AhR-active compounds in sediments of the Haihe and Dagū Rivers, China

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Abstract

Total concentrations of compounds that can cause activation of the aryl hydrocarbon receptor (AhR) in extracts of river sediments from various locations in the Haihe and Dagū Rivers, Tianjin, China were determined by use of the *in vitro* H4IIE-*luc* cell line. AhR-active compounds were isolated from sediments by Soxhlet extraction, and the crude extracts were fractionated using a Florisil column into three Fractions. The response of samples was compared to the TCDD standard and expressed as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (TCDD-EQs). Significant dioxin-like activity was observed in each sample. The TCDD-EQs in crude extracts of sediments (SCEs), as determined with the bioassay were 2–4 times greater than the sum of the TCDD-EQs of the eluent from fractions separated with a Florisil column. The results also showed that Fractions 2 and 3 contained most of the AhR-mediated activity. The results obtained by using the bioassay were compared with those of previous measurements of the concentrations of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) and the structurally-similar AhR-active polychlorinated biphenyls (PCBs). It was determined that sediments from the Dagū River contained greater concentrations of TCDD-EQ than did sediments from the Haihe River except at Jingangqiao (location R3), which is associated with industrial activities in the adjacent densely populated area. The concentrations of TCDD-EQs, based on the EC₂₀ and the relative potency ranges, of SCEs ranged from 330 to 930 and 1200 to 13900 pg TCDD-EQ g⁻¹ dry wt in Haihe and Dagū Rivers, respectively.

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1. Introduction

A large number of chemicals can interact with the aryl hydrocarbon receptor (AhR) such that a cascade

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of biochemical effects occur that ultimately can result in a wide range of adverse effects in wildlife (Giesy and Kannan, 1998). AhR-active compounds, sometimes referred to as dioxin-like compounds because they are structurally similar to the prototypical compound, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), include many subclasses of chemicals, many of which are persistent and bioaccumulative, diaromatic hydrocarbons such as polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs) and polychlorinated naphthalenes (PCNs), as well as polycyclic aromatic hydrocarbons (PAHs) (Giesy et al., 1994a; Villeneuve et al., 2002). There are literally thousands of chemicals that exhibit AhR-mediated activity (Giesy et al., 1994b; Murk et al., 1996) including heterocyclic oxygen- and sulfur-containing compounds for which authentic standards are not available. The PCDD/PCDFs and structurally-similar compounds constitute a group of lipophilic, persistent, ubiquitous, and bioaccumulative environmental chemicals exhibiting a broad spectrum of biological effects (Giesy and Kannan, 1998; Behnisch et al., 2003), some of which include hepatotoxicity, induction of certain types of cancer, thymic atrophy, immunotoxicity, wasting syndrome, reproductive toxicity, and induction of monooxygenase enzymes or porphyria (Poland and Knutson, 1982; Brouwer et al., 1995; Denison and Heath-Pagliuso, 1998; Denison et al., 1998; Van den Berg et al., 1998).

The bioaccumulation of AhR-active compounds in humans and their biomagnification in the food chain (Giesy et al., 1994b; Berggren et al., 1999) is worthy of further study but convenient methods of determination are required. Until about ten years ago, a prohibitively expensive method, namely high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS), was the only option and became the “gold standard” for detecting dioxin-like compounds (Schoeters et al., 2004). However for environmental samples, without prior knowledge of the compounds that might be present or authentic standards for all of the known AhR-active compounds, and the fact that the compounds can interact with one another it is difficult to predict the total AhR-mediated activity of an environmental sample. The uncertainty is exacerbated by the fact that relative potency (REP) values or toxic equivalency factors (TEFs) are not available for all of the classes or compounds that may be present in environmental matrices (Van den Berg et al., 1998). The use of bioanalytical methods can provide a comprehensive measure of total AhR-mediated activity and its associated risk (Behnisch et al., 2003). A bioassay method has the advantage of integrating the toxic potency of all the compounds in a sample and of being more rapid and inexpensive when many samples need to be assessed (Schirmer et al., 2004).

