDs, *etc.* is a planar form.

Methods

We employed PM3 and AM1 calculations in MOPAC 6.0 software to determine the electronic structure of optimized PCDDs.[10] The results of the orbital energies of HOMO and LUMO, absolute electronegativity (χ), and absolute hardness (η), as determined by PM3 calculations for dioxins, are given in Table 1. Particularly, DD geometry was obtained from energy minimization procedures incorporated in the GAUSSIAN 90 program by the RHF/6-31G* method. The geometry of DD is non-planar and the torsional angle (ϕ, in degree) along the O···O axis is determined to be 2.6°. The conformation of DD, a tricyclic system, has been obtained from nematic liquid crystal (NLC) NMR, photoelectron spectroscopic (PES) studies, and X-ray diffraction analysis. The torsional angle along the O···O axis in DD was determined previously to be 16.2°

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and 14.4° by PES and NLC analyses, respectively, and to be 0.0° by the X-ray diffraction study.[11a-c]

The values of absolute hardness (η) and absolute electronegativity (χ) become the following eqs. 1 and 2 in regard to density functional theory, as defined by Parr and Pearson[12]:

$$\chi = -\partial E/\partial N \quad (1)$$

$$\eta = 1/2\,\partial^2 E/\partial N^2 \quad (2)$$

where E is the electronic energy of a molecule and N is the number of electrons. According to Koopman's theorem, the frontier orbital energies can be approximated by

$$-\varepsilon_{HOMO} = I_p\,, \qquad -\varepsilon_{LUMO} = E_a$$

where I_p is the ionization energy (eV) and E_a is the electron affinity (eV). I_p and E_a have been used to calculate the η and χ values of dioxins in this work, since the equations below were given by Pearson

$$\eta = 1/2(\varepsilon_{LUMO} - \varepsilon_{HOMO})\,, \qquad \chi = -1/2(\varepsilon_{HOMO} + \varepsilon_{LUMO})\,.$$

The HOMO–LUMO gap of molecules can be regarded approximately as the excitation energy required to promote the mono-exited configuration of an electron from HOMO to LUMO. Moreover, just the half value of HOMO–LUMO gap is equal to the absolute hardness. The electronegativity is half of HOMO and LUMO sum. Since the experimental values of the I_p and E_a of dioxins, except for DD, have not been reported, we estimated the absolute hardnesses and absolute electronegativities by a semiempirical MO method.

Table 1. Calculated Absolute Hardness and Electronegativity Parameters for Some Dioxins (eV)[a]

No.	Chlorination	$-\varepsilon_{HOMO}$	$-\varepsilon_{LUMO}$	$\Delta\varepsilon$	χ	η
1	1,4,6,9-	9.0419	0.6036	8.4383	4.823	4.219
2	1,3,4,6-	8.9518	0.6728	8.2790	4.812	4.140
3	1,3,6,8-	8.9314	0.7224	8.2090	4.827	4.105
4	1,2,6,8-	8.9007	0.7055	8.1952	4.803	4.097
5	1,3,4,8-	8.9075	0.7107	8.1969	4.809	4.098
6	1,3,4,7-	8.8941	0.7187	8.1754	4.806	4.088
7	1,3,7,8-	8.8519	0.7562	8.0957	4.804	4.048
8	1,2,3,8-	8.8389	0.7452	8.0936	4.798	4.047
9	1,4,7,8-	8.8840	0.7070	8.1770	4.795	4.089
10	1,2,3,7-	8.8371	0.7460	8.0911	4.792	4.046
11	2,3,7,8-	8.7989	0.7841	8.0147	4.792	4.007
12	1,2,3,6-	8.8733	0.7061	8.1672	4.790	4.084
13	1,2,6,7-	8.8946	0.6767	8.2179	4.786	4.109
14	1,2,3,9-	8.8672	0.7013	8.1659	4.784	4.083
15	1,2,7,8-	8.8368	0.7334	8.1034	4.785	4.052
16	1,2,8,9-	8.8843	0.6752	8.2091	4.780	4.105
17	1,2,3,4-	8.8513	0.7092	8.1421	4.780	4.071
18	DD	8.6464	0.1769	8.4694	4.412	4.235
19	1-	8.7515	0.2977	8.4538	4.525	4.227
20	2-	8.7042	0.3674	8.3367	4.536	4.168
21	1,6-	8.8576	0.4082	8.4494	4.633	4.225
22	1,4-	8.8503	0.4188	8.4315	4.635	4.216
23	1,2-	8.7773	0.4626	8.3148	4.620	4.157
24	2,7-	8.7635	0.5227	8.2408	4.643	4.120
25	2,8-	8.7562	0.5256	8.2307	4.641	4.115
26	2,3-	8.7221	0.5326	8.1895	4.627	4.095
27	1,4,6-	8.9473	0.5103	8.4370	4.729	4.219
28	1,6,8-	8.8905	0.5829	8.3076	4.737	4.154
29	1,2,7-	8.8288	0.6026	8.2262	4.716	4.113
30	1,2,3-	8.7882	0.6231	8.1651	4.706	4.083
31	2,3,7-	8.7780	0.6652	8.1128	4.722	4.056
32	1,2,3,6,9-	8.9457	0.7820	8.1637	4.864	4.082
33	1,2,3,4,9-	8.9310	0.7831	8.1479	4.857	4.074
34	1,2,3,6,7-	8.8964	0.8093	8.0871	4.853	4.044
35	1,2,3,6,8-	8.9133	0.8297	8.0835	4.872	4.042
36	1,2,3,7,8-	8.8532	0.8543	7.9989	4.854	3.999
37	1,2,3,4,6,9-	9.0051	0.8546	8.1506	4.930	4.075
38	1,2,3,6,7,8-	8.9082	0.9198	7.9884	4.914	3.994
39	1,2,3,7,8,9-	8.9011	0.9289	7.9723	4.915	3.986
40	1,2,3,4,7,8-	8.9042	0.9229	7.9813	4.914	3.991
41	1,2,3,4,7-	8.8948	0.8232	8.0717	4.859	4.036
42	1,2,4,7,8-	8.9052	0.8245	8.0807	4.865	4.040
43	1,2,3,4,6,7,8-	8.9561	0.9803	7.9758	4.968	3.988

a) At the PM3 level.

Our method, an absolute hardness–electronegativity diagram (η–χ diagram) as a coordinate of biological activity, may be useful for problems of toxicity and biological activity in the field of pharmacology and toxicology. The results show that a soft dioxin is more toxic and a hard dioxin less toxic.

The biological assays for aryl hydrocarbon hydroxylase (AHH) activity, δ-aminolevlinic acid (δ-ALA) synthetase activity, ethoxyresorufin O-deethylase (EROD) activity, body weight loss, thymic atropy *in vivo*, and acute toxic activity were performed as described in the literature,[3-5,14-17] and the data under the same experimental conditions were compared to the calculated values.

