Synthesis of New Steric and Electronic Analogs of 2,3,7,8-Tetrachlorodibenzo-p-dioxin

by Andrew S. Kende* and James J. Wade*

In contrast to the determination of a chemical reaction mechanism, the molecular mechanism for xenobiotic toxicity among polychlorinated dibenzo-*p*-dioxins is a problem of imposing complexity. These compounds are often lethal at such low dosages that identification of *in vivo* localization sites, or of metabolites, is especially difficult. Even though recent work (*1, 2*) has identified specific enzymatic sites of action which parallel the toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and its congeners, we still do not know whether TCDD itself or its metabolite(s) are responsible for the observed biological effects, nor do we know the physical and chemical mechanisms by which parameters such as molecular size, symmetry, planarity, electron distribution, solubility, ionization potential, etc., influence biological activity.

As an entry to this perplexing problem we have initiated synthetic studies to help define structural effects on biological activity in this series. We have begun by taking a look at TCDD, for which Hückel π-electron densities and bond orders are given in Figure 1. Is the activity of this molecule toward our enzymatic assays controlled by a steric fit to an active site, by the electron distribution of the molecule, or by a subtle combination of several factors?

Condensation of 4,5-dimethylcatechol (*3*) with 1,2,4,5-tetrachlorobenzene by using the conditions of Pohland and Yang (*4*) gave 49% of the colorless, crystalline 2,3-dichloro-7,8-dimethyldibenzo-*p*-dioxin [eq. (1)]. This compound is essentially isosteric with TCDD, yet it was entirely devoid of aryl hydrocarbon hydroxylase (AHH) activity.

To study the effect of varying the halogen atoms, three 2,3-dihalo-dibenzo-*p*-dioxins (I, II, III) were prepared by the catechol condensation route [eq. (2)]. The yields were 81, 25, and 41%, respectively.

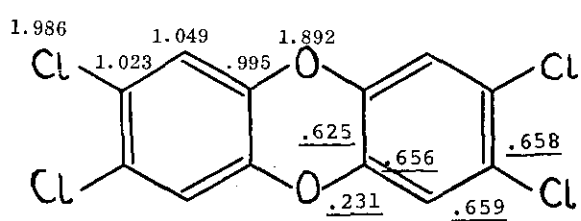

FIGURE 1. Bond orders (underlined) and densities of π-electrons in TCDD based on simple Hückel molecular orbital approximation. These calculations were obtained through the courtesy of Mr. Robert Eilerman by use of a program supplied by Professor K. Morokuma of this department. The parameters used were: $\alpha_o = \alpha_C + 2\beta$, $\alpha_{Cl} = \alpha_C + 2\beta$, $\beta_{C-O} = 0.8\,\beta_{C-C}$, $\beta_{C-Cl} = 0.4\,\beta_{C-C}$.

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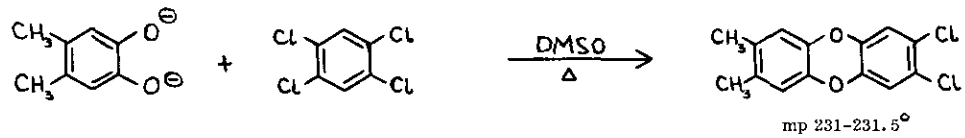
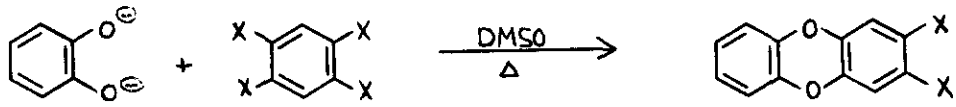


(1)



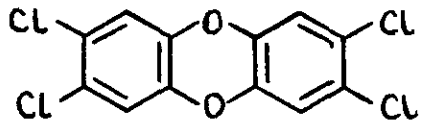
I. X=Cl, mp 163-164°C

II. X=Br, mp 157-158°C

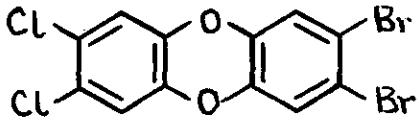
III. X=F, mp 174-176°C

(2)