The bioassay used in this study is based on the ability to activate the AhR-signal transduction pathway (Hilscherova et al., 2000). The H4IIE-luciferase induction assay is an *in vitro* technique that offers a more cost effective alternative for the identification of AhR-active compounds (Sanderson et al., 1996). The assay is also referred to as the chemical activated luciferase gene expression (CALUX) system based on rat hepatoma cells stably transfected with an AhR-controlled luciferase reporter gene construct (Murk et al., 1996; Sanderson et al., 1996; Giesy et al., 2002). Luciferase induction potential is assessed by comparing the response to the sample with the response to TCDD (Villeneuve et al., 2000). This bioanalytical assay has been used to detect and determine the toxicity of complex mixtures of dioxin-like compounds in extracts of environmental samples such as soil, water, food and biota (Villeneuve et al., 2000; Behnisch et al., 2001), but is particularly useful for the rapid screening of total concentrations of TCDD-EQ in sediments (Khim et al., 2001; Hilscherova et al., 2001). The H4IIE-*luc* cell line was developed at Michigan State University (Sanderson et al., 1996). The transfected cells contain an AhR element which controls the luciferase reporter gene. Binding of compounds to the AhR leads to transcription of the luciferase gene and the production of the luciferase enzyme.

While the AhR-active compounds have been found in many parts of the world as persistent environmental contaminants, due to a general lack of instrumentation and trained personnel, until recently this class of compounds was poorly characterized in much of the People's Republic of China (UNEP-Chemicals, 2002). The Haihe River, the largest water system in northern China, flows through the Tianjin area. The Tianjin region has a long history as an industrial city with a population of 9.2 million. Industrial and domestic waste waters from the city and agricultural effluent discharge into the Haihe River which carries the water to the Bohai Sea at Tanggu influx (Yuan et al., 2004). The Dagou River is another very important channel into which wastewater from domestic sources and from industries in Tianjin is discharged to be finally emptied into the Bohai Sea. Some toxic compounds, such as organochlorine pesticides (OCPs), xeno-estrogens (including 4-*tert*-octylphenol, 4-nonylphenol and bisphenol A) and mercury, have been reported in the previously studies in this area (Jin et al., 2004; Shi et al., 2005; Wan et al., 2005; Yang et al., 2005). However, few data are available to indicate the level of contamination of dioxin-like compounds in these rivers. Sediments are the eventual sink for dioxin-like compounds in the aquatic environment (Berggren et al., 1999; Hurst et al., 2004), so eleven sites (Fig. 1) were selected along the Haihe River and the Dagou River for sediment sampling and testing. This paper presents the results of using the H4IIE-*luc* bioassay to determine total concentrations of TCDD-EQ in surficial sediments.

Fig. 1. Map of the sampling locations in Haihe River and Dagu River, Tianjin, China.

2. Materials and methods

2.1. Sample collection

Sediment samples were collected from 11 sites along the Haihe and Dagu River on November 15–16, 2003 (Fig. 1). Sampling sites R1 and R2 are in Beiyun River and Ziya River, which are the two main branches of Haihe River that converge in Tianjin. The R3 sampling site lies in an urban district and is an important crossing point of several branches of the Haihe River. Site R4 is the location where the Haihe River flows out from Tianjin. Sites R5 and R7 are located at the two gates of the Haihe River. Site R8 is in the estuary of the Haihe River. Dagu River and Machangjian River are important drainage rivers in Tianjin. The sampling site R6 is in the Machangjian River just before it pours into the middle reaches of the Haihe River, and sampling sites S1, S2 and S3 are in the Dagu River.

Samples of sediments (<10 cm depth) were collected with a stainless steel grab and then placed into hexane-rinsed glass jars with aluminum foil covering. All sediment samples were immediately transferred to the laboratory and kept at -20°C in the refrigerator until extraction.

2.2. Total sediment extract and fractionation

Sediment extraction and cleanup was performed before the samples were analyzed using the H4IIE-*luc*

bioassay following previously published methods (Khim et al., 2001; Koh et al., 2004). The samples were freeze dried, homogenized using a blender with stainless steel blades, and passed through a $63\text{-}\mu\text{m}$ sieve. Five gram of the freeze-dried sediment was mixed with 10 g of silica (Merck, silica gel 60, column chromatography), and 15 g of anhydrous sodium sulphate (Na_2SO_4) to remove residual moisture. The sediment was then refluxed with a mixture of 300 ml dichloromethane (DCM) and hexane (1:1) for 16 h in a Soxhlet apparatus. Acid-activated copper powder was used to remove the sulfur in the sediment during extraction. The blank consisted of the same amount of anhydrous Na_2SO_4 , silica and copper powder only. After extraction, the solvent-soluble material was back extracted into hexane by rinsing the solvent three times with 15 ml hexane in a separating funnel. The extract was rotary evaporated ($\sim 40^{\circ}\text{C}$) and transferred to 1 ml *n*-hexane.

