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# Identification of Ah Receptor Agonists in Soil of E-waste Recycling Sites from Taizhou Area in China

CHAOFENG SHEN,<sup>†,‡</sup>  
SHENGBIAO HUANG,<sup>†</sup> ZIJIAN WANG,<sup>†</sup>  
MIN QIAO,<sup>‡</sup> XIANJIN TANG,<sup>†</sup>  
CHUNNA YU,<sup>§</sup> DEZHI SHI,<sup>†</sup>  
YOUFENG ZHU,<sup>†</sup> JIYAN SHI,<sup>†</sup>  
XINCAI CHEN,<sup>†</sup> KAREN SETTY,<sup>^</sup> AND  
YINGXU CHEN<sup>\*,†</sup>

Department of Environmental Engineering, Zhejiang University, Hangzhou 310029, China, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China, Department of Drug Metabolism and Pharmaceutical Analysis, Zhejiang University, Hangzhou 310058, China Bren School of Environmental Science and Management, University of California, Santa Barbara, CA 93106-5131

Received May 17, 2007. Revised manuscript received October 29, 2007. Accepted October 31, 2007.

In recent years, increasing concern has surrounded the consequences of improper electric and electronic waste (e-waste) disposal. In order to mitigate or remediate the potentially severe toxic effects of e-waste recycling on the environment, organisms, and humans, many contaminated sites must first be well-characterized. In this study, soil samples were taken from Taizhou city, one of the largest e-waste disposal centers in China, which was involved in recycling for nearly 30 years. The extracts of the samples were assayed for aryl hydrocarbon receptor (AhR)-mediated ethoxyresorufin-O-deethylase (EROD) induction in the rat hepatoma cell line H4IIE. Some of the target AhR agonists, including polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs), were instrumentally analyzed as well. The cause–effect relationship and dose–response relationship between the chemical concentrations of AhR agonists and observed EROD activity were examined. The results showed that soil extracts could induce AhR activity significantly, and the chemically derived 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (TEQ<sub>cal</sub>) were perfectly correlated to bioassay-derived TCDD equivalents (TEQ<sub>bio</sub>;  $R = 0.96$ ,  $P < 0.001$ ), which indicated that the known AhR agonists could account for the observed responses. Among different contributors, PCBs accounted for 87.2–98.2% and PCDD/Fs contributed 1.7–11.6% of TEQ<sub>cal</sub>, while the contribution of PAHs could almost be neglected. Under these conditions, a quantitative dose–effect relationship between

TEQ<sub>PCB</sub> and EROD activity could be evaluated, suggesting that the observed AhR effect was mainly caused by PCBs.

Further source identification by congener profiles analysis showed that the crude dismantling of electric power devices and open burning of electric wires and printed circuit boards may be the main sources of these dioxin-like compounds. This study suggests that the combination of *in vitro* bioassay and chemical analysis is useful to screen, identify, and prioritize AhR agonists in soil from e-waste recycling areas.

## Introduction

Electric and electronic waste, or e-waste, represents an emerging environmental problem, as waste electrical and electronic equipment already constitutes more than 5% of municipal waste and is still growing fast in developed countries (1). However, industrializing countries show the most rapidly increasing consumption rates for electrical and electronic equipment, predicting that large quantities of domestically generated e-waste will become part of the waste stream in the near future (2). Moreover, developing countries such as China and India face a rapidly increasing amount of e-waste that is imported illegally from developed countries for recycling. A report by Toxics Link found that 70% of the e-waste collected at recycling units in New Delhi, India, was actually exported or dumped by developed countries (3). About 50–80% of the e-waste collected for recycling in the western U.S. is being exported to Asia, about 90% of which is sent to China for recycling (4). The recycling and disposal of e-waste in these countries poses a serious environmental threat, since commonly used treatment methods remain rudimentary (2, 4). Persistent toxic substances such as polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs) are emitted into the environment during crude recycling, which may threaten the health of local people and organisms (5–7).

