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Characterization of the H4IIE Rat Hepatoma Cell Bioassay as a Tool for Assessing Toxic Potency of Planar Halogenated Hydrocarbons in Environmental Samples

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■ An in vitro system, the H4IIE rat hepatoma cell bioassay, was characterized for use in assessing the overall toxic potency of PCBs, PCDDs, and PCDFs in extracts from environmental samples. This in vitro bioassay of cytochrome P450IA1 catalytic activity in the H4IIE cells in response to planar halogenated hydrocarbons (PHHs) was repeatable over time and standards were reproducible among laboratories when dosing conditions were similar. Three common extraction/cleanup procedures tested had no adverse affect on the response of the cells and biogenic interferences were not encountered. Comparison of the response of the H4IIE cells to extracts was calibrated against their response to the standard, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). This method of calibration proved to be effective for quantitation of known amounts of PHHs spiked into a sample matrix. The potential utility of this bioassay is as an integrative tool to assess the toxic potency of complex mixtures of PHHs. The results of this bioassay can complement chemical residue analysis and direct the need for such analysis, as well as aid in the interpretation of biological effects data from environmental studies.

Introduction

Planar halogenated hydrocarbons (PHHs) are a group of chemicals with isosteric configurations or structures and include, among other environmental contaminants, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs). PHHs were used industrially for decades or were contaminants of chemical synthesis and entered the environment by both intentional and inadvertent release. The recalcitrant nature of PHHs, along with their inherent toxic properties and a propensity to bioaccumulate, has caused concern that these environmental contaminants may reach concentrations in organisms at the top of the food chain great enough to elicit toxic effects (*1–4*). The problem that scientists face in this respect is the evaluation of PHH residues that occur in the environment. Currently, there are analytical techniques to extract, concentrate, isolate, separate, and quantitate PHHs from environmental samples (*5–9*). However, concentrations of PHHs provide only part of the information necessary to evaluate their potential for adverse effects on fish, wildlife, and humans. This is because PHH congeners each have different toxic potencies (*10–13*) and the complex interactions of synergism, antagonism, and additivity, which are known to occur within mixtures of PHHs (*14–24*), are not understood completely at this time. These interactions are not considered when attempts are made to predict biological effects from concentrations of PHHs alone.

PHHs are proximate isostereomers, which exert their toxic effects through the same biological receptor (*10–12*). Although differing in potency, PHHs elicit the same suite of toxicological effects across many phylogenetic lines (*12*). The characteristic symptoms of PHH poisoning include weight loss (wasting syndrome), thymic atrophy, subcutaneous edema, immune suppression, hormonal alterations, P450IA1-associated enzyme induction, and the reproductive effects of fetotoxicity and teratogenesis (see reviews, refs 25 and 26). Additionally, there are strong correlations between the enzyme induction potency of individual congeners and their potency for causing effects such as weight loss and thymic atrophy (*27–30*). These correlations are significant ($r > 0.90$) for both in vivo enzyme induction potency versus the toxic potency in vivo with rats, and for in vitro enzyme induction potency in H4IIE rat hepatoma cells versus the toxic potency in vivo in rats (*31*). In other words, the response of the H4IIE cells to the individual congeners was predictive of the toxic responses of whole organisms to these PHH congeners. Therein lies the potential utility of this in vitro bioassay as an integrative bioanalytical tool for screening PHH extracts of environmental samples.

The H4IIE cells were derived from the Reuber hepatoma H-35 (*32*) by Pitot and co-workers (*33*). It is a continuous cell line and was characterized with regard to aryl hydrocarbon hydroxylase (AHH) activity by Nebert and coworkers (*34*). Besides excellent growth characteristics and low basal cytochrome P450IA1 activity, they found the H4IIE cells to have inducible AHH enzyme activities. These researchers went on to characterize the AHH induction response of the H4IIE cells to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the prototypic PHH, and suggested that the H4IIE rat hepatoma cell culture bioassay might be useful in detecting TCDD (*35*). They found that the H4IIE cells are exquisite in their response to TCDD with a detection limit of 10 fmol.