The concentrated sediment extract was further fractionated into three fractions (Hilscherova et al., 2001) through a column that contained 10 g of activated Florisil (60–100 mesh size; Sigma, St. Louis, Mo, USA). After the extract was added to the column, hexane was added until 100 ml of hexane was eluted to obtain Fraction 1 that contained the PCBs and a portion of the PCDD/PCDFs. A mixture of hexane containing 20% DCM was next used to elute organochlorine (OC) compounds, PAHs, alkylphenolethoxylates and the remaining PCDD/PCDFs to obtain Fraction 2. Fraction 3 was eluted with 100 ml DCM and contained the most

polar compounds, such as the breakdown products of steroids. The H4IIE-*luc* bioassay was performed on each fraction to calculate the contribution that each of these Fractions made to the overall AhR-mediated activity.

2.3. H4IIE-*luc* assay

The H4IIE cells were cultured aseptically in Dulbecco's Modified Medium (Sigma) with 10% fetal bovine serum (FBS, Logan, UT). Cells were continuously cultured in 100 mm tissue culture plates (Cambridge, MA) under standard conditions (37 °C, 5% CO₂) until 80–90% cell confluence. Cells were then collected and diluted to a final concentration of about 60 000 cells ml⁻¹ with culture medium. To conduct the test 250 µl of cell suspension was added to each well (about 15 000 cells) of a 96-well plate using an Eppendorf repeat pipettor and incubated as before for 24 h until the cell layer reached 60–80% confluence when the medium was replaced with 250 µl of dextran/charcoal-stripped fetal bovine serum (Hyclone, Logan, UT) medium containing the test material. At least six concentrations of the calibration standard or extract were tested and each exposure was in triplicate. Blank controls and 2,3,7,8-TCDD calibration standards (0.1–30 pg well⁻¹) were included on each well plate followed by incubation for 3 days. After addition of 100 µl luciferase assay reagent (LucLite Luciferase Assay Kit; Packard BioScience, Meriden, CT), the luciferase activity was determined using an automated luminometer (BMG LABTECH, Polar-Star, Durham, NC). For calculation of TCDD equivalent (TCDD-EQ) values the luciferase activity of extract was interpolated using the TCDD standard curve (Fig. 2(A)), and was based on the fragmentary parallel slopes (Villeneuve et al., 2000).

2.4. TCDD-EQ calculation

Because complete dose–response curves reached the maximal induction caused by the TCDD standard (TCDD_{max}) and there were significant differences between estimates made based on the EC₅₀ and the EC₂₀ (Fig. 3), activity was calculated based on the EC₂₀ values using REP range and the point of departure (Moore and Caux, 1997; Oris and Bailer, 1997; Putzrath, 1997; Villeneuve et al., 2000). When this method was applied, the H4IIE cells were found to be sufficiently sensitive to determine TCDD-EQs in sediment extracts. Sediment extract equivalents as little as 0.01 µl well⁻¹ (extract from 0.5 mg sediment) were sufficient to induce a significant response relative to the solvent control. When mass of sediment extract equivalents used in the assay was increased, a significant cytotoxicity towards the H4IIE cells was observed at a level of 0.83 µl well⁻¹ or 2.5 µl well⁻¹ (SCE and Fraction 1; Fig. 2(B)). Otherwise, complete dose–response curves were obtained with

Fig. 2. Dose–response curves for the induction of luciferase activity in the H4IIE-*luc* assay. (A) H4IIE-*luc* activity induced by 2,3,7,8-TCDD. (B) H4IIE-*luc* activity induced by sediment crude extract (Total) and fractions of SCE separated by Florisil column chromatography (F1: Fraction 1, F2: Fraction 2 and F3: Fraction 3). Luciferase induction is expressed in terms of the percentage of luciferase activity relative to that of 30 pg well⁻¹ 2,3,7,8-TCDD (TCDD_{max}). Values represent the mean $\pm$ SD; $n = 3$.

most of the Fraction 2 and Fraction 3 sediment extracts (F2 and F3; Fig. 2(B)).