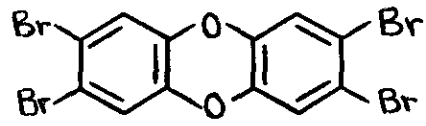
Each of these biologically inactive intermediates was cleanly dihalogenated in the 7,8 positions to yield highly toxic tetrahalo compounds. The position of the substituents in these compounds was established by the melting point and GLC identity of TCDD prepared by the present route and that of a reference sample (Kindly supplied by Dr. A. E. Pohland, FDA). In addition, the tetrabromo compound obtained by us had the identical melting point as that reported by Gilman and Dietrich, who prepared their sample by direct bromination of dibenzo-*p*-dioxin (*5*). The 2,3-dibromo-7,8-dichlorodibenzo-*p*-dioxin (IV) was prepared by two independent routes, namely, by chlorination of the 2,3-dibromo compound (II) or by bromination of the 2,3-dichloro compound (I). Satisfactory mass spectral data (Table 1) were obtained for all new compounds described in this paper, and these plus *GLC* analyses (Table 2) showed that each halodibenzo-*p*-dioxin compound depicted in Figure 2 was at least 98% pure.



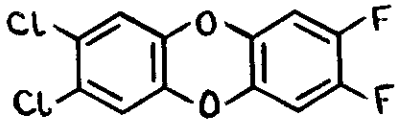
TCDD



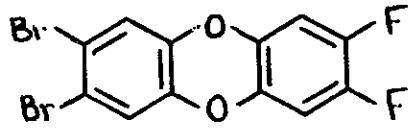
IV, mp 316-317.5°



V, mp 334-336°



VI, mp 223-225°



VII, mp 210-212°

FIGURE 2. Toxic 2,3,7,8-tetrahalodibenzo-*p*-dioxins.

The bioassays of the above tetrahalo compounds were of considerable interest. The brominated compounds IV and V proved to be as active in inducing aryl hydrocarbon hydroxylase as TCDD itself and are presumably of comparable toxicity. On the other hand, the closely related fluorine-containing counterparts VI and VII were an order of magnitude less active in the AHH assay. Moreover, the brominated compound VII was significantly more active than the chlorine analog VI. A summary of structure–activity relationships for compounds in this series is shown in Figure 3.

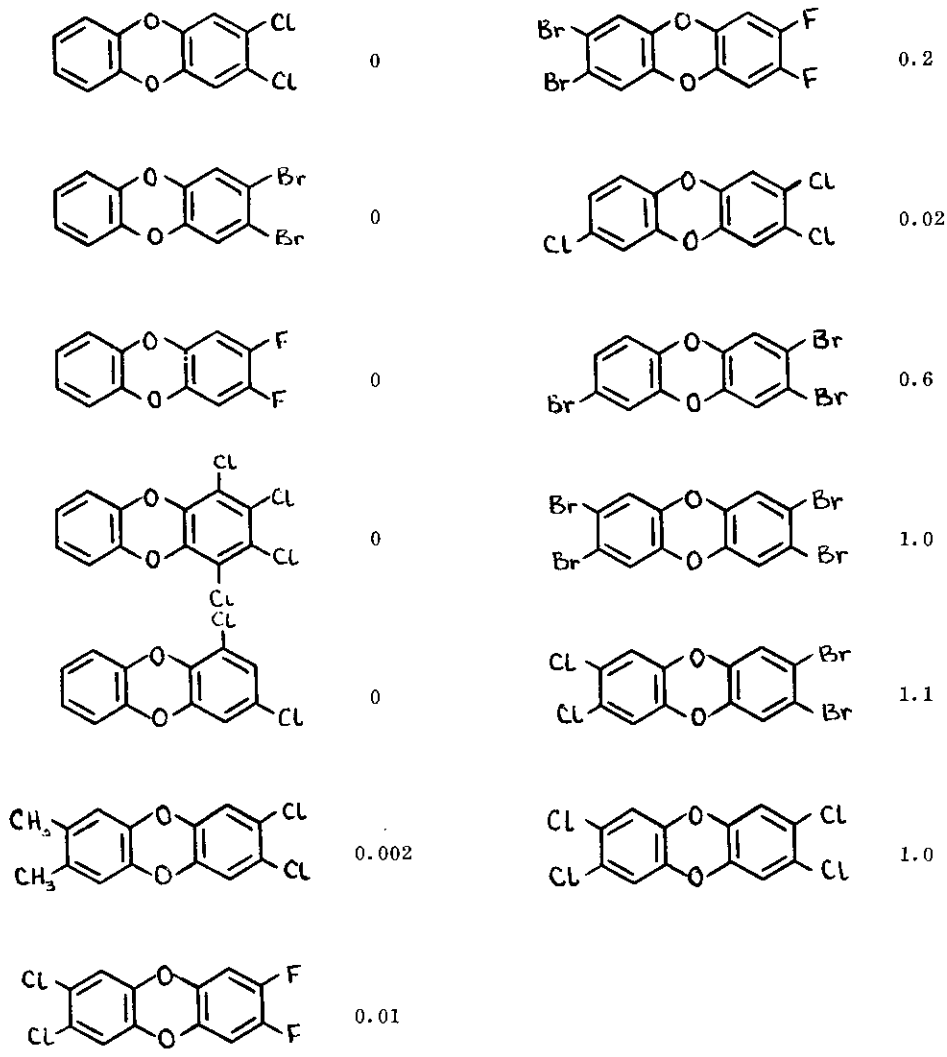
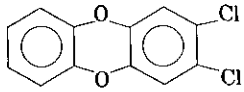
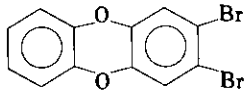
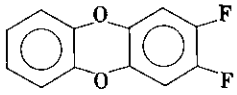
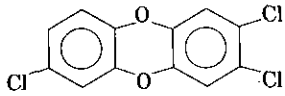
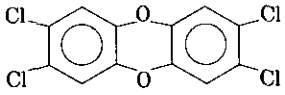
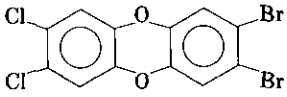
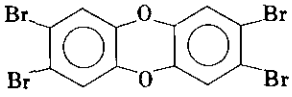
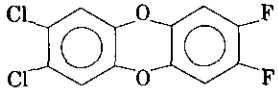
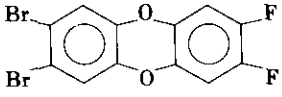
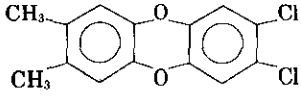
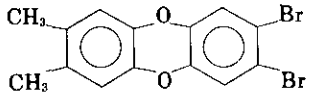
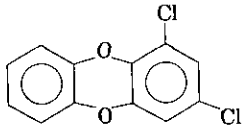
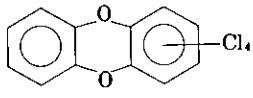
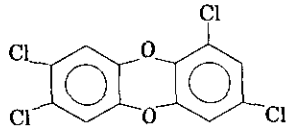