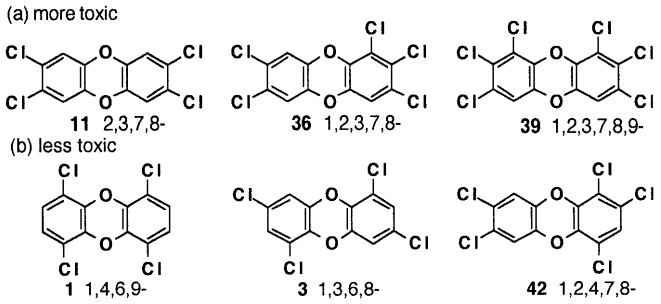
Results and Discussion

Correlation between Hardness and Biological Activities of Dioxins Dioxins are potent inducers of cytocrome P450 and P448 families, *etc.* For example, the potency of the AHH induction response decreases in the following order: 2,3,7,8-tetra-CDD > 1,2,3,7,8-penta-CDD > 1,2,3,-4,7-penta-CDD > 2,3,7-tri-CDD > 2,8-di-CDD. The binding affinity to the Ah receptor of dioxins is larger than that of 1,2,3,7,8,9-hexa-CDD, 1,3,7,8-tetra-CDD, or 1,3,6,8-tetra-CDD, which possess the same number of chlorine substitutions as 2,3,7,8-tetra-CDD.[13] From these relations, it is clear that the rule relies on the relation between the toxicity (and binding affinity) and the number and position of the chlorination pattern, as shown in the Figure below (a part).

In previous studies of the structure–activity relationships of dioxins, the lipophilicity and polarizability of these molecules were shown to play an essential role in the formation of the ligand-AhR binding complex.[14]

We investigated the relationship between the absolute hardness (η) of dioxins and the potency of biological activity based on the effect of the chlorination pattern. Figs. 1(a) and (b) show the correlation curves obtained by plotting the η of dioxins against the logarithm of their induction of AHH and δ-ALA synthetase, respectively, reported by Poland *et al.*[4,14,15] These figure show that the induction of these enzymes by dioxins is in good proportion to the value of η. Hard dioxins such as 2,8-di-CDD, 1,4,6,9-tetra-CDD, 1,2,3,4,7-penta-CDD, and 2,3,7-tri-CDD, *etc.* have less potent biological activity. The soft dioxins had more potent biological activity than the hard dioxins.

Safe, *et al.* reported a dose–response ED_{50} value for EROD response, thymic atrophy, and body weight loss for the 2,3,7-tri-CDD, 2,3,7,8- and 1,3,7,8-tetra-CDDs, 1,2,3,7,8- and 1,2,4,7,8-penta-CDDs, and 1,2,3,4,7,8-hexa-



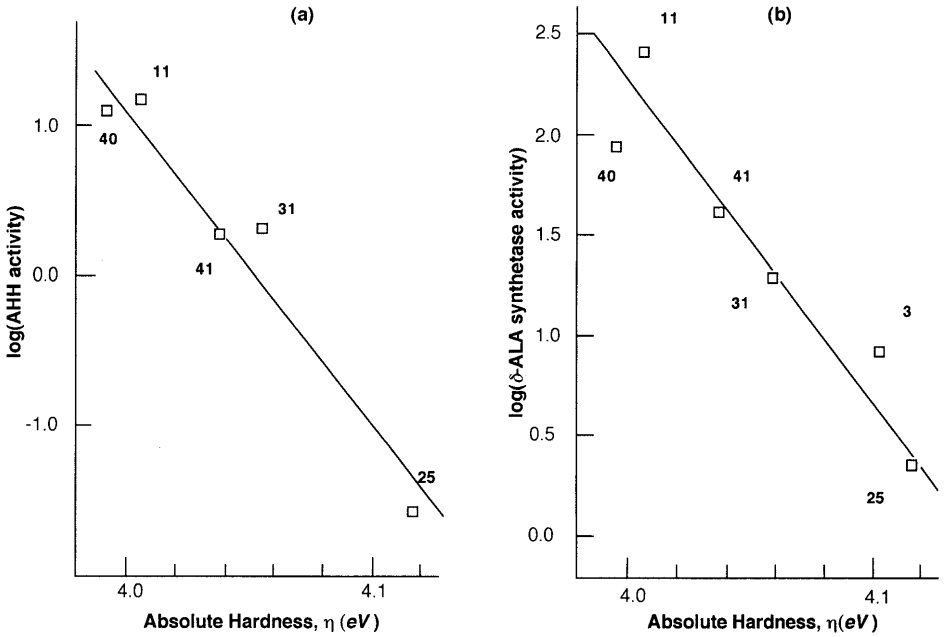


Fig. 1. Plot of Correlation between Activities of (a) AHH and (b) δ-ALA Synthetase in Chick Embryo Liver *vs.* Absolute Hardness of Polychlorinated Dibenzo-*p*-dioxins

3, 1,3,6,8-; **11**, 2,3,7,8-; **25**, 2,8-; **31**, 2,3,7-; **40**, 1,2,3,4,7,8-; **41**, 1,2,3,4,7-CDD.

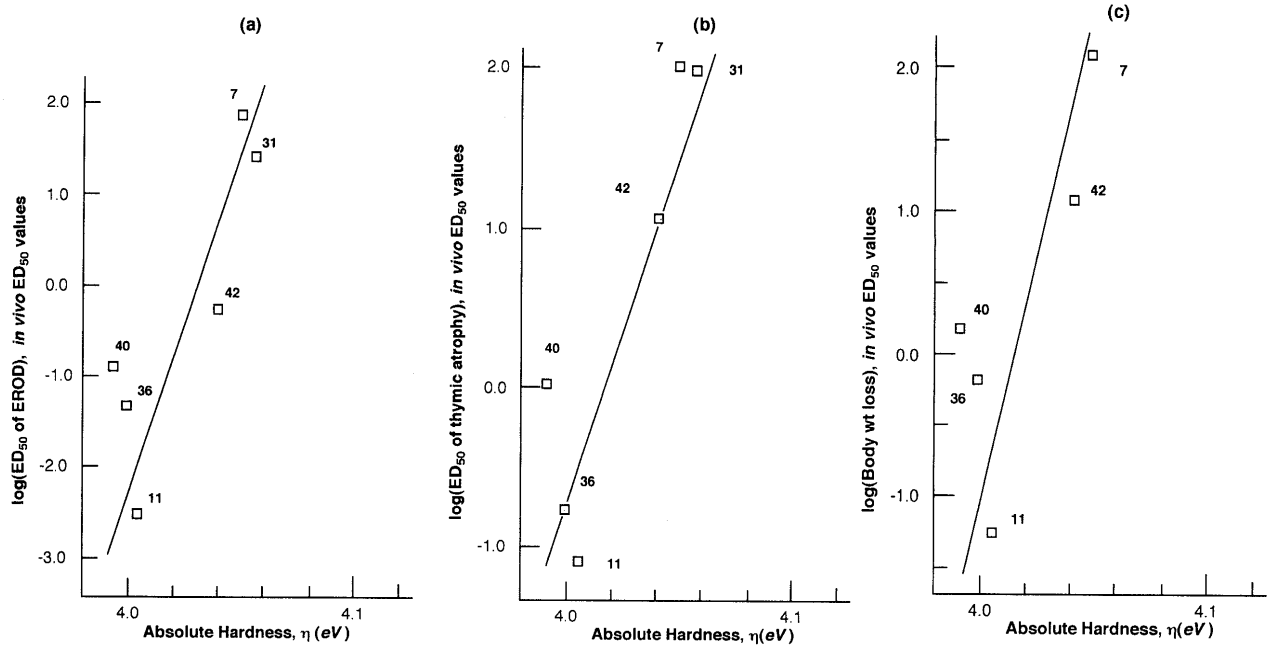


Fig. 2. Plot of Correlation between Activities (ED_{50}) of (a) EROD, (b) Thymic Atrophy, and (c) Body Weight Loss *in Vivo vs.* Absolute Hardness of Polychlorinated-*p*-dioxins

11, 2,3,7,8-; **31**, 2,3,7-; **36**, 1,2,3,7,8-; **40**, 1,2,3,4,7,8-; **42**, 1,2,4,7,8-CDD.