Simultaneous to the developments of the H4IIE bioassay, structure–activity relationships of PHHs indicated that halogen substitution in the lateral positions of the dioxin, furan, or biphenyl molecules imparted a greater receptor affinity, AHH induction potency, and toxicity to these compounds (*10–13*). In particular, a strong correlation between AHH or ethoxyresorufin-*O*-deethylase (EROD) induction potency in vitro in the H4IIE cells and the toxic potency in vivo of individual biphenyl (*29, 36*), dioxin (*37*), and furan (*28, 30*) congeners was observed. These reports were summarized by Safe (*31*). The corre-

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lations of $-\log ED_{50}$ (effective dose for half-maximal induction) for weight loss in rats versus $-\log EC_{50}$ for AHH induction in H4IIE cells and $-\log ED_{50}$ for thymic atrophy in rats versus $-\log EC_{50}$ for AHH induction in H4IIE cells had correlation coefficients of 0.93 and 0.92, respectively (*31*). These strong correlations between in vitro induction potency and in vivo toxic potency were critical validations for the use of this bioassay for prediction of potential toxicity of PHHs.

The first use of the H4IIE bioassay as a tool for assessing complex mixtures of PHHs in extracts was by the U.S. Food and Drug Administration. They performed the initial analytical characterization of the H4IIE bioassay as an environmental extract assay (*37–40*). Isooctane (ISO) as the solvent carrier for extracts or pure compounds optimally enhanced bioassay sensitivity (*39*). A detection limit of 10 pg of TCDD was reported with the isooctane solvent carrier system, with an ED_{50} of 45 pg of TCDD/plate (0.14 pmol/plate, 28 pM). A quantitation limit for this solvent system was not reported; however, in subsequent publications the limit of quantitation was 25 pg of TCDD and the linear response range was 25–500 pg of TCDD/plate (*38, 40*). Thus, the sensitivity of this bioassay system for detection of PHHs had been established.

The H4IIE bioassay has been shown to be a sensitive tool for detection of PHHs in extracts of environmental samples (*38–40*) and much of the initial analytical characterization was performed by these scientists. However, due to improvements in PHH extractions, cleanup, and quantitation techniques in the past 10 years, studies to confirm and expand on the work already done are necessary if this bioassay technique is to be adapted as a bioanalytical tool. In this study we reexamine the isooctane carrier solvent system, detection and quantitation limits, and some reference toxicants. Additionally, we investigate potential endogenous and exogenous interferences of matrices or extraction protocols. We also investigate the quantitative ability of the H4IIE bioassay with spike/bioanalysis experiments. These studies are important because to date investigations of this bioassay have been fragmented and performed in different laboratories. This is the first study to systematically investigate the H4IIE bioassay as a quantitative bioassessment tool for PHHs.

Experimental Section

Extractions and Spike/Bioanalysis Protocols. Three extraction and cleanup protocols were investigated: the method used by FDA scientists in the original bioassay reports (*41*); an improved method used for PCB analysis, which utilizes column extractions with dichloromethane (*6*); and a modification of this latter method, which results in extracts that contain PCBs, PCDDs, and PCDFs in one fraction (*42*). The PAM procedure (*41*), which results in a fraction that contains PCBs without PCDDs or PCDFs, was used in PCB spike/recovery bioanalysis experiments with chicken eggs and with environmental waterbird egg samples. We found extraction efficiencies of the PAM procedure for [^{14}C]-2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153) were 57–61% from fortified chicken eggs. A second, more contemporary extraction/cleanup method with dichloromethane (DCM), which also results in a fraction that contains PCBs without PCDDs or PCDFs, was used in PCB spike/recovery bioanalysis experiments with chicken eggs and fish samples (*6*). This method is routinely used to extract environmental samples for PCB-congener-specific analysis. The DCM method results in a fraction that contains PCBs (90–98% recovery efficiency) without PCDDs, PCDFs, polar pesticides, or most organochlorine pesticides (*6*). A series of TCDD spike/recovery bioassay experiments were conducted to ensure that PCDDs and PCDFs were not cocontaminants in the resultant PCB fraction of the PAM or DCM methods. We were unable to detect any activity when the TCDD-fortified chicken egg extracts of either the DCM or PAM procedures were tested in the H4IIE bioassay (spike concentrations up to 1000 pg of TCDD/g; data not presented). Last, we assessed a modified version of the DCM procedure in which an acidic silica gel (AS) column cleanup was used after GPC. This AS procedure was previously described (*42*). The resultant fraction contains PCBs with recovery efficiencies of 90–100% for all quantifiable PCBs. External standardization of the AS procedure for PCDD/PCDF recovery efficiency had not been described previously. Therefore, duplicate 10-g portions of chicken egg homogenates were spiked with 7×10^3, 21×10^3, 70×10^3, 210×10^3, or 700×10^3 DPM [^{3}H]TCDD (specific activity approximately 45 Ci/mmol). The resultant recovery efficiencies (±SD) were 95.4 ± 4.0, 93.2 ± 1.0, 96.3 ± 0.7, 97.7 ± 3.3, and 103.5 ± 3.5%, respectively, with an average of 97.2 ± 4.2%. It should be noted that none of the bioassay results were corrected on the basis of external standard recovery efficiencies.

