SURROGATES FOR DIOXIN ANALYSES: ANALYTICAL, ELISA AND TOXICOLOGICAL ASPECTS

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Introduction

The polychlorinated dibenzodioxins and dibenzofurans or polychlorinated biphenyls have undesirable mammalian toxicities such that preparation and disposal of these standard solutions require strict adherence to regulatory guidelines. Because some of the handling and disposal requirements for handling these compounds are so stringent, not all analytical laboratories have the capability or the interest in doing these types of analyses. The purpose of this paper is to outline an approach for the use of analytical standards for the analysis of *2,3,7,8*-tetrachloro dibenzo-*p*-dioxin (TCDD) that have all the requisite analytical characteristics of TCDD and its congeners by instrumental and ELISA methodology but have toxicities that are less than the standards currently employed.

The approach to the development of nontoxic analytical surrogates for TCDD analysis depends on the close interaction of the analytical aspects of the issue with the ability to test the surrogates in a system that allow the detection of TCDD activity. The design of these standard with functionalities that hopefully will provide reduced toxicity is based on the literature background on pesticides that have developed around these persistent organic pollutants and the physical-chemical characteristics that result in persistence and in some cases their bioaccumulation in aquatic species and humans [1]. The most difficult aspect of this work will be the finding the balance between utility in both conventional analytical techniques and ELISA and also possess reduced toxicity which will be advantageous with respect to handling and disposal. With respect to the synthesis of these analogues the same procedures reported in the initial reports of development of an ELISA assay for TCDD were used [2] and others investigating the biological activity of these analogues [3-5].

For the selection of surrogate substituents, other than immunoassay, these analogues were evaluated in two assays that evaluates AhR activity: Gel-shift assay [6,7] and transformed mouse hepatoma cells responsive to AhR agonists [8]. Lipiphilic charateristics and retention indices on GC are two important factors and evaluated for the development of surrogates for dioxin analysis. The first factor is estimated by calculation of Kow, and the later one is based on the relative GC retention time compared to C22 hydrocarbon with an index 2200 as a standard reference. Finally,

the selected compounds were also compared with the prediction of molecular modeling study, which is based on monoclonal TCDD antibody.

Material and Methods

Synthesis: The surrogate analytical standards were prepared by the routes described in the AhR SAR papers of Romkes, *et al* [3,4] *and* Denomme, *et al.*[5].

Analytical Data: The GC/MS results were generated on a Trio-2 GC/MS system (VG Masslab, Atrincham, UK) using 70 ev electron ionization. A 30 meter DB1 column (0.25 mm I.D., 0.25 μm film) using helium at a linear velocity of 30 cm/s was employed. Samples were dissolved in tetrahydrofuran (THF) and splitless injections of 1 μl were made. The column was programmed from 80° C (1 min hold) to 150° C at 20 degrees/min followed by an increase to 300° C at 10 degrees/min. As mentioned previously, the retention indices for the various chlorinated dibenzo-*p*-dioxin isomers were estimated by the method of Donnelly *et al* [9].

Bioassay and ELISA: Gel-Shift and AhR Assays were performed following the methods demonstrated in Helferich and Denison [6], Denison and Yao [7] and Garrison *et al* [8]. ELISA format and condition were described in Shan *et al.* [10].

Molecular Modeling Studies were performed using a CAChe WorkSystem as described previously [11]. Minimum energy conformations were calculated using Allinger's standard MM2 force field augmented to contain force field parameters for case not addressed by MM2. For determination of the electronic properties of the modeled compounds, the electronic wave function for all compounds was calculated by solving the Schrodinger equation using the Extended Huckel approximation.

Results and Discussion

Surrogates design. A search of the literature provided references that have evaluated the structure-activity relationships of dichloro- and tetrachlorobenzodioxins in a variety of systems that respond to these analogues [3-5]. The substituents in these publications sought to cover a range of electronic, steric, lipophilic and hydrogen bonding parameters for the purposes of development of a Quantitative Structure Activity Relationship (QSAR) for AhR activity. Carboxylic ester (methyl or propyl) or a methyl in either of these series in the 2-position of either 7,8-dichlorodibenzo-*p*-dioxins or 3,7,8-trichloro appeared to appropriate to test the concept of whether these analogues fit the preceding criteria for an analytical surrogate. These two substituents (carboxylic esters and methyl) based on the data in these publications were less active than TCDD *in vivo* and *in vitro* assays. Ultimately, the choice of a substituents should include a consideration of the facility of radiolabeling for matrix recovery studies which are essential to the validate any method using these surrogate analogues.