CDD in the immature male Wistar rat. These results also show good correlation of the biologic responses(logarithm of ED_{50}, μmol/kg) *in vivo* with the η value for dioxins, as shown in Figs. 2(a), (b), and (c). Therefore, the value of η as a single parameter may play an important role in elucidaing the correlation of the structure–activity relationship for biological potency and the chlorination pattern required in the toxicity.

The LD_{50} values of dioxins have been reported to cause acute toxicity in guinea pigs and mice, and 2,3,7,8-tetra-CDD is the most toxic of these compounds.[17] Figures 3(a) and (b) show the correlation curves obtained by plotting the η of dioxins against the logarithmic LD_{50-30} and LD_{50} values, respectively. The results indicate that: i) soft dioxins have small absolute hardness values, and potent toxicity; and ii) the absolute hardness can be used to predict the toxic potency for dioxin. Thus, the values of η are also important parameters for understanding the correlations.

Dioxin Hardness Since the MO method as used in this work is much detailed to the optimized structure, total energies, η value, and χ value, we examined the correlation

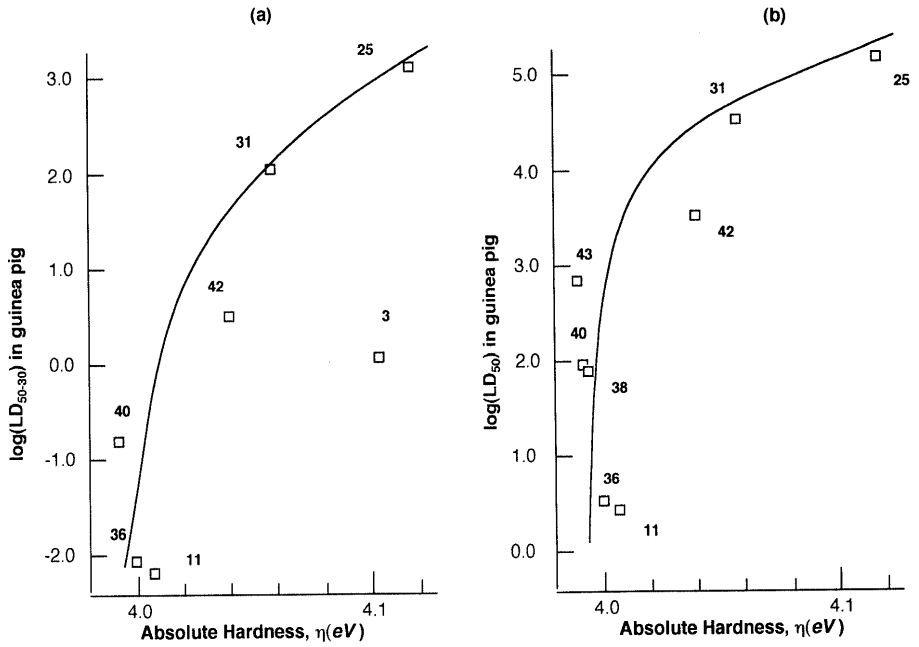


Fig. 3. Plot of Correlation between (a) LD_{50-30} and (b) LD_{50} Values of Acute Toxicity in the Guinea Pig *vs.* Absolute Hardness of Polychlorinated Dibenzo-*p*-dioxins

3, 1,3,6,8-; **11**, 2,3,7,8-; **25**, 2,8-; **31**, 2,3,7-; **36**, 1,2,3,7,8-; **38**, 1,2,3,6,7,8-; **40**, 1,2,3,4,7,8-; **42**, 1,2,4,7,8-; **43**, 1,2,3,4,6,7,8-CDD.

between the physical quantities and the chlorination pattern of dioxins. The chemical property of prototype DD is relatively soft(as acid) compared to benzene because its values of η and χ are 4.234 and 4.412, respectively. If the number of chlorine substituents is equal, the dioxin with chlorine substituted at the C-2 position is more soft than when it is substituted at C-1, as shown in Fig. 4. Therefore, we found that chlorine substitutions in lateral positions such as C-2, C-3, C-7, and/or C-8 on the DD decrease the value of absolute hardness, but substitution at the lengthwise positions such as C-1, C-4, C-6, and/or C-9 increases the value.

Figure 5 shows that if the same number of chlorines is substituted at various positions, the values of χ are invariable, but the η values shift greatly. In dioxins substituted with four chlorine substitutions, for example, the gap in value of hardness of 1,4,6,9-tetra-CDD(η= 4.219) and 2,3,7,8-tetra-CDD(η=4.007) shows a typical trend in the difference between lateral and lengthwise chlorine substitution on DD skeleton. The 1,2,6,7-, 1,2,3,6-, and 1,2,8,9-tetra-CDDs, *etc.*, with substituted chlorine on the adjacent carbon have lower χ values than those with substituted chlorine on no adjacent carbon such as 1,4,6,9- and 1,3,6,8-tetra-CDDs, *etc.* Obviously, the highly toxic dioxins are only distributed within the encircled area on the η–χ diagram. The values of η are affected by the chlorination pattern, and its values of lateral chlorine substituents are lower than those of other substituents.

Figure 6 shows the η–χ diagram, given as a the coordinate of η and χ, of 30 important dioxins. From Fig. 6, in dioxins with the same number of chlorine substituents at various positions on the DD molecule, the values of χ are mostly invariable but the values of η change greatly. For example, the values of η in **32**—**36** change obviously where the distribution of the χ value is restricted to about

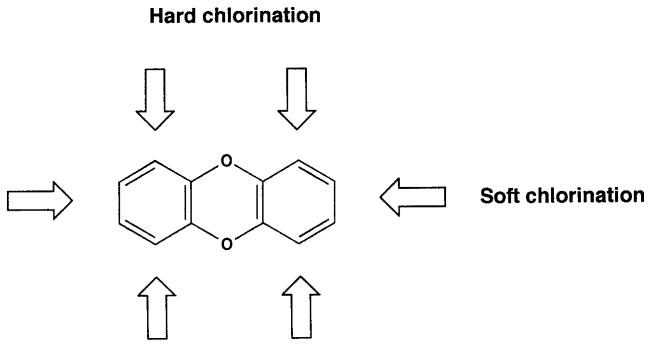


Fig. 4

4.85. Since the more toxic dioxins, 2,3,7,8-tetra-, 1,2,3,7,8-penta-, and 1,2,3,6,7,8-hexa-CDDs, are distributed within the encircled area in the figure, it is likely that the toxicity and induction responses are controlled by the absolute hardness more than by absolute electronegativity. These findings indicate that the electronic structures of dioxins are an important element, in addition to shape, in generalizing the structure–activity relationship.

The hardness is a measure of the resistance to change in electron density around the molecule. According to this concept, the η is half the value of the energy gap between HOMO and LUMO in Eq. 2, and a soft molecule has a small HOMO–LUMO gap. Therefore, we concluded that the soft dioxins are more toxic than the hard dioxins. The hardness concept was introduced through the HSAB principle and has been applied in explaining and in understanding a variety of chemical problems. Neverthless, we think that the concept can be applied to the understanding of the molecular mechanism in the biological expression of toxicity and induction responces.