A series of experiments were performed in which "clean" samples were spiked with a PCB (3,3′,4,4′-tetrachlorobiphenyl, congener 77) or TCDD, extracted, and then analyzed with the H4IIE bioassay. The sample matrix used in these studies consisted of chicken eggs from a retail store because many of the samples we are currently analyzing are bird eggs. The first spike/bioanalysis experiment was PCB 77 spiked into chicken eggs at 0.1, 0.5, 1.0, 5.0, 10, 50, and 100 μg/g, extraction with either the PAM (*41*) or DCM (*6*) procedure, followed by bioanalysis of the extracts. A second spike/bioanalysis experiment consisted of a TCDD spike of 0.01, 0.1, 0.5, 1.0, or 10 ng/g into chicken eggs. These eggs were then extracted with the AS protocol (*42*), and extracts were subjected to bioanalysis.

PHH standards tested in this study consisted of TCDD, 2,3,7,8-tetrachlorodibenzofuran (TCDF, Ultra Scientific, Hope, RI) and four PCB congeners (3,3′,4,4′-tetrachlorobiphenyl, PCB 77; 3,3′,4,4′,5-pentachlorobiphenyl, PCB 126; 2,3,3′,4,4′-pentachlorobiphenyl, PCB 105; and 2,3,3′,4,4′,5′-hexachlorobiphenyl, PCB 156, Ultra Scientific). Purity of all PHH congeners was confirmed by mass spectral analysis by Jay W. Gooch, Chesapeake Biological Laboratory, University of Maryland, Solomons, MD. All PCB congeners and TCDD were >99% pure. 2,3,7,8-TCDF contained an impurity, 1,2,7,8-TCDF, at 0.84–1.59%, which was not considered to be significant based on the relatively low potency of 1,2,7,8-TCDF as compared to 2,3,7,8-TCDF.

Cell Culture and Bioassay Procedure. The H4IIE rat hepatoma cells were obtained from the American Type Culture Collection (ATCC No. CRL 1548). Cells were cultured in Dulbecco's modified Eagle's medium (D-MEM) base (Sigma, D5030) supplemented with 1× glutamine, 1.5× vitamins (Sigma, M6895), 2× nonessential amino acids, 1.5× essential amino acids, 1 mM pyruvate, 1000 mg/L D-glucose, 2200 mg/L sodium bicarbonate, 15% fetal bovine serum (Gibco, 200-6140AJ), and 50 mg/L gentamicin. These conditions provided optimal growth and EROD induction potential of the H4IIE cells. Stock cultures were grown in 75-cm^2 flasks at 37 °C in a humidified 95:5 air–CO_2 atmosphere. New cultures were started from frozen cells after nine or less passages.

The bioassay conditions were slight modifications of previous reports (*36–40*). Cells, trypsinized from stock