It has been proven that 2,3,7,8-substituted PCDD/Fs, several PCBs, and some PAHs with dioxin-like structures can act in a similar way through binding to the aryl hydrocarbon receptor (AhR) and subsequently induce the expression of target genes such as CYP1A1 (8). The difficulty of characterizing complex mixtures of these dioxin-like compounds for risk evaluation, has led to the conceptual development of toxic equivalency factors (TEF) (8, 9). Accordingly, the total dioxin-like toxic potency of a compound mixture can be expressed as a single toxic equivalency (TEQ<sub>cal</sub>). The TEQ<sub>cal</sub> value has been proven to be a significant indicator in risk assessment for humans and wildlife (8, 10).

When applying the TEF method to field conditions, instrumental analysis of the target compounds is still fundamental. However, chemical analyses are used to identify and quantify only those compounds for which analytical techniques and standards are available and do not account for interactions among the chemicals in complex mixtures, thus providing little information on their biological effects (11). As a result, *in vivo* and *in vitro* bioassays have been developed to estimate the total biological activity of all dioxin-like compounds in complex environmental matrices and integrate possible interactions among them (11). Numerous studies have utilized bioassays based on ethoxyresorufin-O-deethylase (EROD) induction, such as rat hepatoma cell line H4IIE, human hepatoma cell line HepG2, the fish liver cell line, and others, to evaluate the relative potency of a compound in inducing the CYP1A enzyme system (11, 12). The EROD bioassay predicts the dioxin-like effect on the

\* Corresponding author. Phone: +86-571-8697-1159. Fax: +86-571-8697-1898. E-mail: yxchen@zju.edu.cn.

<sup>†</sup> Department of Environmental Engineering, Zhejiang University.

<sup>‡</sup> Chinese Academy of Sciences.

<sup>§</sup> Department of Drug Metabolism and Pharmaceutical Analysis, Zhejiang University.

<sup>^</sup> University of California, Santa Barbara.

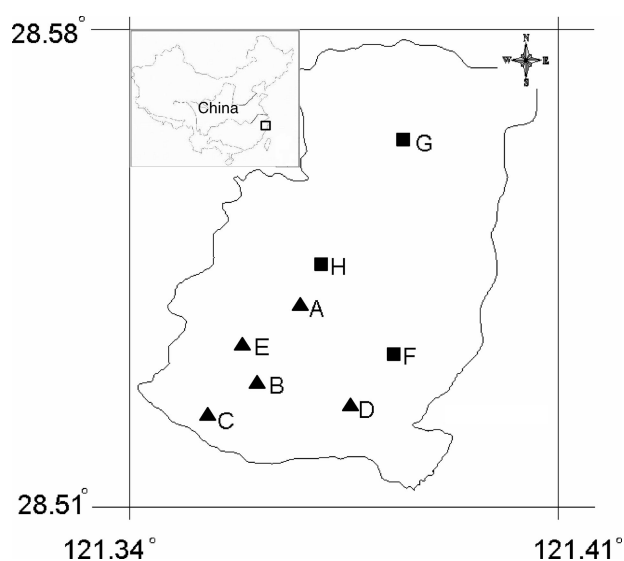
basis of the relationship between induction of this enzyme and *in vivo* toxicity. This type of enzymatic assay has undergone a number of significant improvements since its initial stages, and it has been proven to be a sensitive biomarker for characterizing the existence of Ah-active components in complex environmental mixtures. The comparison between bioassay-derived potency and theoretical potency (based on chemical analysis of suspect compounds) is an effective means of characterizing the causative agents or potential toxic compounds present. However, the limited studies currently available have provided mainly qualitative comparisons.