Dioxin–AhR Binding Model Previously, we showed that the extent of toxicity and induction responses by

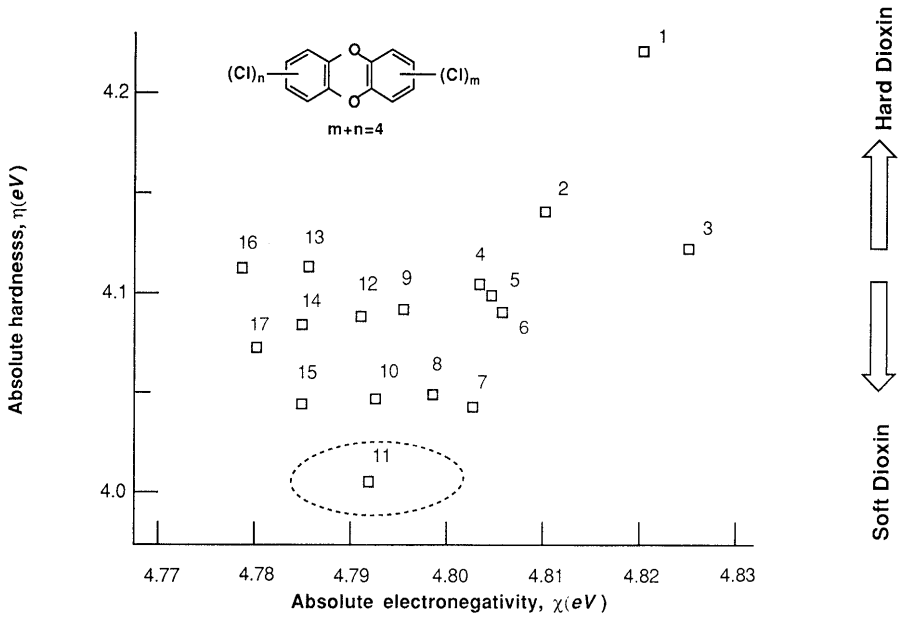


Fig. 5. Absolute Hardness–Electronegativity Diagram for Several Tetrachlorinated Dibenzo-*p*-dioxins

1, 1,4,6,9-; **2**, 1,3,4,6-; **3**, 1,3,6,8-; **4**, 1,2,6,8-; **5**, 1,3,4,8-; **6**, 1,3,4,7-; **7**, 1,3,7,8-; **8**, 1,2,3,8-; **9**, 1,4,7,8-; **10**, 1,2,3,7-; **11**, 2,3,7,8-; **12**, 1,2,3,6-; **13**, 1,2,6,7-; **14**, 1,2,3,9-; **15**, 1,2,7,8-; **16**, 1,2,8,9-; **17**, 1,2,3,4-CDD.

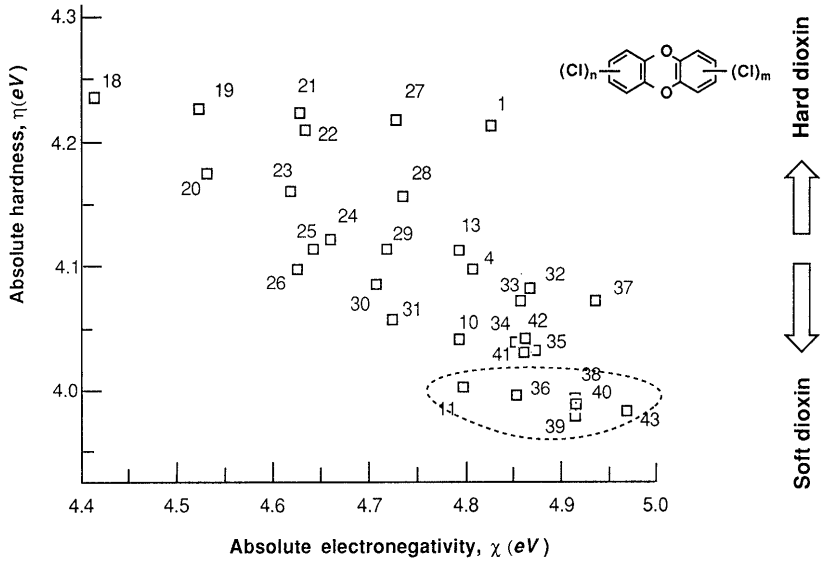


Fig. 6. Absolute Hardness–Electronegativity Diagram for Several Dioxins

Highly toxic dioxins are distributed in the dashed circle. **1**, 1,4,6,9-; **4**, 1,2,6,8-; **10**, 1,2,3,7-; **11**, 2,3,7,8-; **13**, 1,2,6,7-; **18**, DD; **19**, 1-; **20**, 2-; **21**, 1,6-; **22**, 1,4-; **23**, 1,2-; **24**, 2,7-; **25**, 2,8-; **26**, 2,3-; **27**, 1,4,6-; **28**, 1,6,8-; **29**, 1,2,7-; **30**, 1,2,3-; **31**, 2,3,7-; **32**, 1,2,3,6,9-; **33**, 1,2,3,4,9-; **34**, 1,2,3,6,7-; **35**, 1,2,3,6,8-; **36**, 1,2,3,7,8-; **37**, 1,2,3,4,6,9-; **38**, 1,2,3,6,7,8-; **39**, 1,2,3,7,8,9-; **40**, 1,2,3,4,7,8-; **41**, 1,2,3,4,7-; **42**, 1,2,4,7,8-; **43**, 1,2,3,4,6,7,8-CDD.

dioxins can be estimated from the value of η. In cells, the interaction with the binding site on the AhR of dioxins is the initial step in the toxic effects; therefore, we have proposed a model of equilibrium in a biochemical system between dioxins and AhR as expressed in Eq. 3.

$$\text{PCDDs}+\text{AhR}\rightarrow[\text{PCDDs}\cdot\text{AhR}]\rightleftarrows([\text{PCDDs}^{\delta+}\text{AhR}^{\delta-}]\leftrightarrow [\text{PCDDs}^{\delta-}\text{AhR}^{\delta+}])\rightarrow\text{move into nucleus} \quad (3)$$

where [PCDDs·AhR] is the PCDDs–AhR complex, and δ is the partial electron density. As the quantity of η is a measure of the stability of the complex, the small η of dioxins reflects the high reactivity between AhR and dioxins. This indicates that the small η of dioxins results in moving the equilibrium to the right-hand side in Eq. 3.

When dioxins interact with the binding site on AhR, an electron will transfer from molecule (C) of the lower χ to molecule (B) of the higher χ, so that C is an electron donor. Whether the dioxin is an electron donor or acceptor is of great importance in understanding the direction of flow of an electron transfer. The approximate quantity of electron transfer (ΔN) and stabilization energy (ΔE) has been given by the following equations,[20]

$$\Delta N=\frac{\chi_C-\chi_B}{2(\eta_C+\eta_B)} \qquad \Delta E=-\frac{(\chi_C-\chi_B)^2}{4(\eta_C+\eta_B)} \quad (4)$$

where B is the molecule which interacted with the dioxin on the binding site of AhR, and C is the dioxin.

The AhR from C57BL/6J mouse consists of 805 amino

acids, and has a molecular weight of about 90 kDa.[18] Its characteristics are caused by an abundance of aromatic and hydrophobic amino acids in its sequence; aromatic amino acids comprise 8.4% of the AhR. Accordingly, when the dioxin–AhR complex forms, the dioxins will easily interact with the aromatic and hydrophobic amino acid residues, and electrons will be transferred from the amino acids to the dioxin at the binding site. According to the model of interaction in Eq. 3, we calculated the ΔN and ΔE of the binding model system with the phenylalanine (Phe), triptophane (Trp), and histidine (His) of dioxins, respectively, as listed in Table 2. The Phe ($\chi=4.688$, $\eta=4.914$) and Trp ($\chi=4.230$, $\eta=4.230$), except for His ($\chi=4.935$, $\eta=4.894$), generally act as electron acceptors for dioxins, because they have larger absolute electronegativity than the dioxins.