flasks at confluency, were seeded in Petri dishes (15 × 100 mm) at 0.8 × 10^6/plate in 10 mL of D-MEM. After a 24-h incubation, the cells were dosed with extract, an appropriate control, or reference compound in 100 μL of isooctane. There was no effect of dosing volume between 10 and 150 μL of isooctane when either an extract or standard (TCDD) was tested. Dosed cells were incubated for 72 h, rinsed with phosphate-buffered saline (PBS), and then harvested with cell scrapers (Gibco) into Tris–sucrose (0.05–0.2 M) buffer, pH 8.0 (*37*). Cells were then centrifuged for 10 min at 5000*g* and resuspended in Tris–sucrose buffer, and protein was determined in duplicate (*43*). Duplicate EROD determinations, by a modification of the spectrofluorometric method of Pohl and Fouts (*44*), were made with 100-μL aliquots of the standardized (1 mg of protein/mL) cell suspensions. Briefly, this method has a final reaction volume of 1.25 mL consisting of 1.0 mL of NADPH generator system (5 mM glucose 6-phosphate, 5 mM $MgSO_4$, 3.5 mM NADP, and 1.6 mg of bovine serum albumin/mL in 0.1 M HEPES buffer, pH 7.8), 0.1 mL of 25 units/mL glucose-6-phosphate dehydrogenase (G6PDH), 0.1 mL of cell suspension (100 μg of protein), and 0.05 mL of 15 μM ethoxyresorufin (ER) in methanol. The reaction mixtures (less the ER) were preincubated 10 min at 37 °C, after which reactions were initiated by the addition of the ER at 10-s intervals. After 10 min, the reactions were stopped by the addition of 2.5 mL of cold methanol, again at 10-s intervals. Proteins were allowed to flocculate for 5 min at 37 °C and then the samples were centrifuged at 5000*g*, 4 °C, for 10 min. Resorufin in the supernatant was determined spectrofluorometrically (550-nm excitation, 585-nm emission) against a standard curve, which was calibrated with a resorufin standard each bioassay. EROD specific activity was calculated as picomoles of resorufin formed per milligram of protein per minute.

Along with each set of extracts, appropriate standards were analyzed on the same day. All environmental extracts were calibrated against a TCDD standard curve for calculation of "TCDD equivalents" (TCDD-EQ) in the extract. The effective doses for half-maximal EROD induction (ED_{50}) were calculated by probit analysis (*45*). Calculations of extract potency for each sample were made according to eq 1 as reported by Sawyer et al. (*46*), where

$$\text{extract potency} = \text{TCDD } ED_{50}/\text{extract } ED_{50} \quad (1)$$

TCDD ED_{50} is in picograms per plate, extract ED_{50} is in microliters per plate, and extract potency is in picograms of TCDD-EQ per microliter. The calculations to TCDD-EQ in an environmental sample were not corrected for extraction efficiencies of the various extraction methods. Variance estimates were calculated according to eq 2 and

$$CV_T = [(CV_E)^2 + (CV_S)^2]^{1/2} \quad (2)$$

an additive model of variance (*47*), where CV_T is the coefficient of variation for TCDD-EQ, CV_E the coefficient of variation for extract ED_{50}, and CV_S the coefficient of variation for standard ED_{50}. Standard deviations (SD) were obtained by multiplying the fractional CV_T by the estimated TCDD-EQ of the sample or extract. Goodness of fit test of a normal distribution for TCDD ED_{50} values was according to Kolmogorov-Smirnov (*47*).

Results and Discussion

The H4IIE bioassay has traits that make it a particularly useful technique for the determination of PHHs in environmental extracts. The basal EROD activity of the H4IIE cells ranges from 0.5 to 5.0 pmol/mg·min. Isooctane, which gave optimal response and sensitivity in previous reports (*37–39*), was also an ideal carrier in our studies. Solvent controls had no induction over basal EROD in the H4IIE cells, nor was there any apparent toxicity as determined by cell viability or cellular protein. The dosing volume of extracts or standards was constant at 100 μL (1% media volume). However, there was no effect of dosing volume on the inductive response of the H4IIE cell cultures when volumes between 10 and 100 μL of extracts or standards were tested. The use of isooctane as a carrier is also compatible with most chemical residue analysis techniques for PHHs.

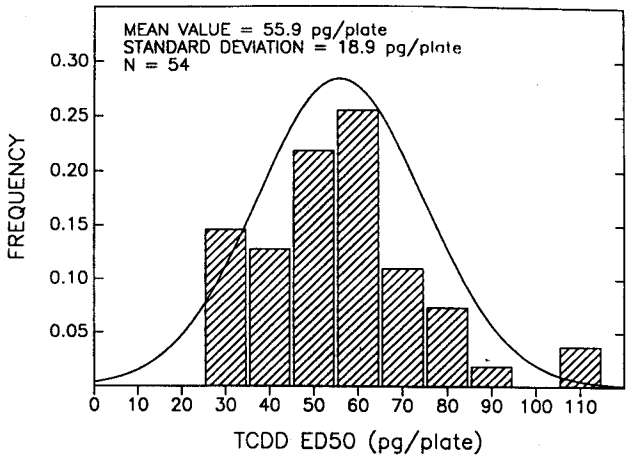

Figure 1. Frequency distribution of TCDD ED_{50} values in the H4IIE bioassay.