2.5. Cytotoxicity to H4IIE-*luc* cells

In addition to the H4IIE-*luc* assay the compounds of interest and environmental mixtures were tested for cytotoxicity towards the H4IIE-*luc* cells using a LIVE/DEAD® Viability/Cytotoxicity Kit (L-3224) (Molecular Probes, Invitrogen, Eugene, OR). Live cells were determined with the polyanionic dye calcein, which is retained within live cells, and produces an intense

Fig. 3. Comparison between TCDD-EQs in the H4IIE-*luc* assay calculated based EC20 and EC50 values.

uniform green fluorescence under UV. Dead cells were identified by use of ethidium homodimer-1 (EthD-1) dye, which binds to occult DNA when cells are lysed, and produces a bright red fluorescence under UV. Only dose ranges of sediment extracts that did not cause cytotoxic effects were used in the subsequent bioassay.

2.6. Chemical analysis

Sediments collected from the same locations on July 7–12, 2003 had been previously analyzed for AhR-active PCDD/PCDFs and PCBs by HRGC/HRMS (Zhang, 2004). Ten PCDDs, seven PCDFs and twelve dioxin-like PCBs in the sediment samples were determined. The analysis used H4IIE-*luc*-specific TEFs to calculate TEQ (e.g. Sanderson et al., 1996; Khim et al., 2001; Koh et al., 2004) based on concentrations of PCDD, PCDF and AhR-active PCB congeners to all the dioxin-like activity in the sediment samples.

3. Results and discussion

3.1. H4IIE-*luc* assay

Concentrations of TCDD-EQs were determined by comparing the dose–response relationship of the extract of a known mass of sediment to a TCDD standard curve (Villeneuve et al., 2000). Mean values of luciferase response/TCDD_{max} response measured in three replicate wells were used to generate the dose–response curves. The dose–response curve of TCDD used as a standard in the H4IIE-*luc* bioassay was sigmoidal in appearance (Fig. 2(A)). Luciferase induction was reproducible with coefficients of variation (CV) less than 15%. The limit of detection (LOD) and EC₅₀ for luciferase induction

by TCDD were 0.16 pg well⁻¹ (0.05 fmol well⁻¹) and 2.52 ± 0.49 pg well⁻¹ (0.80 ± 0.16 fmol well⁻¹), respectively. Typical results obtained with the H4IIE-*luc* analysis of sediment extracts and fractions are given (Fig. 2(B)).

3.2. AhR-mediated activity of crude extracts of sediments

Eleven SCEs were analyzed at six dilutions. At SCE concentrations greater than 11% (0.28 µl extract well⁻¹) that were used for screening, all sediment extracts exhibited cytotoxicity towards the H4IIE cells. However, at SCE concentrations from 0.49% to 3.7% (0.01–0.09 µl extract well⁻¹) there were significant responses in the H4IIE cells without any observed cytotoxicity. Response magnitude presented as percentage of the maximum response was observed for a 30 pg well⁻¹ 2,3,7,8-TCDD (%TCDD_{max}) with the greatest value for each sample shown in Fig. 4. The greatest magnitude of activity observed for SCEs was 63.5% TCDD_{max}, for location S1. Only two SCEs yielded a response greater than 50% TCDD_{max} (locations R3 and S1) and one lower than 20% TCDD_{max} (location R7).

The TCDD-EQs, based on the EC₂₀ and the REP ranges of all SCEs, are shown (Table 1). Concentrations of TCDD-EQs in sediments from the Dagū River were greater than those in sediment from the Haihe River, except for one location (Jingangqiao: Location R3).

According to reports on environmental quality in Tianjin, during 2002, the total industrial effluents from the area were 2.2 × 10⁸ t. One municipal sewage treatment plant (STP) effluent (capacity of treatment, 2.6 × 10⁵ t d⁻¹) discharged into the Dagū River and finally entered into the Bohai Sea. The Dagū River and another drainage river have been reported to receive

Fig. 4. Maximal luciferase induction in the H4IIE-*luc* assay elicited by the sediment crude extracts (SCEs). Response magnitude is expressed as percentage of TCDD_{max}.