Table 2 shows that the potent toxic dioxins, 2,3,7,8-tetra-CDD, 1,2,3,7,8-penta-CDD, 1,2,3,7,8,9-hexa-CDD, 1,2,3,7,8,9-hexa-CDD, *etc.* have large ΔN values, and that the less toxic dioxins, 1,2,4,7,8-penta-CDD, 2,3,7-tri-CDD, 1,3,6,8-tetra-CDD, *etc.* have small values on interaction with Phe and Trp. The increasing ΔN value is roughly proportional to the increasing order of toxicity of the dioxins, suggesting that the ΔN value plays a dominant role in the interaction with AhR of dioxins. The soft dioxins generally have a large ΔN value, so that the chemical property of AhR would be a soft enzyme.

Formation of the dioxin–AhR complex is an example of a reversible molecular recognition process. In our model, the hardness of dioxins is roughly proportional to the stabilization energy of the interaction between dioxins and the AhR. Moreover, the ΔE value should be equal to the magnitude of binding affinity with the AhR of dioxins. The ΔN value expresses the degree of polarization of charge in dioxin–AhR complexes, as shown in Fig. 7. For example, in 2,3,7,8-tetra-CDD, electrons will flow from Phe to the dioxin in the binding site, since its ΔN value is positive (+0.0058). The result means that the chemical property of this complex includes not only the charge transfer but also ionic binding, and toxic dioxins have a large ΔN value. As a result, the 2,3,7,8-tetra-CDD charges transfer to δ- in the binding site of AhR. Next, for the

Table 2. Values of ΔN and ΔE Calculated for Reaction of Dioxins with Some Amino Acids

Chlorination	Phenylalanine		Tryptophan		Histidine		Ah receptor binding affinity (M)[a]
	ΔN	$-\Delta E$	ΔN	$-\Delta E$	ΔN	ΔE	
2,3,7,8-	0.00581	3.01×10^{-4}	0.0341	9.58×10^{-3}	-8.006×10^{-3}	5.706×10^{-4}	1.0×10^{-8}
1,2,3,7,8-	0.00930	7.70×10^{-4}	0.0379	0.0118	-4.528×10^{-3}	1.823×10^{-4}	7.9×10^{-8}
1,2,3,4,7,8-	0.01267	1.43×10^{-3}	0.0416	0.0142	-1.155×10^{-3}	1.186×10^{-5}	2.8×10^{-7}
2,3,7-	0.00019	3.16×10^{-6}	0.0297	7.30×10^{-3}	−0.01187	1.2617×10^{-3}	7.1×10^{-8}
1,2,3,4,7-	0.00953	8.14×10^{-4}	0.0380	0.0119	-4.229×10^{-3}	1.597×10^{-4}	6.4×10^{-6}
1,2,4,7,8-	0.00986	8.72×10^{-4}	0.0384	0.0122	-3.891×10^{-3}	1.353×10^{-4}	1.1×10^{-6}
1,3,7,8-	0.00645	3.73×10^{-4}	0.0347	9.94×10^{-3}	-7.299×10^{-3}	4.763×10^{-4}	7.9×10^{-7}
2,3,6-	0.00143	1.83×10^{-5}	0.0291	7.03×10^{-3}	−0.01187	1.262×10^{-3}	2.2×10^{-7}
1,3,6,8-	0.00077	5.33×10^{-4}	0.0358	0.0107	-5.972×10^{-3}	3.210×10^{-4}	—
2,8-	−0.00026	6.19×10^{-5}	0.0246	5.06×10^{-3}	−0.01629	2.391×10^{-3}	3.2×10^{-6}
1-	−0.00893	7.29×10^{-4}	0.0174	2.57×10^{-3}	−0.02245	4.596×10^{-3}	$>1.0\times10^{-4}$
DD	−0.01510	2.09×10^{-4}	0.0107	9.76×10^{-4}	−0.02862	7.477×10^{-3}	—

a) Taken from ref. 3.

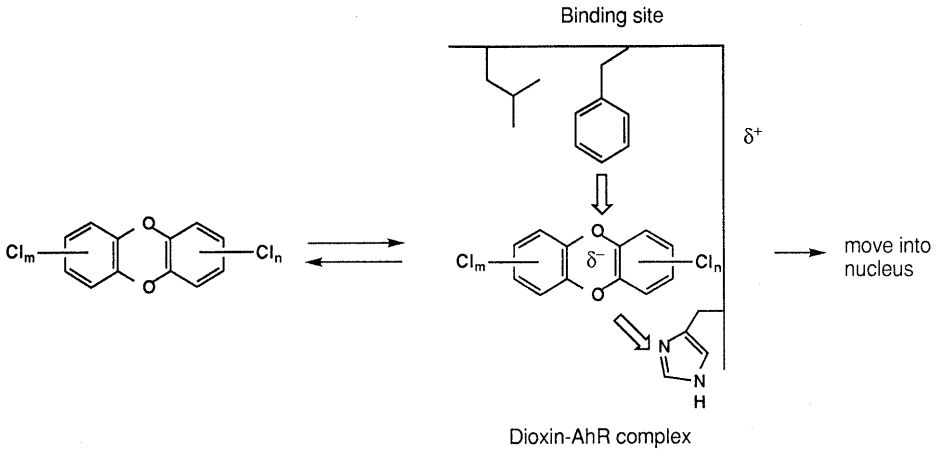


Fig. 7. Illustration for Polarization and Binding Model of Dioxin-AhR
(⇨), electron transfers from Phe to dioxin.

DD of the prototype, electrons will flow from DD to Phe, so that DD acts as a electron donor, but 2,3,7,8-tetra-CDD acts as an electron donor for Phe. Nevertheless, His will act as an electron acceptor for dioxins. Predictions for either an electron acceptor or donor must be considered in comparison with the χ of the dioxins and amino acids.

Since the binding affinity parallels the ΔN value in the dioxin amino acid complex, its value will play a dominant role in explaining the potency of toxicity and induction responses of dioxins. The polarization of charges of dioxin–AhR complexes may accelerate the transfer of the complex from the cytosol to the nucleus.

"Bend⇄Plane" Equilibrium Investigating the effects of conformation change dependence on the torsional angle of dioxins, we have defined the angle hardness (η_ϕ). The torsional angle is defined as the dihedral angle for C14–C13–O5–C12, as listed in Table 4. The results are summarized in Table 3. The values of η_ϕ are given as a function of the torsional angle fixed for dioxin. In DD, 1,4,6,9-tetra-CDD, 2,3,7-tri-CDD, and 2,3,7,8-tetra-CDD, the values of η_ϕ are low within barriers of about 2—3 kcal/mol with the ϕ from 0° to 25°. This means that these dioxins have a conformational flexibility. Although the conformation of the minimum for DD, 2,3,7-tri-CDD, and 2,3,7,8-tetra-CDD is probably planar, it can be expected that they easily yield a bended form since the equilibrium is given by a bent⇄plane. Our calculations assume that 1,4,6,9-tetra-CDD, 1,2,3,7,8,9-hexa-CDD, and octa-CDD, *etc.*, with chlorine substituted in the lengthwise positions, have been shown to be of a bent conformation. They could be expected to easily assume a bent form, as shown in figure below.