Sensitivity of the H4IIE bioassay is quite exquisite for PHHs and TCDD in particular. The limit of detection was 10 pg of TCDD (31 fmol) per plate in our studies, which is the same as that reported by others (*35*, *37–39*). The coefficients of variation for within bioassay variance of TCDD ED_{50} estimates were generally small with an average of 3.70%. The coefficients of variation associated with extract ED_{50} estimates are generally in the range of 5–15%. Precision of this bioassay, therefore, is fairly high with the final estimates of TCDD-EQ in environmental samples having coefficients of variation between 10 and 20%. However, this type of precision is only observed when a TCDD standard curve is run with each set of environmental samples. Our average ED_{50} for TCDD over a 2-year period and 54 standard curves was 55.9 pg/plate (0.17 pmol/plate), also very similar to the 45 pg/plate (0.14 pmol/plate) ED_{50} reported previously with a similar solvent system (*39*). This demonstrates the reproducibility of the H4IIE bioassay, even among different laboratories. The ED_{50} values for TCDD in this system followed a normal distribution (p = 0.80, coefficient of skewness = 0.0, coefficient of kurtosis = 3.0) with a range of 30–115 pg/plate and standard deviation of 18.9 pg/plate (Figure 1). This corresponds to a coefficient of variation among bioassays of 33.8%. If the average TCDD ED_{50} value with its 34% CV were used, resultant estimates of TCDD-EQ would have CV = 40–50%. Therefore, in bioanalytical applications of the H4IIE bioassay, it is important to run a TCDD standard with each set of environmental extracts.

Another test of reproducibility with this bioassay is interlaboratory comparisons of other PHH standards. For this purpose we tested TCDF, PCB 77, PCB 126, PCB 105, and PCB 156, in addition to TCDD (Table I). A comparison of PHH ED_{50} values from different laboratories is given (Table II). ED_{50} values from this study were very close to those reported by others (*39*) when isooctane was used as a carrier solvent (Table II), even when different

Table I. ED_{50} Values and Relative Potencies of Selected PHHs for EROD Induction in the H4IIE Bioassay[a]

compd	ED_{50}, pg/plate ± SD	ED_{50}, pmol/plate ± SD	relative potency
TCDD	$5.59 \pm 1.89 \times 10^1$	0.17 ± 0.06	1.0
TCDF	$8.08 \pm 0.16 \times 10^3$	26.4 ± 0.52	6.4×10^{-3}
PCB 126	$2.48 \pm 0.02 \times 10^3$	7.59 ± 0.07	2.2×10^{-2}
PCB 156	$1.13 \pm 0.03 \times 10^6$	3120 ± 89.9	5.5×10^{-5}
PCB 77	$2.74 \pm 0.10 \times 10^6$	9370 ± 341	1.8×10^{-5}
PCB 105	$7.34 \pm 0.81 \times 10^6$	22500 ± 2480	7.6×10^{-6}

[a] All bioassays carried out in duplicate except TCDD where n = 54, r = 2–3. ED_{50}, effective dose for half-maximal EROD induction; potencies are calculated relative to TCDD as (TCDD ED_{50}, pmol/plate)/(compound ED_{50}, pmol/plate).