Nowadays, the pollution of dioxin and dioxin-like compounds is of great concern in China (13, 14). The majority of the research to date has been primarily based on chemical analysis, while the integrated ecological effects and the respective contribution of different pollutants have not been well illustrated. Little information on dioxins and related chemicals in e-waste recycling areas has been reported, though previous research has been done regarding pollutant concentrations in Guiyu, a village in the southeast of China (5, 7, 15). This site attracted international attention after the Basel Action Network described it in a public report (4). Relevant research about Taizhou city, the other of the two largest primitive e-waste recycling centers in eastern China, is very limited, even though it has a longer disposal history by 10 years, and the area involved in crude dismantlement is 6 times larger (about 300 km<sup>2</sup>) than Guiyu. Moreover, the e-waste in Taizhou includes many items which are scarce in Guiyu, including electric power electromotors, capacitors, transformers, and their components. The potentially hazardous recycling processes in Taizhou included the unprotected manual removal of printer cartridge toner, the open burning of wires and printed wiring boards to recover metals, the dismantling of electric power appliances, and the use of strong acid baths to retrieve gold from chips and other components (Figure S1, Supporting Information). These unregulated salvaging operations and optional dumping of the residual waste have resulted in severe pollution (6). Accordingly, there is a large data gap surrounding how the environment is influenced by the growing e-waste recycling industry.

The objective of this study was to perform an investigation to characterize soil pollution from dioxins and related chemicals, using Taizhou as a case study of e-waste recycling. The *in vitro* bioassay was used to evaluate the integrated potency of AhR-active compounds in the soil. The target chemicals were instrumentally analyzed to determine whether known ligands could account for the responses observed. Finally, the congener profiles of contributors were further characterized in order to identify the pollution sources.

## Experimental Section

**Sample Collection and Preparation.** Surface soil samples (to a depth of 20 cm) were collected from crop lands very close to eight e-waste recycling sites in Fengjiang town of Taizhou city in September 2005, with the aid of a global positioning system (Figure 1). Sites A to E were relatively large-scale recycling plants, while sites F to H were simple household workshops (Figure S1, Supporting Information). Sites I to K were chosen as controls and were located in the towns of Jinjing, Pengjie, and Huanglang in Taizhou city, respectively, about 15 km east of Fengjiang town (not shown in Figure 1), and there was no e-waste recycling industry in this area. Large areas of rice, vegetables, grapes, or oranges were located near the disposal sites. Five subsamples were mixed to obtain about 1000 g representative samples. All of the surface soils were collected using acid-washed stainless steel scoops and were immediately transferred to prewashed aluminum boxes.



**FIGURE 1.** Map of study area and sampling locations: (▲) large-scale e-waste recycling plants; (■) simple household e-waste recycling workshops.

The samples were then air-dried at room temperature, sieved through a 1 mm sieve, and homogenized. A total of 20 g of the samples were immersed in 200 mL of acetone/dichloromethane (1:1, v/v) for 24 h and Soxhlet-extracted for 48 h with activated copper for desulfurization. The extracts were concentrated to about 2 mL on a rotary evaporator (Büchi R-200, Switzerland) and were solvent-exchanged to hexane. The concentrated extracts were evaporated to near dryness under a gentle stream of nitrogen gas, redissolved in 0.5 mL of dimethyl sulfoxide (DMSO), and stored at -20 °C prior to the EROD bioassay.