Table 1
TCDD-EQs obtained using H4IIE-*luc* assays of sediment crude extracts (SCEs)

Sampling site code	Sampling site name	Latitude/Longitude	pg TCDD-EQ g ⁻¹ dry wt (REP range ^a % TCDD _{max})
R1	Beiyun River	39°13.673'N 117°06.425'E	693.9 (6.5–26.3)
R2	Ziya River	39°10.523'N 117°05.945'E	501.2 (–5.7–24.5)
R3	Jingang Bridge	39°08.918'N 117°11.148'E	6834 (11.7–52.4)
R4	Haihe Bridge	39°04.346'N 117°18.648'E	926.6 (11.2–29.2)
R5	Erdao Gate	39°01.341'N 117°26.759'E	679.9 (–1.7–25.7)
R6	Machangjian River	38°58.789'N 117°32.909'E	676.6 (0.6–22.3)
R7	Haihe Gate Front	38°59.190'N 117°42.773'E	331.7 (2.3–16.9)
R8	Haihe Gate Back	38°59.190'N 117°42.773'E	545.2 (3.9–22.8)
S1	Xinzhuangzi River	38°57.792'N 117°28.745'E	13890 (–8.1–63.5)
S2	Mid-Dagu River	38°58.329'N 117°35.983'E	3798 (14.8–47.2)
S3	Down-Dagu River	38°57.817'N 117°41.978'E	1182 (5.8–36.4)

^a REP range includes the response of the well where the dilution used is cytotoxic towards the H4IIE-*luc* cells.

65% of the sewage effluent from Tianjin including domestic wastewater (Jin et al., 2004). This is a potential source of TCDD-EQ. The present study is the first investigation using in vitro bioassay of dioxin-like compounds in sediments collected in this area. The results indicate that the dioxin-like activities in SCEs collected in the Tianjin area are similar to previous reports of sediments from UK estuaries (1.1–154 ng TCDD-EQ g⁻¹ dw) (Hurst et al., 2004) and sediment from the Czech River Basin (0.782–22.3 ng TCDD-EQ g⁻¹ dw) (Hil-scherova et al., 2001).

3.3. AhR-mediated activity of fractions

Six dilutions of each of the three Fractions were tested with the H4IIE cell line. Five samples were selected for further analysis. The greatest response of each Fraction of those selected samples is shown (Fig. 5). PCBs and some of the PCDD/PCDFs were eluted in F1, which are non-polar compounds that have relatively great AhR-mediated activity. Due to their small concentrations AhR-active compounds in F1 contributed only a small proportion of the total TCDD-EQs (Table 2). Concentrations of TEQs calculated for sediments based on concentrations of each PCDD/PCDF and coplanar PCB congener and their respective TEF (Van den Berg et al., 1998) for locations S2 and S3, were 285 and 893 pg TEQ/g dw, respectively. Of the three fractions, only F1 caused significant cytotoxicity to H4IIE cells. The range of cytotoxic concentrations was from 11% to 100% (0.28–2.5 µl extract well⁻¹). These results indicate that the cytotoxic compounds were non-polar and concentrated in F1. It was unknown whether the cytotoxic compounds could affect the response of H4IIE cells to the PCDD/PCDFs and PCBs in the valid concentration wells.

The greatest induction of luciferase activity was caused by fractions 2 (F2) and 3 (F3) for these five sam-

Fig. 5. Maximal luciferase induction in the H4IIE-*luc* assay elicited by individual fractions separated by Florisil column chromatography (F1: Fraction 1, F2: Fraction 2 and F3: Fraction 3). Response magnitude is expressed as percentage of TCDD_{max}.

ples. The magnitudes of induction were 2- and 12-fold greater than that of F1 but no samples elicited a response higher than the TCDD_{max}. F2 contained PAHs, their derivatives, OC pesticides and a portion of the PCDD/PCDFs. Some PAHs, such as benzo(a)pyrene (BAP), dibenz(a,h)anthracene (DBA), benzo(a)anthracene (BA), benzo(k)fluoranthene (BkF), benzo(b)fluoranthene (BbF), chrysene (Chr), and indeno(1,2,3-c,d)pyrene (IdP), are a class of chemicals that can significantly contribute to the induction-TEQs. In the study area, the concentrations of PAHs have been reported to be 800–1200 µg kg⁻¹ soil (Wang et al., 2003). This indicates that the relatively great concentrations of PAHs could be responsible for the AhR-mediated activity in F2. The