substrates were used to monitor P450IA1 catalytic activity of the cells. A large part of this stability is attributable to the fact that a standardized stock of H4IIE cells is available at ATCC. Discrepancies begin to appear among ED_{50} values when comparisons are made among solvent carrier systems (*39*, *48*, *49*). Isooctane increases the sensitivity of the H4IIE bioassay toward TCDD as compared to dimethyl sulfoxide (DMSO) (*39*). ED_{50} values for TCDD are 4–10 times less when isooctane was the carrier as compared to when DMSO was used (Table II). However, greater sensitivity was not seen with the isooctane carrier solvent system for all PHHs. There appears to be little effect of carrier solvent on TCDF or PCB 156 potency and DMSO seems to result in greater bioassay sensitivity for PCB 126, PCB 105, and PCB 77 as compared to isooctane (Table II). This phenomenon of apparent differential sensitivity caused by the carrier solvent system may be due to PHH solubility differences. It should also be mentioned at this point that the similarities in potency noted above are based on ED_{50} values. If effective concentration values (EC_{50}) are compared, there is not a good agreement between values from different laboratories. The effective concentration for half-maximal induction, EC_{50}, is calculated by normalizing the dose per plate to the volume of media in the plate (ED_{50}/media volume) and has been used by some researchers (*36*, *48*). The size of Petri dish and volume of media used varied among all laboratories, but the cell densities were fairly constant between 0.8×10^6 and 1.0×10^6 cell/plate. The fact that ED_{50}s and not EC_{50}s are similar among laboratories, along with the similarity in cell seeding rates, suggests that most of the PHH dose is effectively reaching the cells. However, radiotracer studies are required to understand if differential solubilities can explain this phenomenon of differential PHH potencies in the H4IIE bioassay. This also has implications on calculations of relative potency factors of PHHs based on their H4IIE cell induction potency. If ED_{50} values are more reliable and consistent estimates of PHH induction potency, as they appear to be, perhaps ED_{50} values instead of EC_{50} values should be used in calculation of H4IIE-derived potency factors of individual PHH congeners relative to TCDD. These potency factors are being used with increasing frequency (*50*), in particular to calculate TCDD-EQ from chemical residue analysis (*51*, *52*).

Use of the H4IIE bioassay for the determination of TCDD-EQ in environmental samples requires a knowledge of potential endogenous and exogenous interferences caused by the matrix or extraction protocols. To address these issues we examined matrix and procedural blanks and performed spike/bioanalysis studies. Characterization of extraction protocols was done to ensure that the fractions known to contain PHHs induced EROD in the H4IIE cells and fractions containing pesticides did not contain measurable amounts of inducible materials. The three extraction procedures tested, PAM (*41*), DCM (*6*), and AS (*42*), showed no induction with procedural blanks or pesticide fractions and significant induction with PHH fractions from these methods (data not presented). The PAM characterization was similar to results reported by previous authors using this method (*38*). Matrix blanks (unfertilized chicken eggs, fertilized 10-day-old chicken eggs, salmon eggs, and rainbow trout flesh), with ≤0.01 μg of total PCBs/g, caused no EROD induction in the H4IIE cells at 1–3 g·equiv of sample/plate. This indicated that endogenous substances in these matrices did not cause false positive responses in the H4IIE bioassay. Because *p,p'*-DDE is a major cocontaminant of PCBs in these extraction procedures, we exposed the H4IIE cells to 10, 100, 1000, or 10 000 ng of *p,p'*-DDE/plate. There was no EROD induction or cytotoxicity, as measured by cell growth, at any dose of *p,p'*-DDE.

To assess the ability of the H4IIE bioassay to quantitate PHHs in biological samples, PHH spike/bioanalysis studies were conducted. The information to be gained by these experiments is 3-fold. First, the actual induction magnitude and dose–response of the extract may be compared with that of the pure congener. Second, the slopes of the extracted and pure congener dose–response curves may be compared in a situation where only a single compound is present. Third, a threshold for detection inclusive of both extraction and bioassay efficiency may be estimated. PAM (*41*) and DCM (*6*) extraction methods were used in combination with the H4IIE bioassay to assess quantitation of PCB 77 and AS (*42*) extraction methods were used to assess quantitation of TCDD.

Extracts of PCB 77 spiked chicken eggs produced a dose–response curve that was in good concordance with that seen when PCB 77 was added directly to the cell cultures (Figure 2). There were similar slopes in all three cases, indicating no extraction or matrix effects on the dose–response curves. ED_{50} values calculated for the extracts varied less than 25% compared to the standard. Correction for the extraction efficiency of each method

Table II. Comparison of Reported ED_{50} Values (pmol/Plate) in the H4IIE Bioassay for Selected PHHs

ref	system	assay	PHH					
			TCDD	TCDF	PCB 77	PCB126	PCB156	PCB105
Bradlaw and Casterline (*39*)	ISO	AHH	0.14	13.0	10250	6.00		
	DMSO	AHH	1.54					
Sawyer and Safe (*36*)	DMSO	AHH	0.77		281	1.92	16600	700
	DMSO	EROD	0.64		708	1.98	7170	960
Sawyer and Safe (*48*)	DMSO	AHH		15.6			3540	
	DMSO	EROD		8.1			4000	
Zacharewski et al. (*49*)	DMSO	AHH	0.73					
	DMSO	EROD	0.51					
this study	ISO	EROD	0.17	26.4	9370	7.60	3120	22500