Inhibition Studies (ELISA). Table 1 showed results of ELISAs performed using polyclonal and monoclonal antibody formats. Each compound was evaluated based on two parameters: I_{50} and inhibition curve slope. Compared with TCDD, compounds **2**, **6**, **7**, and **8** showed similar responses in both ELISA systems, demonstrating the analog's potential as a surrogate standard for TCDD.

Cell-based bioassay. Selected compounds (Table 2) were tested for Ah receptor-mediated response in Gel-shift and luciferase activity assays. The results indicate that the response of these analogs is over an order of magnitude less than that of TCDD for these assays.

Chromatographic data and molecular modeling. According to the retention indices and estimated octanol-water partition coefficients (K_{ow}) listed in Table 3, compounds **2** and **7** showed very close physical and chromatographic characteristics to our target compound TCDD. In addition, The molecular modeling studies also predicted that TCDD, compounds **2**, and **7** are very similar in geometric and electronic structure which would make the analog a surrogate standard for TCDD.

In conclusion, rational design, synthesis and intensive screening of ELISA, bioassay, and chromatography resulted in two compounds **2** and **7** as surrogate standards for TCDD. These two compounds not only have identical response of TCDD in ELISA and GC system, also possess much lower Ah receptor-mediated responses compared with TCDD, indicating they might be much less toxic than TCDD. Finally, molecular modeling studies of TCDD and these two compounds independently predicted the above experimental results. These two compounds might be suitable surrogates for TCDD analysis and other related studies.

Acknowledgements

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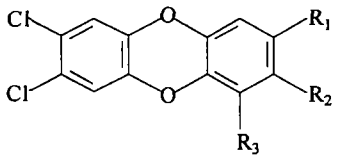
Table 1 Summary of TCDD surrogate screening test by ELISA and bioassay



Compd	R_1	R_2	R_3	pAb 7598		mAb DD3	
				Slop	I_{50}(ppt)	Slope	I_{50}(ppb)
TCDD	Cl	Cl	H	0.92	28	0.80	1.28
1	H	CH_3	H	0.66	954	0.83	61.4
2	Cl	CH_3	H	0.90	36	0.72	1.33
3	Cl	H	NH_2	0.99	35	0.77	4.61
4	Cl	OH	H	0.57	64	1.0	35.6
5	Cl	NH_2	H	0.98	88	0.46	6.42
6	Cl	$COCH_3$	H	0.99	32	1.20	10.7
7	Cl	$COOCH_3$	H	1.12	32.1	0.87	3.30
8	Cl	$COOC_3H_7$	H	1.0	129	0.54	5.71
9	Cl	$NHCOCH_3$	H	1.20	56	--	>200
10	Cl	CH_2CH_2COOEt	H	0.66	202	--	>200

Table 2 AhR Activity of TCDD Analytical Surrogates

Compd	R_1	R_2	R_3	Gel-Shift (I_{50}, nM)	Relative Activity[a/]	Luciferase Activity (I_{50}, nM)	Relative. Activity
1	H	CH_3	H	1.0	0.2	98	0.0001
2	Cl	CH_3	H	0.8	0.25	0.99	0.01
7	Cl	CO_2CH_3	H	10.0	0.02	1.0	0.01
8	Cl	$CO_2C_3H_7$	H	--	--	3.47	0.003
TCDD	Cl	Cl	H	0.2	1.0	0.009	1.0
a/ TCDD Activity = 1.0 for both assays							

Table 3 Retention Indices and Estimated Octanol-Water Partition Coefficients (K_{ow})

Compd	R_1	R_2	R_3	M.W.	Retention Index	Estimated Log K_{ow}
1	H	CH_3	H	266	2097	6.30
2	Cl	CH_3	H	300	2315	7.21
7	Cl	CO_2CH_3	H	344	2646	6.65
8	Cl	CO_2n-C_3H_7	H	372	2737	7.97
TCDD	Cl	Cl	H	320	2435	7.26 (6.54-6.95)[a/]
Octachlorodibenzo-*p*-dioxin				456	3417	8.80
a/ Marple *et al.*[12].						