FIGURE 3. Structure–activity relations for AHH induction in chick embryo liver. Activities are expressed relative to TCDD activity = 1.0. Bioassays were carried out by Dr. Alan Poland, Pharmacology Department, University of Rochester.

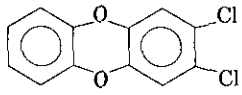
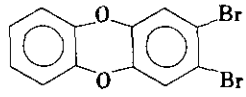
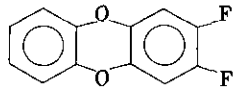
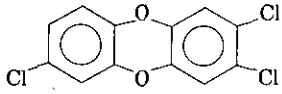
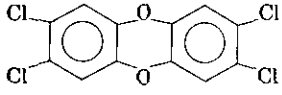
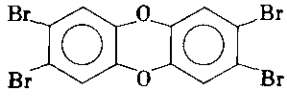
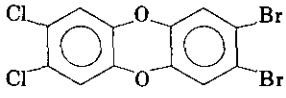
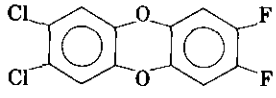
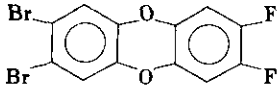
Table 1. Mass spectral data for substituted dibenzo-*p*-dioxins.[a]

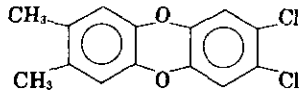
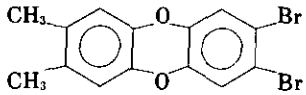
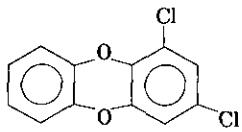
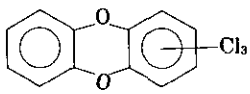
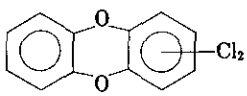
Compounds	Peaks
	254(64), 252(100), 191(7), 189(19).
	344(48), 342(100), 340(53), 263(4), 261(4), 182(36).
	220(100), 191(6), 173(16), 164(16).
	290(34), 288(100), 286(100), 253(10), 251(10), 225(28), 223(46).
	324(48), 322(100), 320(74), 259(23), 257(50), 194(23).
	414(32), 412(85), 410(100), 408(38), 349(4), 347(5), 345(4), 305(3), 303(6), 301(3).
	504(19), 502(69), 500(100), 498(69), 496(19), 423(9), 421(23), 419(23), 417(9), 342(9), 340(18), 338(9).
	290(66), 288(100), 227(6), 225(18), 162(10).
	380(50), 378(100), 376(52), 299(3), 297(3), 271(7), 269(7), 218(32), 188(5).
	282(65), 280(100), 267(13), 265(22).
	372(48), 370(100), 368(52), 357(7), 355(14), 353(7), 291(3), 289(3), 210(16).
	254(68), 252(100), 217(5), 189(24), 161(6), 126(20).
	324(52), 322(100), 320(81), 259(13), 257(13), 194(10).