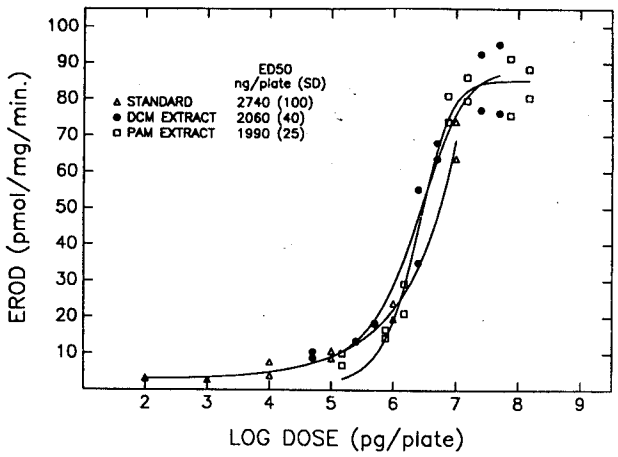

Figure 2. Dose–response relationship of pure and extracted PCB 77 in the H4IIE bioassay. DCM (*6*) and PAM (*41*) extraction protocols were tested with clean chicken eggs fortified with PCB 77 at seven concentrations. Dose to the cells was calculated based on 50 μL (5%) of a 1-mL extract/plate, spike concentration in chicken eggs, and 100% extraction efficiency.

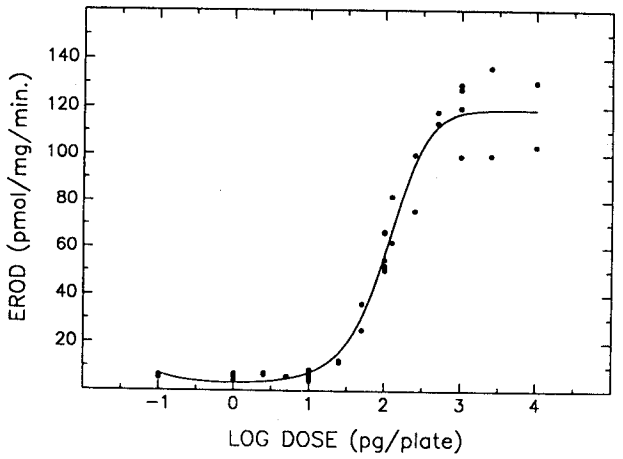

Figure 3. Dose–response relationship of pure and extracted TCDD in the H4IIE bioassay. The AS (*42*) extraction protocol was tested with clean chicken eggs fortified with TCDD. Dose to the cells was calculated based on a dosing volume (1, 10, 25, or 100 μL) of a 1-mL extract/plate, spike concentration in chicken eggs, and 100% extraction efficiency.

could reduce these differences among ED_{50} values.

Spike/bioanalysis experiments with TCDD and the AS extraction procedure indicated that the H4IIE bioassay could accurately predict extract potency. Extracted TCDD produced a similar dose–response curve in the H4IIE cells compared to that of the standard (Figure 3). Slopes of the curves were not significantly different, indicating no extraction or matrix effect on the response of the bioassay. Extract potency was calculated from observed ED_{50} values for each spike concentration, as would be done with environmental extracts, and these were compared with the known concentration of TCDD in the extract (Figure 4). The nominal concentrations of the extracts were 0.1, 1.0, 5.0, 10, and 100 pg of TCDD/μL. The extract at 0.1 pg of TCDD/μL was below the limit of quantitation, however, the bioassay predicted extract potency within a factor of 2 for the other concentrations. Predictions of extract potency by the ED_{50} method were linear between 1.0 and 100 pg of TCDD/μL and the regression slope of observed versus expected was not different from 1.0, the ideal. It is clear from this set of experiments that the H4IIE bioassay can accurately and precisely determine the potency of PHH extracts. Comparison of extract and

