CROSS-REACTIVITY ON PROCEPT ARYL HYDROCARBON BASED POLYMERASE CHAIN REACTION DIOXIN ASSAY

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Introduction

The Procept Rapid Dioxin Assay (Eichrom Technologies, Inc.) is an Aryl hydrocarbon-Receptor (AhR) based assay which utilizes Polymerase Chain Reaction (PCR) to quantify levels of polychlorinated dibenzo-*p*-dioxins and furans (PCDD/F) in samples.[1] Previous work[2] has shown the cross-reactivity for the 17 PCDD/F congeners that have been assigned a toxicity equivalent factor (TEF) by the World Health Organziation[3] (WHO) and several polychlorinated biphenyls (PCB) and polycyclic aromatic hydrocarbons (PAH). This work will extend the study of cross-reactivity to additional PAH, PCDD/F, brominated dioxins and furans, pesticides and other compounds similar in structure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD).

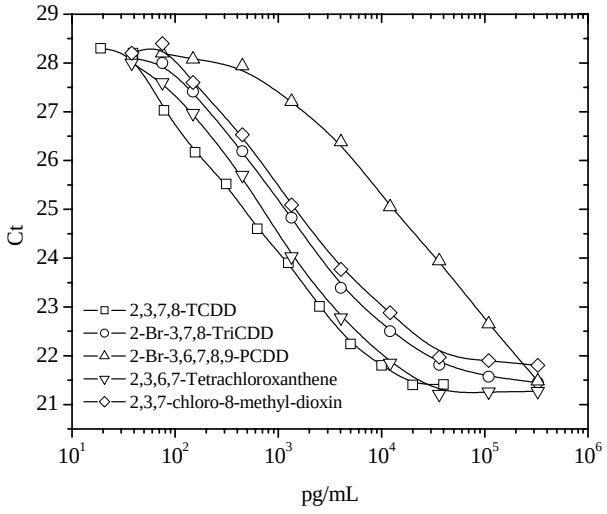


Figure 1. Dose-response curve for Procept Assay

Materials and Methods

The Procept Rapid Dioxin Assay was obtained from Eichrom Technologies, Inc. and Hybrizyme Corporation. Analytical standards were obtained from Cambridge Isotope Laboratories, Accustandard, Inc., Wellington Laboratories and SPEX Certiprep Group. Solvents were obtained from Sigma Aldrich and were of HPLC grade. Deionized water was obtained from a Milli-Q2 water purification system. PCR reagents were obtained from Stratagene, Inc..

Cross-reactivity values were obtained by measuring the response of the various compounds over a wide range of concentrations (10 pg/mL to 100 µg/mL). From the resulting sigmoidal dose-response curve, the EC_{50} value for the compound was determined as the effective concentration where the response falls halfway between the bottom and top plateau of the dose-response curve. The cross-reactivity for the compound was then calculated by dividing the EC_{50} value for 2,3,7,8-TCDD by the EC_{50} value for the compound of interest. Repeat determinations of selected cross-reactivity values showed agreement within 10%.

Results and Discussion

Typical dose-response curves for several compounds on the Procept assay are depicted in Figure 1. The concentration of the compound, in heptane, is on the x-axis, while the Threshold cycle (Ct) is on the y-axis. Ct is the output of the PCR instrument and is the number of PCR temperature cycles at which the fluorescence of a sample reaches a threshold value. In the Procept assay, the Ct of a sample is inversely proportional to the TEQ of the sample. By comparing the Ct values for a standard curve vs. the Ct value of an unknown sample, the TEQ of the sample can be determined.

Table 1. Response for Bromo/chloro Dioxins and Furans and Dioxin-like Compounds on Procept Assay