Compound	Peaks
	324(54), 322(100), 320(84), 287(5), 285(5), 259(19), 257(19), 194(11).

[a] Mass spectra were obtained at 70 eV with a Hitachi–Perkin–Elmer RMU–6E instrument by use of the direct inlet method and chamber temperatures of 110–160°C. The intensity of each peak relative to the base peak (100) is given in parentheses.

Table 2. Melting point, GLC, and UV data for substituted dibenzo-*p*-dioxins.[a]

Compound	Mp, °C	GLC data		UV data	
		Column temp, °C	R_t, min	λ_{max}	ϵ
	159–160	220	5.2	299	3020
	157.5–158	200	18.3	308	3900
	174–176	200	5.1	296	4140
	153–158	230	5.1	304	3460
	305–307	230	8.3	306	6030
	334–336	230	31.8	308	
	316–317.5	230	17.8	307	4600
	223–225	200	6.5	300	3900
	210–212	200	11.4	301	4600

Compound	Mp, °C	GLC data		UV data	
		Column temp, °C	R_t, min	λ_{max}	ϵ
	231–231.5	220	11.5	301	4200
	229–230	230	11.6	302	4800
	113.5–114.5	220	4.1	296	3100
		200	12.2 (66%) 13.6 (34%)		
		200	6.9 (64%) 7.7 (36%)		

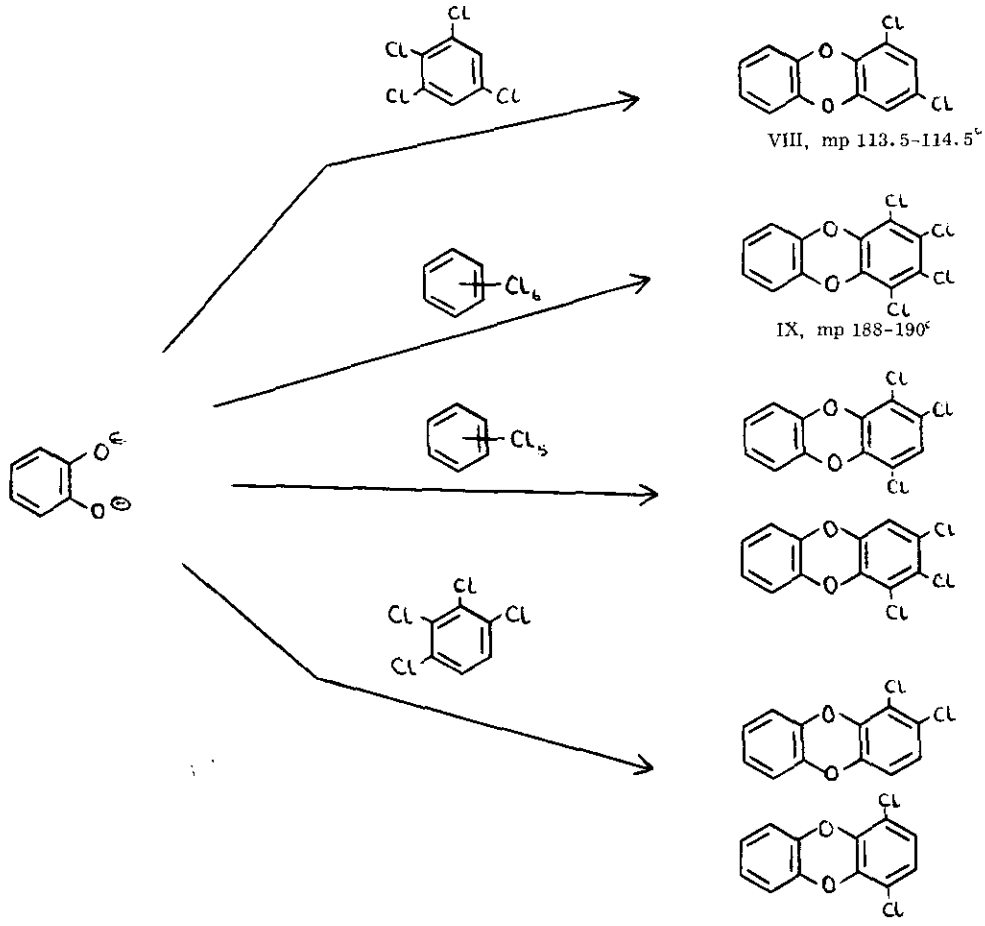
[a] Melting points were determined on a Mel-temp apparatus and are uncorrected. Ultraviolet (UV) spectra were obtained in chloroform on a Cary 118 Spectrophotometer. GLC data were obtained with a Perkin–Elmer 900 instrument with a hydrogen flame detector with the use of a 6 ft x 1/8 in. 15% SE 30 column at a nitrogen flow rate of 30 ml/min.