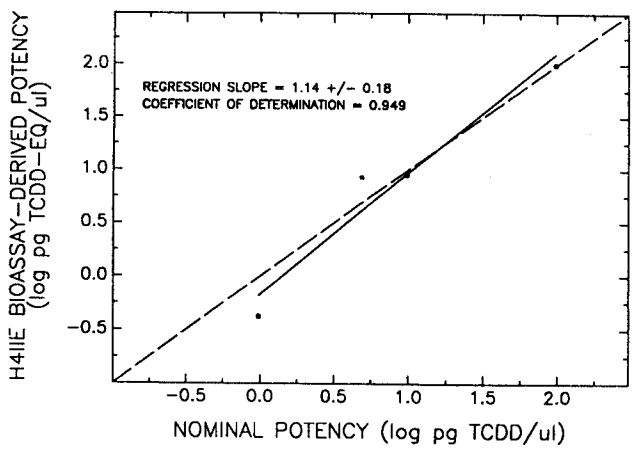

Figure 4. Observed versus expected potency of TCDD in spike/bioanalysis experiments. The nominal potency (pg of TCDD/μL) of extracts was based on the TCDD spike to the chicken egg sample and an assumption of 100% extraction efficiency. H4IIE bioassay derived potency (pg of TCDD-EQ/μL) based on eq 1 for each of four extracts.

Table III. H4IIE Bioassay-Derived TCDD-EQ in Environmental Samples from the Great Lakes[a]

sample/site	TCDD-EQ, pg/g ± SD
chinook salmon, Lake Michigan	
dorsal muscle	26.7 (2.9)
eggs	115.8 (5.8)
double-crested cormorant eggs	
Green Bay, Lake Michigan	344.1 (25.6)
Beaver Is., Lake Michigan	94.0 (7.9)
Caspian tern eggs	
Green Bay, Lake Michigan	281.2 (23.8)
Saginaw Bay, Lake Huron	415.7 (48.1)
Beaver Is., Lake Michigan	49.7 (2.2)
black-crowned night heron eggs, Saginaw Bay, Lake Huron	221.8 (19.7)

[a] All samples collected 1986–1987, composited, and extracted according to ref 41, except chinook salmon, which were extracted according to ref 6.

standard ED_{50}s from the H4IIE bioassay is a simple and accurate method of calculating potencies and associated error estimates for PHH extracts.

H4IIE bioassay derived TCDD-EQ were determined for some fish and wildlife samples taken from the Great Lakes (Table III). The samples were extracted and extracts subjected to H4IIE bioanalysis. The range of TCDD-EQ found in these samples is reflective of the values we have observed in environmental samples in this region. The precision of these measurements is also typical of the analytical precision of this bioassay when environmental samples are tested (CV_T = 5–15%). The H4IIE-derived TCDD-EQ calculations for environmental samples may be compared with biological effects data and chemical residue analysis from the samples to help interpret these data. The utility of this bioassay system is to aid chemical residue analysis and act as a data reduction tool to help understand the complex interactions of PHHs. Studies are currently underway to determine the validity of using this mammlian bioassay system to predict the toxic potency of PHH mixtures to avian and fish species.

Summary

Previous studies have used the H4IIE bioassay to estimate the potency of individual PHHs (*36*, *37*), assess environmental extracts of PHHs (*37*, *46*, *48*, *49*), and address the complex interactions of synergism, antagonism, and additivity (*18–21*, *24*). This is impossible to do by chemical

residue analysis alone. The H4IIE bioassay has been shown to be a sensitive bioanalytical tool (*35*, *37–40*, *48*, *49*) with potential for predicting the toxic effects of PHHs in whole organisms (*31*). In this study we demonstrate the reproducibility of the H4IIE bioassay among laboratories and its repeatability over time within a laboratory. We also provide experimental data of its ability to quantitatively predict known concentrations of PHHs in biological extracts. The potential utility of this bioassay is as an integrative tool, which can complement chemical residue analysis and biological effects data from environmental studies. The H4IIE bioassay can also be used to screen or prioritize chemical residue analysis and thereby save valuable time and funds.

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Registry No. TCDD, 1746-01-6; PCB 126, 57465-28-8; PCB 156, 69782-90-7; PCB 77, 32598-13-3; PCB 105, 32598-14-4; TCDF, 51207-31-9.

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