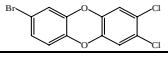
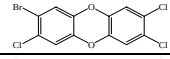
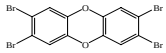
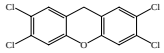
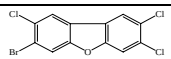
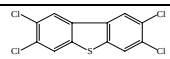
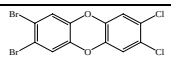
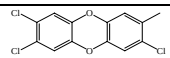
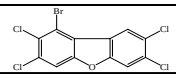
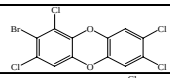
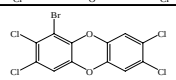
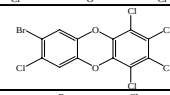
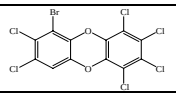
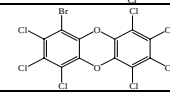
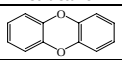
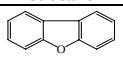
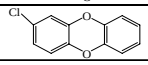
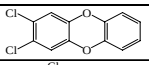
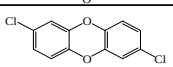
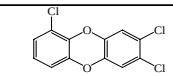
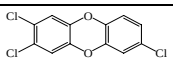
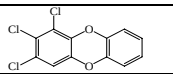
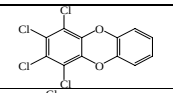
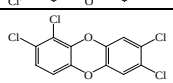
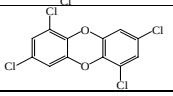
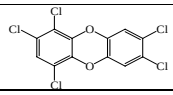
Structure	compound	Procept Response	compound	Procept Response	Structure
	2-Br-7,8-DiCDD	$<10^{-6}$	2-Br-3,7,8-TriCDD	0.5	
	2,3,7,8-TBrDD	0.3	2,3,6,7-TCl-xanthene	0.7	
	3-Br-2,7,8-TriCDF	0.4	2,3,7,8-TCl-thiophene	0.2	
	2,3-Br-7,8-DiCDD	0.4	2,3,7-Cl-8-methyl-dibenzo-p-dioxin	0.3	
	1-Br-2,3,7,8-TCDF	0.3	2-Br-1,3,7,8-TCDD	0.6	
	1-Br-2,3,7,8-TCDD	0.4	2-Br-3,6,7,8,9-PCDD	0.05	
	1-Br-2,3,6,7,8,9-HxCDD	0.06	1-Br-2,3,4,6,7,8,9-HpCDD	0.00004	

Table 2. Response of non-2,3,7,8 Polychlorinated Dibenzo-*p* -Dioxins and Furans on Procept Assay

structure	compound	Procept Response	compound	Procept Response	Structure
	dibenzo-p-dioxin	$< 10^{-6}$	dibenzo-p-furan	$< 10^{-6}$	
	2-MCDD	$< 10^{-6}$	2,3-DiCDD	$< 10^{-6}$	
	2,7-DiCDD	$< 10^{-6}$	1,7,8-TriCDD	0.004	
	2,3,7-TriCDD	0.08	1,2,3-TriCDD	0.006	
	1,2,3,4-TCDD	0.007	1,2,7,8-TCDD	0.9	
	1,3,6,8-TCDD	0.001	1,2,4,7,8-PCDD	0.3	

The cross-reactivities on the Procept assay for several brominated, mixed brominated/chlorinated dibenzo-*p*-dioxins and furans and several dioxin-like compounds are listed in Table 1. The cross-reactivity of these compounds is typically very similar to the corresponding chlorinated analogue.

The cross-reactivities on the Procept assay for several non-2,3,7,8 polychlorinated dibenzo-*p*-dioxins and furans are listed in Table 2. Several of the non-2,3,7,8 polychlorinated dibenzo-*p*-dioxins have significant response on the Procept assay. Typically, those compounds with chlorine substitution at three of the four 2,3,7,8 positions exhibit the highest response, with those compounds with chlorine substitution at less than three of the 2,3,7,8 positions exhibiting little or no response on the assay.

The cross-reactivities on the Procept assay for several polycyclic aromatic hydrocarbons (PAH) are listed in Table 3. Since many of these compounds exhibit a significant response on the Procept assay and can be present in much larger quantities than PCDD/F compounds, sample preparation methods for the analysis of PCDD/F by the Procept assay must efficiently remove PAH compounds from the PCDD/F fraction.[2,4,5]