In addition to the compounds already discussed, several other halogenated dibenzo-*p*-dioxins were prepared by variants of the catechol condensation. Thus catechol itself reacts cleanly with 1,2,3,5-tetrachlorobenzene or with hexachlorobenzene to give 1,3-dichlorodibenzo-*p*-dioxin (VIII) or 1,2,3,4-tetrachlorodibenzo-*p*-dioxin (IX), respectively [eqs. (3)]. The condensations with 1,2,3,4-tetrachlorobenzene or pentachlorobenzene also occur readily but each yields two products, as would be expected. None of of the compounds in eq. (3) showed any AHH-inducing activity.

The condensation of 4-chlorocatechol (*6*) with 1,2,4,5-tetrachlorobenzene gives 2,3,7-trichlorodibenzo-*p*-dioxin (X), which is two orders of magnitude less active than TCDD in the AHH assay. The condensation with 4,5-dichlorocatechol (*6*) also proceeds readily, and we have obtained the new 1,3,7,8-tetrachlorodibenzo-*p*-dioxin (XI) and also TCDD itself by condensation with 1,2,3,5- or 1,2,4,5-tetrachlorobenzene, respectively [eqs. (4)–(6)]. This last reaction provides an alternative, one-step synthesis of TCDD.

This preparation of TCDD, or the two-step sequence proceeding through I as described earlier, each provides a facile synthesis of TCDD in nearly 40% yield. Both offer an advantage for the microscale preparation of uniformly labeled ^{14}C-TCDD, since labeled 1,2,4,5-tetrachlorobenzene is commercially available (Mallinckrodt Chemical Works). Alternative but less direct paths to labeled TCDD have been reported (*7*, *8*).

The scope of the condensation reaction of substituted catechols with polyhalobenzenes has not yet been fully delineated, but its utility for making new TCDD derivatives, including penta-, hexa- and heptachlorodibenzo-*p*-dioxins, is already evident. In addition, preliminary studies reveal the feasibility of carrying out the mono- and dinitra-



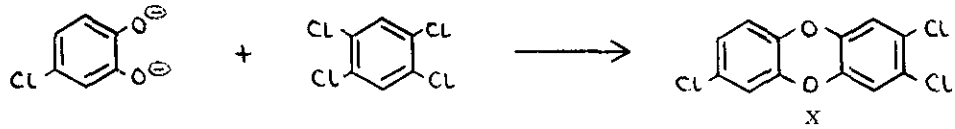
(3)

tion of the 2,3-dihalodibenzo-*p*-dioxin system by using nitronium tetrafluoroborate. This in turn would offer an entry to a new set of 2,3,7-tri- and 2,3,7,8-tetrasubstituted dibenzo-*p*-dioxins through Sandmeyer reactions on aminodibenzo-*p*-dioxin intermediates. Further studies of such extensions of our synthetic methodology are in progress.

A concise summary of our present knowledge of structure activity relationships for some two dozen dibenzo-*p*-dioxin derivatives (*1*) leads to the following rules: (1) two halogen substituents at positions 2, 3 and one halogen at position 7 are minimum structural requirements; (2) bromine as a substituent is more active than chlorine which is more active than fluorine; (3) at least one hydrogen atom must remain on the dibenzo-*p*-dioxin nucleus.