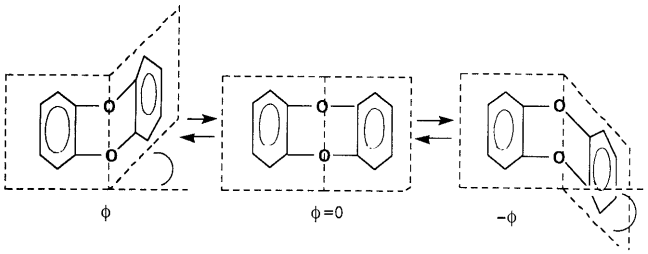
Table 3. Calculated Angle Hardness (η_ϕ) of Several Dioxins with Changes in Torsional Angle (ϕ)

Torsional angle (ϕ)[a]	Dibenzo-*p*-dioxin η_ϕ (eV)	1,4,6,9-Tetra-CDD η_ϕ (eV)	2,3,7-Tri-CDD η_ϕ (eV)	2,3,7,8-Tetra-CDD η_ϕ (eV)
0	4.235	4.219	4.056	4.007
5	4.239	4.223	4.059	4.011
10	4.251	4.235	4.068	4.020
15	4.271	4.256	4.082	4.036
20	4.299	4.278	4.101	4.059
25	4.336	4.288	4.123	4.088
30	4.380	4.300	4.148	4.090
35	4.435	4.315	4.175	4.162
40	4.492	4.332	4.202	4.204

a) In deg.

The absolute hardnesses of dioxins is influenced by a change in torsional angle. The effects of this influence may help to elucidate the expression of dioxin toxicity, as described in a previous section. We consider that these bend planar conformations of dioxins explain the planarity (or coplanarity) required for toxicity.

Dioxin–H_2O Complex Dioxins have oxygens that can potentially hydrogen bond with the binding site of AhR. McKinney *et al.*[21] indicated that the lower binding affinity with AhR and the biological activity of highly substituted dioxins such as 1,2,3,7,8,9-, 1,2,3,6,7,8-hexa-CDDs, and octa-CDD, *etc.*, as compared to the 2,3,7,8-tetra-CDD, may be due to steric blocking of the stabilizing hydrogen bonding. For the complexes with H_2O and the dioxins with lateral substitution such as 1,4,6,9-tetra-, 1,2,3,7,8,9-



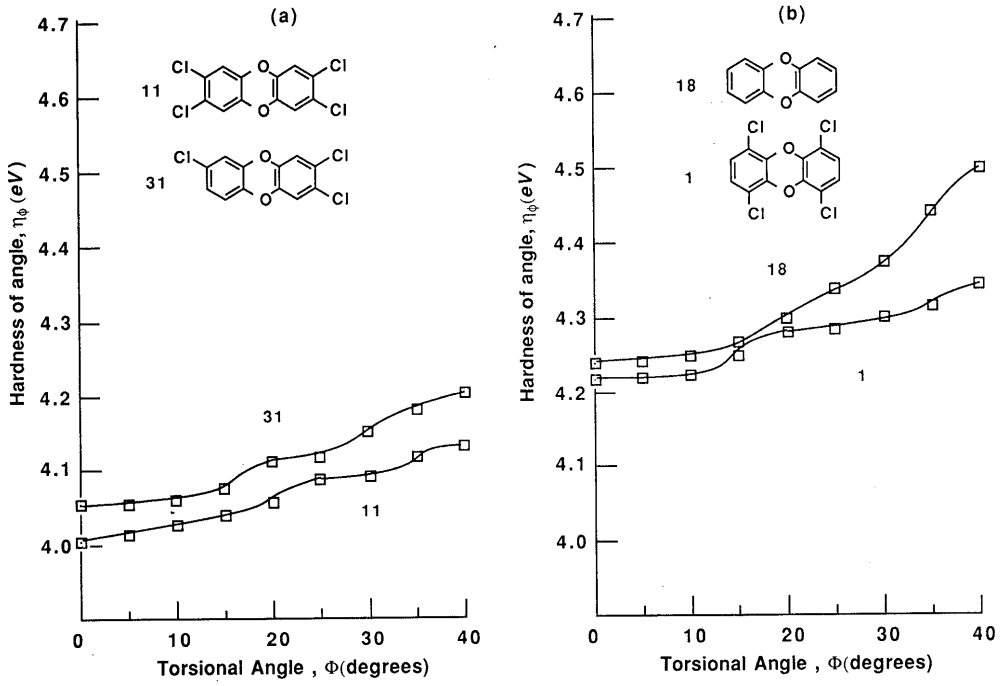
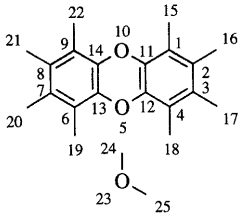


Fig. 8. Correlation Curve for Hardness of Angle *vs.* Torsional Angle(ϕ) of (a) Toxic Dioxins, 2,3,7,8-Tetra-CDD, 2,3,7-Tri-CDD, and (b) Less Toxic Dioxins, DD, 1,4,6,9-Tetra-CDD

Table 4. Absolute Hardness (η), Torsional Angles (ϕ), and H---O Distances for Several Dioxin–Water Complexes[a]



Compounds	Torsional angle ϕ (in deg.)		Distance (Å) O5–H24	Structure[b]
	C14–C13–O5–C12	C12–C11–O10–C14		
Dibenzo-*p*-dioxin–H_2O	−1.7	−1.5	2.23	Linear
1,6-DCDD–H_2O	−9.9	−9.6	2.33	Bifurcated
1,9-DCDD–H_2O	−19.3	−16.3	2.33	Bifurcated
2,8-DCDD–H_2O	−0.3	0.0	2.25	Linear
2,3,7-Tri-CDD–H_2O	−2.4	−2.2	2.26	Bifurcated
2,3,7,8-TCDD–H_2O	−0.90	−0.73	2.26	Linear
1,3,6,8-TCDD–H_2O	−10.9	−11.4	2.33	Bifurcated
1,4,6,9-TCDD–H_2O	−24.4	−23.3	2.37	Bifurcated
1,2,4,7,8-PCDD–H_2O	−20.1	−18.1	2.34	Bifurcated
1,2,3,7,8-PCDD–H_2O[c]	−2.2	−2.9	2.35	Bifurcated
[d]	−8.1	−8.6	2.29	Bifurcated
1,2,3,7,8,9-HCDD–H_2O[c]	−21.3	−17.7	2.35	Bifurcated
[d]	−14.8	−16.3	2.27	Linear
1,2,3,4,6,7,8,9-OCDD–H_2O	−26.9	−25.2	2.34	Bifurcated

a) At the AM1 level. *b*) Structure for hydrogen bonding of PCDD–H_2O complex. *c*) Cl side. *d*) H side.

hexa-, and octa-CDDs, we have found that their ϕs are assumed to be −24.4°, −21.3°, and −26.9°, respectively; their conformation has a stable bent form. However, the conformation of 2,3,7,8-tetra-CDD consists of a planar form. Therefore, if dioxins form a hydrogen bond with the ligand H–O–H in the binding site, a difference in its conformation is expected, and the recognition process will be inhibited or accelerated by the change in ϕ of the dioxins by the formation of hydrogen bonding.