Table 2

Comparison of sediment crude extracts (SCEs) and fractions analyzed by H4IIE-*luc* assay (TCDD-EQ) and chemical analysis (HRGC–HRMS; TEQ)

	H4IIE- <i>luc</i> -TCDD-EQs					TEQ _{HRGC–HRMS} ΣTEQ PCDD/PCDFs + PCBs
	SCEs	ΣF1 + F2 + F3	F1 ^a	F2	F3	
R3	6834.2	5592.6	168.3	4741.3	683.0	6.76
R8	545.2	187.1	79.4	84.7	23.0	19.13
S1	13892.1	3718.2	190.3	2794.4	733.5	21.93
S2	3797.8	2270.3	152.7	1028.8	1088.8	284.97
S3	1182.5	527.2	129.4	200.5	197.3	892.59

^a The maximal luciferase induction was less than EC₂₀ relative to TCDD_{max}.

third fraction (F3) also elicited significant AhR-mediated luciferase activity. For some samples (S2 and S3), maximum induction in F3 was greater than that caused by F2. The chemicals that caused the dioxin-like activities in polar F3 are unknown. The sums of the TCDD-EQs from the fractions were significantly less than the TCDD-EQs for total extract. This may suggest antagonistic interactions among compounds. Furthermore, the Florisil column could have adsorbed some of the compounds that could elicit dioxin-like activity (Hilscherova et al., 2001).

The TCDD-EQs derived by H4IIE-*luc* bioassay are significantly correlated with TEQs ($R^2 = 0.75$, Fig. 6). Concentration of TCDD-EQs estimated by use of the H4IIE-*luc* assay calculated were greater than the concentration of TEQ predicted from the individual PCDD/PCDF and PCB congeners. The most likely cause of the difference between the two methods of measuring AhR activity in the sediments is the presence of

AhR-active compounds other than those measured in this study. Of those unknown compounds capable of producing dioxin-like responses in the H4IIE-*luc* assay that may be present in the samples the most likely are the PAHs (Hilscherova et al., 2001; Hurst et al., 2004). The compounds responsible for dioxin-like activity in F3 are unknown. They may include polar compounds that are relatively weak AhR agonists, which may be of either natural or synthetic origin. Recently, some studies have suggested additional types of AhR ligands and inducers with a wide structural variety, some of which could have been present in our sediment samples at concentrations sufficient to cause the observed induction (Denison et al., 1998). Also, studies with marine sediments have documented the presence of unknown AhR-active compounds in the most polar fractions of sediment extracts (Khim et al., 1999).

4. Conclusions

In vitro H4IIE-*luc* bioassay has been shown to be a useful tool for assessing the dioxin-like activity of complex environmental mixtures. The dioxin-like activities in sediments from Haihe River and Dagou River were significant. Most of the AhR-mediated, dioxin-like activity is attributable to the PAHs in F2 and unidentified polar AhR-active compounds in F3. The H4IIE-*luc* bioassay is a relatively rapid, inexpensive and sensitive method for detecting and screening the occurrence of AhR-active compounds in complex mixtures before the application of the more expensive and time-consuming instrumental analyses. Application of the bioassay to screen environmental matrices in China will allow more effective use of the relatively limited capacity for analyses by HRGC–HRMS. It also can provide much information on the biological effects of complex mixtures that chemical analysis cannot provide. However, the application of the bioassay cannot quantify specific compounds. Some unknown cytotoxic chemicals may affect the results of bioassay so caution must be applied when assessing the risks posed by TCDD-EQs in sedi-

Fig. 6. Relationship between toxic equivalents determined by H4IIE-*luc* (TCDD-EQ_{H4IIE-*luc*}) and toxic equivalents (TEQs) is the sum of TEQ_{PCDD/PCDFs+PCBs} (based on instrumental analysis and WHO-TEF) and TEQ_{PAH} (based on H4IIE-*luc* bioassay).

ment. To provide an estimate of the relative level of contamination, it is reasonable to use bioassays combined with instrumental analytical approaches.

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