**H4IIE-EROD Bioassay.** The details of the EROD bioassay have been described previously (16, 17). In short, the H4IIE rat hepatoma cells were seeded into 96-well plates at a density of  $1.5 \times 10^3$  cells/well. After 24 h of incubation (about 70–80% confluence), the culture medium was replaced with a fresh medium containing either sample solutions or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) standard solutions. Five concentration levels of test solutions were prepared by sequential 2-fold dilution of each raw soil extract. For each concentration level, three replicates and blanks were performed on the 96-well plate. The final concentration of the control solvent DMSO was less than 0.5%, and no cytotoxicity was observed. The TCDD standard solutions were also included on each plate as positive controls and for calibration purposes. Standard solutions (0.007, 0.014, 0.027, 0.055, 0.110, 0.219, 0.438, 0.875, 1.750, 3.500, 14.000 ng/mL TCDD,  $n = 14$ ) were prepared by the serial dilution of a TCDD stock solution in order to yield a full dose–response curve of EROD induction. The cells were exposed for another 72 h. Then, the culture medium was replaced with 100  $\mu$ L of fresh medium containing 10  $\mu$ M 7-ethoxyresorufin and 10  $\mu$ M dicumarol. The reaction was terminated with 130  $\mu$ L of methanol after incubation for 1 h. After being shaken, 100  $\mu$ L of suspension from each well was transferred to a new plate for fluorescence measurement with a microplate reader at a 535 nm excitation and a 590 nm emission wavelength. The protein concentration was determined on the basis of Bradford's method (18). The standard curve of resorufin was prepared simultaneously to calculate the EROD enzyme activity expressed as mean picomoles of resorufin per minute per milligram of microsomal protein [pmol/(min·mg)].

The data were analyzed by a sigmoid nonlinear curve-fitting module. The program is a four-parameter logistic equation (16, 17, 19). The bioassay-derived TCDD equivalents

(expressed as TEQ<sub>bio</sub>) in soil samples were then obtained by comparing values to the TCDD dose–response curve created using the standard solution on the same plate. If necessary, an extract of the soil sample was diluted to fit the linear part of the TCDD dose–response curve. Control solutions extracted in the same manner, but without any soil sample were used to exclude EROD induction during the working procedure because of experimental conditions. The mean solvent control response was subtracted from both sample and standard responses on a plate-by-plate basis. The detection limit of the EROD assay was 18.5 fg TCDD/well, which corresponds to an activity equal to three standard deviations below the mean solvent control response.

**Chemical Analysis.** For PCDD/Fs and PCBs analysis, Soxhlet extracts were cleaned up by multilayered columns and were analyzed by high-resolution gas chromatography/high-resolution mass spectrometry on an Agilent 6890 gas chromatograph coupled with an Autospec Ultima mass spectrometer (Waters Micromass, Manchester, United Kingdom) (20). The extracts were passed through a silica gel/aluminum oxide column to obtain PAH fractions by elution with 70 mL of dichloromethane/hexane (3:7, v/v). A gas chromatograph interfaced with a mass spectrometer (Agilent 5973, United States) was used to quantify PAH concentrations. All procedures were subjected to strict quality control measures to meet EPA requirements for field research. Phenanthren-d10 and <sup>13</sup>C-labeled surrogate standards were spiked to detect recoveries. Detailed information regarding quality assurance and control has been discussed elsewhere (20, 21).

The TEQs of PCDDs, PCDFs, and PCBs were calculated by multiplying the measured concentration by the corresponding TEF proposed by the World Health Organization (8). PAHs such as benzo[k]fluoranthene (B[k]F), benzo[a]pyrene (B[a]P), benzo[b]fluoranthene (B[b]F), chrysene (Chry), benz[a]anthracene (B[a]A), indeno[1,2,3-*cd*]pyrene (I[cd]P), and dibenz[a,h]anthracene (D[ah]A) are commonly metabolized by cytochrome P4501A1 activity and have been reported to show dioxin-like responses *in vitro* (22, 23). In the present study, relative potencies (REPs) of PAHs related to TCDD were used to estimate TEQs contributed by individual PAHs. REPs were obtained from an *in vitro* bioassay using recombinant H4IIE cells (22, 24). The TEQ<sub>cal</sub> was obtained as the sum of TEQs from the individual compounds (including PCBs, PCDD/Fs, and PAHs) by assuming an additive effect when dioxin-like chemicals mixed.

**Statistical Analysis.** Means were compared using an independent-samples T test, and linear regression (as based on Pearson correlation coefficients) was used with SPSS version 13.0 software. The significance level was  $P < 0.05$ .

