Organohalogen Compounds, Volume 60, Pages 231-234 (2003)

A COMPARISON OF TOXICITY EQUIVALENCY (TEQ) RESULTS OBTAINED USING RAPIDSCREEN AND THE DIPS-CALUX BIOASSAY WITH EPA METHOD 8290 AND 1668A CALCULATED TEQS

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Introduction

Toxic Equivalency (TEQ) describes the toxicity of a sample in terms of one representative compound. Among dioxins and furans, Toxicity Equivalency Factors, or TEFs, are used to describe the relative toxicity of 16 specific congeners in comparison to 2,3,7,8-TCDD. The TEQ of a sample is typically determined from the sum of each congener's concentration (or detection limit) multiplied by its TEF.

Congener	TEF
2,3,7,8-TCDD	1.0
1,2,3,7,8-PeCDD	0.5
OCDD	0.001
2,3,7,8-TCDF	0.1
1,2,3,7,8-PeCDF	0.05
2,3,4,7,8-PeCDF	0.5
OCDF	0.001
3,3',4,4'-PCB	0.0005
3,3',4,4',5-PCB	0.1
3,3',4,4',5,5'-PCB	0.01

Table 1: List of Dioxins, Furans, and PCBs monitored by the RapidScreen Method and their corresponding EPA 1989 TEFs

While HRGC/HRMS is the definitive analytical method used for determining the TEQ of an environmental, food or serum sample at part-per-trillion levels (ppt), it is time consuming and expensive. Cost barriers may undermine the thoroughness of a sampling or monitoring program. Screening methods for the TEQ of samples from a variety of matrices are becoming increasingly important due to concerns about dioxin/furan/PCB contamination. Screening methods promise cost-effective, more rigorous sampling and monitoring by greatly reducing the number of samples that have to be tested using full-scale quantitative methods.

The RapidScreen method for TEQ[1] developed by Triangle Laboratories, Inc., USA and the DIPS-CALUX Bioassay developed by Xenobiotic Detection Systems, Inc., USA[2], are two methods available to screen samples from a variety of matrices for dioxin toxicity. The RapidScreen method uses isotope dilution HRGC/HRMS to screen samples as positive or negative based on a threshold TEQ. Seven dioxin/furan congeners (shown in Table 1) that contributed 62% to an environmental sample's overall 8290 measured TEQ 77% of the time[3], and three PCB congeners are used in the calculations. The DIPS-CALUX bioassay quantitates the TEQ based on the activation of aryl hydrocarbon receptor (AhR)-mediated gene transcription.

Screening assays need to be evaluated against fully validated methods before they can be used reliably. The incidence of false positives and negatives provides a good comparison statistic for

evaluating screening methods. In this study, samples were analyzed using both RapidScreen and CALUX and the results were compared with those obtained using the EPA reference methods.

Methods and Materials

Soil samples from a contaminated site were used in this study. The reference methods used were SW-846 8290 for dioxins and furans, and Method 1668A for PCBs. Both of these are isotope dilution methods whose standard procedures are documented elsewhere[4,5]. Method-approved procedures were followed to estimate the amounts in pg/g of the target analytes. The EPA 1989 TEFs were then used to calculate the TEQ from dioxins and furans, and PCBs.

RapidScreen Method: Briefly, soil samples were spiked with a solution containing specified amounts of six isotopically ($^{13}C_{12}$) labeled PCDDs/PCDFs and three PCBs. The soil samples were solvent extracted and purified using a modified carbon column. The extracts were dried by vacuum centrifuge and 1µl aliquots were analyzed using HRGC/HRMS. A positive or negative result was determined using the isotope dilution method in the calculations. The RapidScreen validation is currently in process with the Office of Solid Waste, EPA.

DIPS-CALUX Bioassay: Different aliquots of the homogenized samples were also analyzed by Xenobiotic Detection Systems, Inc., USA. using the DIPS-CALUX Bioassay. One gram samples were extracted, and then purified using acid silica and carbon columns to remove interfering compounds. The assay also incorporated a column-based separation of the dioxins and furans from the PCBs, providing independent TEQs for these two sets of compounds.

Results and Discussion

Comparison of Screening Methods

A summary of the results for the ten soil samples analyzed is shown in Table 2. The samples encompassed TEQs ranging from 0.8-50 ppt. All results were compared using the 8290 TEQ and 1668A TEQ as a reference. To facilitate comparison between the RapidScreen and the CALUX assays, the CALUX TEQ values were converted to a positive/negative screening result using the RapidScreen TEQ criterion of a 4 ppt TEQ threshold and these are shown in parentheses next to the CALUX TEQs.

The CALUX assay screened each sample analyzed as positive with the lowest TEQ measured being 8.74 for a sample that had a reference TEQ of 0.96. This result is indicative of interfering compounds not being removed during the sample cleanup procedure. The RapidScreen method, which reports a positive or negative result based on a threshold of 4 ppt TEQ, identified seven out of the ten samples correctly.

RapidScreen presents a false positive result for samples 005 and 006 due to the presence of interfering compounds not removed during the purification procedure. The RapidScreen procedure uses a carbon column based cleanup that can, if overloaded, result in the presence of planar aromatic compounds in the extract. These compounds may produce responses in the analyte channels and result in positive interferences.