According to the model described above, we calculated differences in ϕs when the dioxins bind with coordinated H_2O in AhR. Table 4 shows the results of some calculations by the AM1 method for such complexes. The geometry optimized with the H_2O of dioxins such as DD, 1,6-di-, 1,2,3,7,8-penta-, and 2,3,7,8-tetra-CDDs is most a certainly planar form, nevertheless, the geometry of 1,6-di-, 1,9-di-, 1,4,6,9-tetra-, 1,2,3,7,8,9-hexa-, and octa-CDD–H_2O complexes are bent forms. These trends in torsional angle are in good correlation with the chlorination pattern required for toxicity and induction responses, since the increase in ϕ decreases the η value in the absolute hardness of dioxin–H_2O complexes. For instance, as the ϕ(C14–C13–C5–C12) of octa-CDD is about −27°, as shown in Fig. 9(12(a,b)), the non-planarity is very evident. Their structures, fully optimized for hydrogen bonding complexes, are described as bifurcated structures. However, the complexes with H_2O of the lateral substituted dioxins as compared to the 2,3,7,8-congeners have a linear structure, as shown in Figs. 9(10(a,b) and 13(a,b)). This is an important difference.

The distances between the hydrogen bond O···H–O–H of the 2,3,7,8-tetra-CDD–H_2O and 1,4,6,9-TCDD–H_2O complexes were estimated to be 2.26 and 2.37 Å, respectively. The linear structure of the hydrogen bonding is produced by a hydrogen bonding interaction between the oxygen atom of water and the hydrogen atom at C-4 on 2,3,7,8-tetra-CDD, as shown in Fig. 9(10a). The distance of H_2O···H in the complex is 2.26 Å. The results for the other dioxins are summarized in Table 4. We can assume characteristic differences in the geometry for hydrogen bonding with the coordinate of water of the toxic and less toxic dioxins in the binding site model. The formation of a dioxin–H_2O complex is based on the solvation. The thermodynamic data for the formation of the complexes are given in Table 5. Since all the heat of formation (ΔH_f^{298}) is a negative value, the formation is exothermic. The solvation energy (E^{298}) of 2,3,7,8-tetra-CDD- and 1,4,6,9-tetra-CDD–H_2O complexes is 62.45 and 61.74 kcal/mol, respectively. This result shows that the 2,3,7,8-tetra-CDD–H_2O is more stable than the 1,4,6,9-tetra-CDD–H_2O complex, and that a 2,3,7,8-tetra-CDD–H_2O complex can be easily formed.

Obviously, our model can account for the differences in magnitude of the toxicity and induction responses due to the differences of both the ϕ and $E^{solv.}$ based on the formation of the complexes, but not on the positions and number of chlorine substituents. Furthermore, the η values of 1,4,6,9-tetra-, 1,2,3,7,8,9-hexa-, and octa-CDDs solvated with H_2O by hydrogen bonding, as compared with 2,3,7,8-tetra-CDD, are larger than that of the reactants, since their conformation is a more bent form. This means that since the H_2O- complexes of the dioxins of the bent form have unstable energy, inclusion to the AhR is inhibited. As a result, the equilibrium in Eq. 3 moves to the left-hand side, and their dioxins show less toxicity and lower induction responses as compared with the dioxins of chlorine substituents with lateral substitution.

We consider that the toxic potency and induction

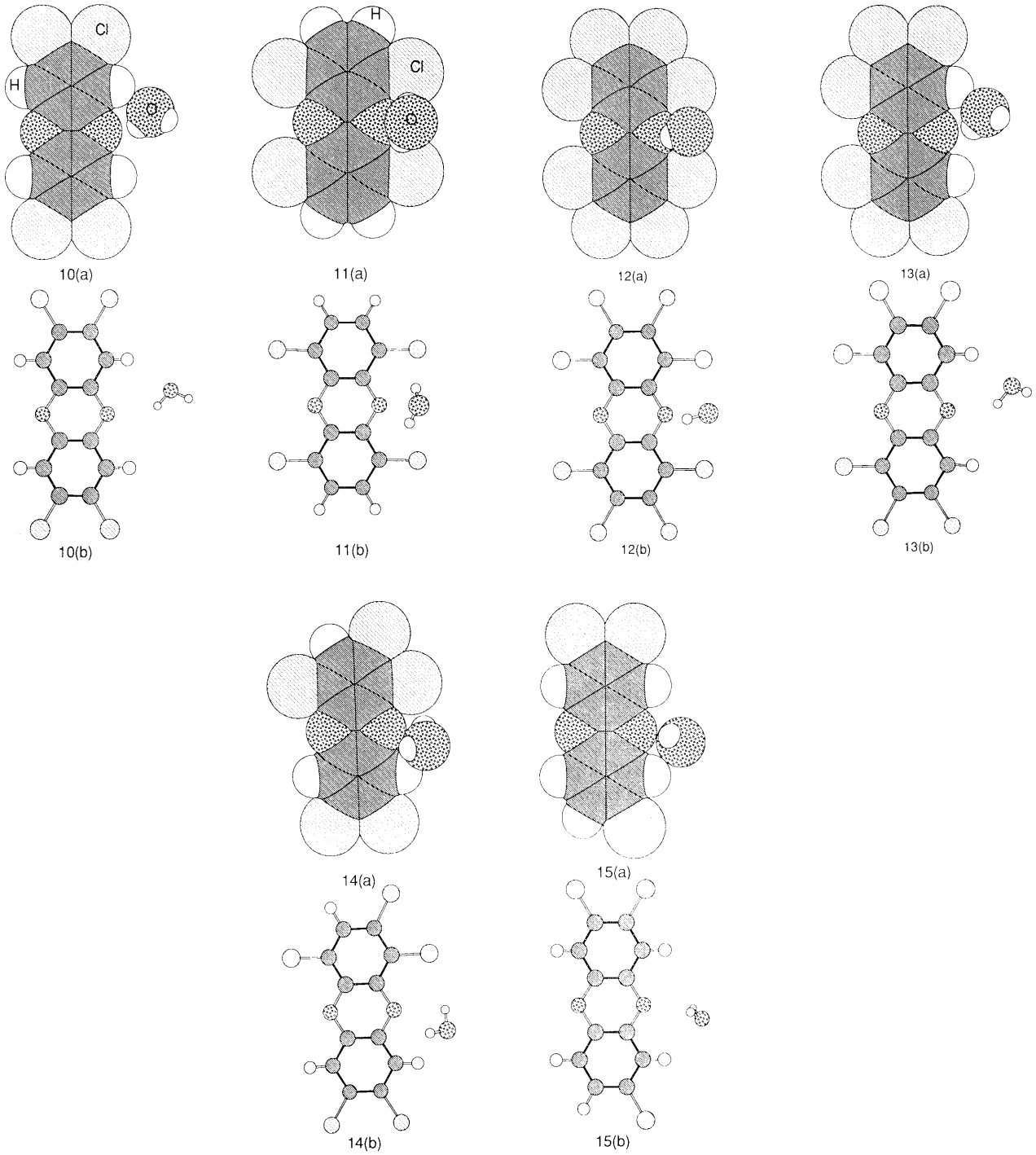
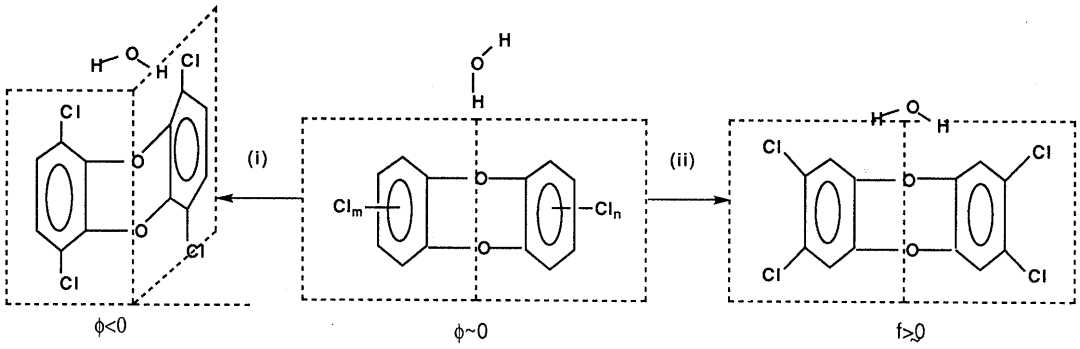