It is noteworthy that no amount of halogen substitution on only one ring leads to biological activity. There is the strong temptation then to postulate a bidentate active site in which a polarizable and strong carbon–halogen dipole must present itself at each end. Given such a site, how would the above structure–activity relationships rationalize a chemical mechanism whereby irreversible chemical binding between toxic compound and protein or nucleic acid could occur?

Three mechanistic variants can be discussed: the aryne mechanism [eq. (7)], the arene oxide mechanism [eq. (8)], and the *o*-quinone mechanism [eq. (9)]. There is no direct evidence for any one of these.



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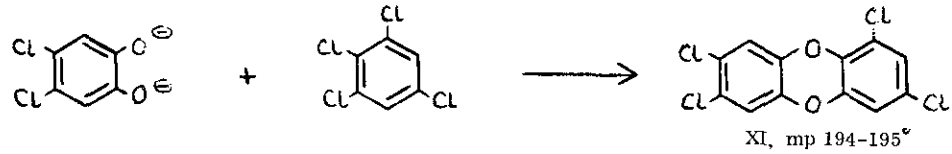
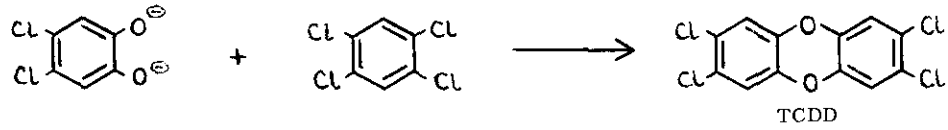
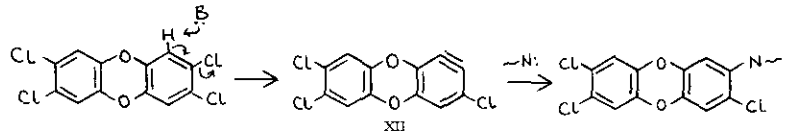


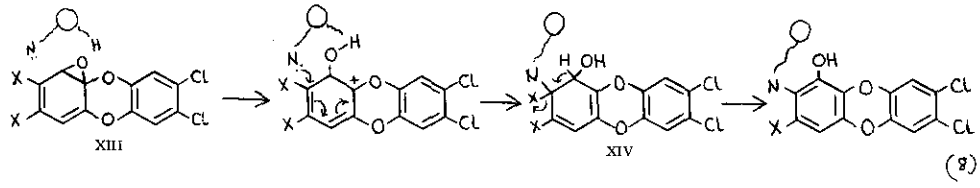
(5)



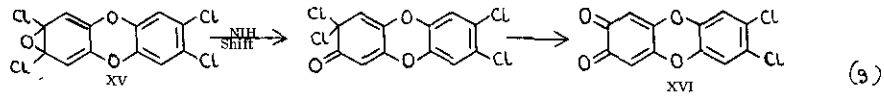
(6)



(7)



(8)



(9)

It has been suggested (A. Streitwieser, private communication) that loss of HCl by deprotonation at C-1 of TCDD could lead to a benzyne [XII, eq. (7)] which would react irreversibly with a cell nucleophile. This hypothesis parallels the F <Cl<Br toxicity sequency with rate of aryne formation (*9*).

The extreme generality of arene oxide formation in the metabolism of halobenzenes (*10*) suggests an intermediate such as XIII [eq. (8)]. Opening of XIII by simultaneous coordination and displacement by an ambident molecule (a purine or pyrimidine?) would yield the dihydroaromatic in-

termediate XIV. Where the halogen X is Br or Cl, loss of HX would bind the nucleophile N irreversibly to the aromatic residue; where X is F (or another poor leaving group) loss of H^+ and N would regenerate the unbound dioxin system.

Both of the above mechanisms ignore the apparent necessity for the 2,3-dihalo system. To rectify this we postulate a different arene oxide intermediate, namely XV [eq. (9)], in which an NIH shift (*10*) of Br or Cl (not observed for F) could lead to an *o*-quinone (XVI) which would possess exceptional mesomeric stabilization and could serve as a lethal and irreversible Michael acceptor toward cell nucleophiles.

These extremely speculative hypotheses suggest specific experimental approaches. These include the study of deuterium isotope effects (1,4,6,9-D_4-TCDD) on toxicity, the examination of Cl^{36} loss from labeled TCDD, or the independent synthesis of quinone XVI or a phenolic precursor. We hope that such studies, together with the search for localization sites and metabolic products by using ^{14}C-TCDD, will provide more precise clues to the molecular mechanism of toxicity in this series.

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