Sample	8290 TEQ (pg/g)		1668A TEQ (pg/g)	Total TEQ (pg/g)	DIPS Calux TEQ (pg/g)		RapidScreen 4ppt TEQ	
	Total	RS-CON			D/F only	Total	D/F only	Total
001	7.1	5.75	0.121	7.22	40.9 (+)	42.4 (+)	+	+
002	48.6	40.12	0.165	48.77	310.7 (+)	314.52 (+)	+	+
003	30.4	25.26	0.174	30.57	328.9 (+)	333.06 (+)	+	+
004	22.3	18.08	0.195	22.50	236.9 (+)	240.68 (+)	+	+
005	1.5	1.13	0.143	1.64	9.1 (+)	10.6 (+)	+	+
006	2.0	1.48	0.174	2.17	12.6 (+)	13.24 (+)	+	+
007	0.8	0.50	0.162	0.96	8.1 (+)	8.74 (+)	-	-
008	1.9	1.35	0.156	2.06	23.1 (+)	23.74 (+)	-	-
009	4.3	3.02	0.09	4.39	21.4 (+)	22.04 (+)	-	-
010	3.46	2.50	0.487	3.95	25 (+)	25.64 (+)	-	-

Table 2: A comparison of TEQ results measured using the 8290 method with the RapidScreen method and the CALUX Bioassay. The RS CON column refers to the sum of the 8290 TEQ contributions of the seven analytes used by RapidScreen. The RapidScreen positive or negative result is estimated based on a 4 ppt TEQ.

Samples 009 and 010 are estimated by the reference methods as having TEQs statistically identical (assuming a 20% error) to the screening threshold of 4 ppt. Sample 009 has a reference TEQ of 4.3 ppt, and is screened by RapidScreen as a negative result. Sample 010 with a TEQ of 3.95 ppt screens negative. RapidScreen estimates TEQ levels based on a set of congeners that have been shown in recent work[3] to contribute between 60-80 % of the total TEQ. The TEQ using the seven "RapidScreen congeners" (RS-CON TEQ) quantitated by 8290 is also shown in Table 2 and shows that for Sample 009, the RS-CON TEQ is 3.02 ppt, which is below the 4 ppt threshold flagged by RapidScreen. Sample 010, which also has the 4 ppt mark within its error bounds, shows a RapidScreen negative for an RS-CON TEQ of 2.5. These two samples are indicative of RapidScreen's performance at or near the threshold value. The only false negatives seen are what can be termed as borderline false negatives.

A comparison of the CALUX TEQs with the 8290 and 1668A computed TEQs

A comparison of the TEQs computed by the reference methods to the CALUX determined TEQs is shown in Figure 1 and indicates that the CALUX assay determined TEQ is 6-10 times higher on all the samples than the reference TEQ. This result indicates that there are high levels of positive interferences over a range of TEQs in the CALUX assay. In an isotope dilution HRGC/HRMS method like RapidScreen, positive interferences are typically caused by compounds eluting with the target analytes, which also have ions of a mass to charge ratio identical (within mass resolution) of the target analyte. In the CALUX bioassay, all compounds that bind to the AhR receptor gene will count towards TEQ. These include ubiquitously present polycyclic aromatic hydrocarbons (PAHs), and halogenated naphthalenes and benzenes[6]. While the AhR response to PAHs is much smaller than that for dioxins and furans, their presence in samples at levels that are

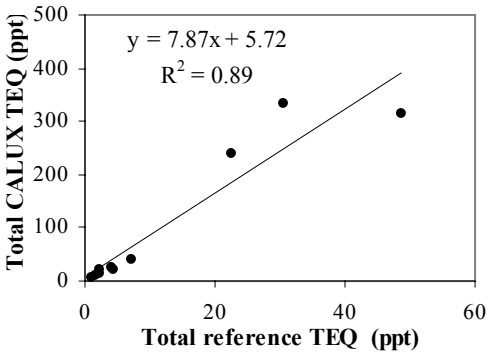

Figure 1: A comparison of total TEQs computed using the 8290 and 1668A reference methods and the CALUX screen

orders of magnitude higher than the target analytes can lead to positive interferences. Since the CALUX assay does not resolve compounds chromatographically, it is wholly dependent on the sample cleanup procedure to eliminate all interfering compounds prior to analysis, and not just interfering compounds that cannot be chromatographically resolved from the target analytes. This may lead to an increased probability of positive interferences. The soil samples for this study were from a contaminated site, which likely contained high levels of compounds that could have caused responses in the AhR receptors.

In this sample set, a linear correlation is seen between the CALUX TEQ and the reference TEQ, with the slope of the regression line in Figure 1 indicating that the CALUX TEQ is approximately 8 times the reference TEQ. As all these samples were from the same location, this correlation is indicative of the presence of interfering compounds whose levels are in proportion with the target analyte levels in the sample, leading to a scaling of the TEQ values for each sample.

In conclusion, the CALUX screen seemed to significantly overestimate the TEQ of the samples used in this study due to the presence of interfering compounds. The RapidScreen method provides a more reliable screening tool for the TEQ of the samples due to the sample purification procedures employed and the use of the isotope dilution technique.

Acknowledgements
This work was supported by Triangle Laboratories, Inc. The authors would like to thank Dr. P. Albro, Dr. J. Chad Roper, for their scientific guidance and input.

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