Fig. 9. Optimized Structures (AM1) for 2,3,7,8-Tetra-CDD–H_2O (**10**), 1,4,6,9-Tetra-CDD–H_2O (**11**), Octa-CDD–H_2O (**12**), 1,2,3,7,8,9-Hexa-CDD–H_2O (**13**), 1,2,4,7,8-Penta-CDD–H_2O (**14**), and 2,3,7-Tri-CDD–H_2O (**15**)

Table 5. Kinetic Data of Dioxin–H_2O Complexes

Complex	$\Delta H_f^{298,solv.}$ (kcal/mol)	ΔH_f^{298} (kcal/mol)	$E^{solv.}$ (kcal/mol)
2,3,7-Tri-CDD–H_2O	−77.46	−14.87	62.58
2,3,7,8-Tetra-CDD–H_2O	−82.01	−19.56	62.45
1,4,6,9-Tetra-CDD–H_2O	−74.03	−12.29	61.74
1,2,3,7,8-Penta-CDD–H_2O	−84.25	−21.34	62.92
Octa-CDD–H_2O	−87.40	−26.23	61.17

responses of dioxins are probably controlled by the magnitude of binding affinity in the apparent interaction with AhR of dioxins associated with the η value, η_ϕ value, and bent $\rightleftarrows$ planar form. The η and ϕ of dioxin–H_2O complexes is also important. The results are listed in Table 6. In fact, Fig. 11, derived from the plotting of the η and χ values shows that the toxic potency of dioxins is a meaningful correlation with absolute hardness for the dioxin–H_2O complexes. This diagram satisfactorily suggests that the magnitude of the toxicity and induction responses depends on the η and χ values of dioxin–H_2O complexes, which form after an interaction with the



(i) 1,9-di-, 1,4,6,9-tetra-, 1,2,3,7,8,9-hexa-, and octa-CDDs, etc.

(ii) 2,8-di-, 2,3,7-tri-, and 2,3,7,8-tetra-CDDs, etc.

Fig. 10

Table 6. Calculated Absolute Hardness (η) and Absolute Electronegativity (χ) for Several Dioxin–Water Complexes

No.	Complex	η	χ	No.	Complex	η	χ
44	1,6-DCDD–H_2O	4.236	4.792	**51**	1,2,3,7,8—TCDD–H_2O	4.039[b]	5.158
45	1,9-DCDD–H_2O	4.271	4.872			4.050[c]	5.183
46	2,8-DCDD–H_2O	4.130	4.703	**52**	1,2,4,7,8-PCDD–H_2O	4.115	5.183
47	1,4,6,9-TCDD–H_2O	4.297	5.149	**53**	1,2,3,7,8,9-HCDD–H_2O	4.090[b]	5.345
48	2,3,7-Tri-CDD–H_2O	4.090	4.973			4.062[c]	5.151
49	1,3,6,8-TCDD–H_2O	4.142	5.078	**54**	1,2,3,4,6,7,8,9-OCDD–H_2O	4.118	5.537
50	2,3,7,8-TCDD–H_2O	4.048	4.954	**55**	Dibenzo-*p*-dioxin–H_2O	4.229	4.403

a) At the AM1 level. *b*) Cl side. *c*) H side.

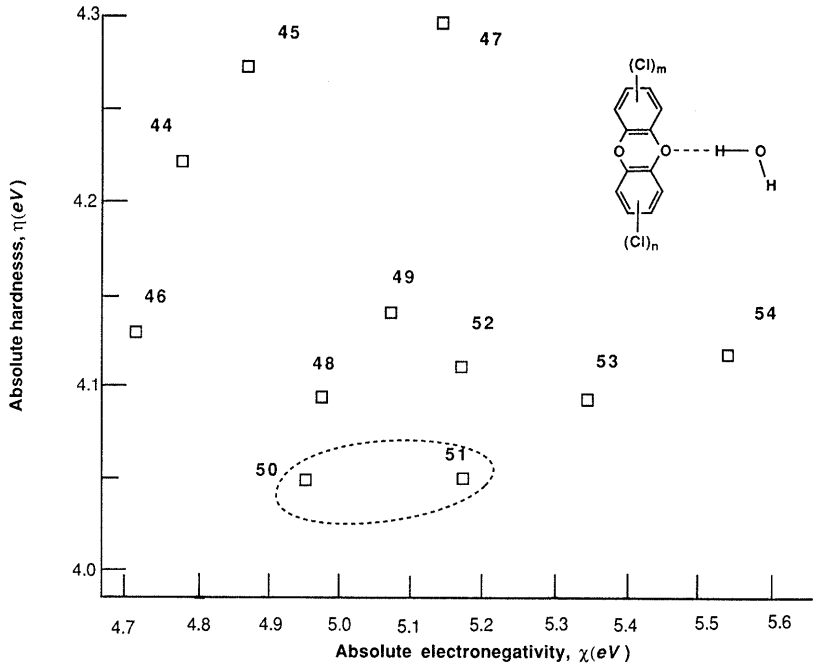


Fig. 11. Absolute Hardness–Electronegativity Diagram for Several Dioxin–H_2O Complexes

More toxic dioxins are distributed within dotted circle. **44**, 1,6-DCDD-; **45**, 1,9-DCDD-; **46**, 2,8-DCDD-; **47**, 1,4,6,9-TCDD-; **48**, 2,3,7-tri-CDD-; **49**, 1,3,6,8-TCDD-; **50**, 2,3,7,8-TCDD-; **51**, 1,2,3,7,8-PCDD-; **52**, 1,2,4,7,8-PCDD-; **53**, 1,2,3,7,8,9-HCDD-, and **54**, 1,2,3,4,6,7,8,9-OCDD–H_2O.

binding site of the dioxin in AhR.

Conclusions

We found an example of structure–biological activity relationships which conformed to the hardness concept regarding the biological activity and toxicity of dioxins. The values of absolute hardness and electronegativity will be meaningful as measurements for predicting the magnitude of the toxicity and induction responses for polyhalogenated hydrocarbons. In the structure–toxicity relationships of dioxins, we showed that the model of the dioxin–H_2O complex plays a dominant role in elucidating the correlation between dependence on the chlorination pattern and the binding affinity with the binding site of

the AhR.

The quantity of electron transfer (ΔN) calculated from Eq. 4 is correlated with binding affinity with the side chain of amino acid residue in the binding site of dioxins. The dioxins act as not only electron donors but also as electron acceptors. Whether the action of the dioxins is as an electron donor or acceptor depends on the species of amino acid residues. In fact, the soft dioxins, 2,3,7,8-tetra-CDD and 1,2,3,7,8-penta-CDD, *etc.* easily interact with phenyl ring of Phe as an electron acceptor, whereas the hard dioxins, DD and 2,8-di-CDD, *etc.*, act as electron donors.

The plotting of an η–χ diagram would be especially important as a measure for predicting the relative magnitude of toxic ability and induction responses of halogenated hydrocarbons in the fields of toxicology. The diagram is the coordinate of activity which assesses information regarding the correlation between the position of chlorine and biological activities. This method would be a more universally applicable model where the η value is different according to molecular size and shape.

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