Table 3. Response of PAH Compounds on Procept Assay

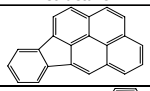
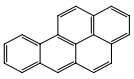
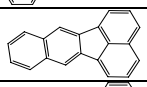
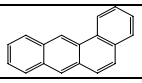
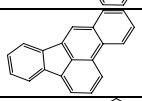
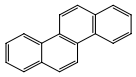
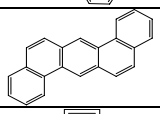
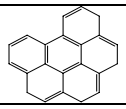
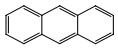
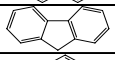
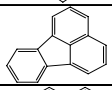
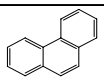
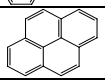
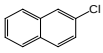
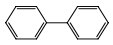
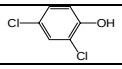
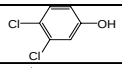
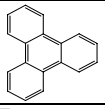
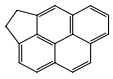
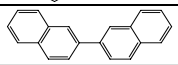
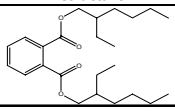
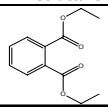
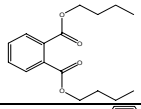
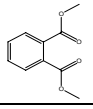
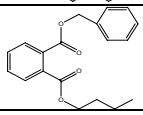
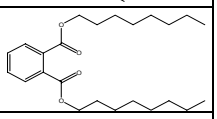
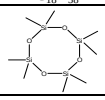
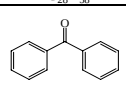
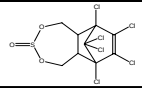
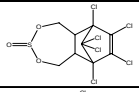
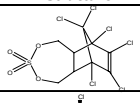
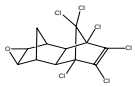
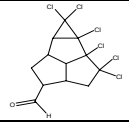
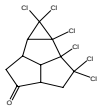
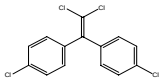
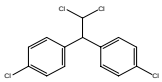
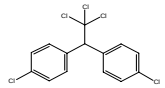
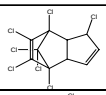
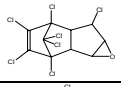
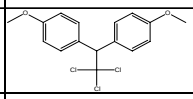
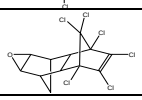
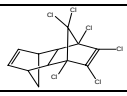
structure	compound	Procept Response	compound	Procept Response	Structure
	Indeno(1,2,3-cd)pyrene	0.8	Benzo(a)pyrene	0.1	
	Benzo(k)fluoranthene	0.5	Benzo(a)anthracene	0.05	
	Benzo(b)fluoranthene	0.6	Chrysene	0.04	
	Dibenzo(ah)anthracene	0.3	Benzo(ghi)perylene	0.004	
	acenaphthylene	No response at 10 ppm	anthracene	No response at 10 ppm	
	fluorene	No response at 10 ppm	naphthalene	No response at 10 ppm	
	fluoranthene	No response at 10 ppm	phenanthrene	No response at 10 ppm	
	pyrene	No response at 10 ppm	acenaphthene	No response at 10 ppm	
	2-methylnaphthalene	No response at 10 ppm	2-chloronaphthalene	No response at 10 ppm	
	biphenyl	No response at 10 ppm	2,4-dichlorophenol	No response at 10 ppm	
	3,4-dichlorophenol	No response at 10 ppm	toluene	No response at 10 ppm	
	triphenylene	0.001	cyclopenta[c,d]pyrene	0.0002	
	2,2'-binaphthyl	0.05			

Table 4. Response of Miscellaneous Compounds on Procept Assay

structure	compound	Procept Response	compound	Procept Response	Structure
	bis-(2-ethylhexyl)phthalate	< 7 x 10^{-7}	diethylphthalate	< 7 x 10^{-7}	
	di-n-butylphthalate	< 7 x 10^{-7}	dimethylphthalate	< 7 x 10^{-7}	
	butylbenzylphthalate	< 7 x 10^{-7}	di-n-octylphthalate	< 7 x 10^{-7}	
$C_{10}H_{22}$	decane	< 9 x10^{-7}	eicosane	< 9 x10^{-7}	$C_{20}H_{42}$
$C_{12}H_{26}$	dodecane	< 9 x10^{-7}	docosane	< 9 x10^{-7}	$C_{22}H_{246}$
$C_{14}H_{30}$	tetradecane	< 9 x10^{-7}	tetracosane	< 9 x10^{-7}	$C_{24}H_{50}$
$C_{16}H_{34}$	hexadecane	< 9 x10^{-7}	hexacosane	< 9 x10^{-7}	$C_{26}H_{54}$
$C_{18}H_{38}$	octadecane	< 9 x10^{-7}	octacosane	< 9 x10^{-7}	$C_{28}H_{58}$
	octamethyl cyclotetrasiloxane	no measurable response at 2000 ppm	benzophenone	< 1 x10^{-6}	

Most work with the Procept assay has focused on the measurement of PCDD/F from soil and sediment. GC-HRMS analysis of PCDD/F fractions isolated from soil extracts prepared for analysis by the Procept assay, have shown the presence of several other types of compounds, including alkanes/alkenes (from solvents), phthalates and silicones (from sample preparation reagents). The cross-reactivities of these compounds on the Procept assay are listed in Table 4. Additionally, since high levels of pesticides may also be present in some soil samples, the cross-reactivities of several pesticides on the Procept assay have been measured and are listed in Table 5. No response was observed for any of the pesticide compounds from 200 pg/mL to 2000 µg/mL.

Table 5. Response of Pesticide Compounds on Procept Assay
(No Response for any of these compounds from 200 pg/mL to 2000 µg/mL)

Structure	Compound	Structure	Compound	Structure	Compound
	Endosulfan I		Endosulfan II		Endosulfan sulfate
	apha-BHC		beta-BHC		delta-DHC
	Endrin		Endrin Aldehyde		Endrin Ketone
	p,p'-DDE		p,p'-DDD		p,p'-DDT
	Heptachlor		Heptachlor epoxide		Methoxychlor
	Dieldrin		aldrin	**No reponse for any of these compounds from 200 ppt to 2000ppm**	

Acknowledgement

The authors wish to thank Steve Billets of the US EPA and Amy Dindal of Battelle for their assistance, providing GC-HRMS analyses, brominated standards and helpful discussion.

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