## Results and Discussion

**TEQ<sub>bio</sub> from EROD Assay.** Significant responses were observed in the H4IIE cells from the EROD assay for all soil extracts of eight e-waste recycling sites (Table 1). The TEQ<sub>bio</sub> ranged from 91.0 to 383.0 pg/g dw with a mean value of 227.4 pg/g dw, which was greater than that of control sites by over 41 times ( $P < 0.05$ ). Since the control areas without e-waste disposal were also in Taizhou city, and thus had other similar natural and industrial conditions with the study area, it was quite possible that improper recycling had caused severe contamination of the local soil with dioxin-like compounds. The TCDD equivalents of soil samples from the simple household workshop sites (F to H) were significantly higher than those of the large-scale recycling plants (site A to E;  $P < 0.05$ ). The small workshops were downstream of the industrial chain of e-waste recycling sites and dealt with residual components of e-waste supplied by the large-scale plants. However, the residue was difficult to dismantled, and

more crude operations were utilized, which might result in more severe pollution.

In the present study, a full dose–response curve but no apparent cytotoxicity was observed for every extract, implying that the EROD assay with wild-type H4IIE cells was applicable. However, AhR activity still might be underestimated due to the inhibition of CYP1A1 activity at high ligand concentrations (11). Recently, several recombinant cell bioassay systems have been developed, using luciferase- or fluorescence-based reporters regulated by dioxin-responsive elements upon interaction with the AhR (11, 25–27). These techniques are more sensitive and lack the inherent problems and thus gain popularity.

It also has to be stressed that the TEQ<sub>bio</sub> levels that have been obtained in this study merely give information on the potential for gene induction, and this does not necessarily reflect the toxicity. In fact, CYP1A1 induction has little to do with dioxin-like toxicity per se but is merely in parallel with the disruption of other biological processes. The TEQ<sub>bio</sub> derived from the *in vitro* bioassay represents an integrated response in comparison to chemical analysis. However, it may not substitute for *in vivo* study to accurately predict the toxicity of a complex mixture, especially in abiotic matrices such as soil. That is because the *in vitro* assay does not account for the chemical- and matrix-specific fate, transport, and bioavailability of the compounds, or the species differences, selective uptake, pharmacokinetics, tissue distribution, biotransformation, and responses through different mechanisms (11). Therefore, additional analysis by *in vivo* animal studies must be carried out, and factors such as bioavailability should be specifically considered before any definitive conclusions regarding the toxicity of the soil can be made.

**Concentration Levels of PCBs, PCDD/Fs and PAHs.** The concentrations of PCBs, PCDD/Fs, and PAHs are shown in Table 1. The average concentration of 12 dioxin-like PCBs from e-waste recycling sites was 167.7 ng/g dw, which was greater than that from control sites by over 15 times ( $p < 0.05$ ). The PCBs level was 1–2 orders of magnitude higher than those obtained from some other areas in China, such as Beijing, Shenyang, Wuhan, Hong Kong, and Tibet (14, 28), but were at the same level as those found at some heavily polluted sites from China (29, 30) and other countries (31, 32), though information from China on soil PCB concentration is still very limited up to now. The PCB levels were statistically higher for sampling sites from simple household recycling workshops when compared with samples from large-scale recycling plants ( $P < 0.05$ ). Among the congeners, tetrachlorinated biphenyls PCB81 and PCB77 were most abundant, though they are not as persistent as the high chlorinated congeners in biota. PCB126 with the highest TEF was detectable in all of the samples from e-waste recycling sites.

The levels of total PCDD/Fs ranged from 271.0 to 932.5 pg/g dw at e-waste disposal sites, with PCDDs accounting for 65.1–82.8% of the total and PCDFs accounting for the remaining 17.2–34.9%. The average PCDD/Fs concentration at recycling sites was statistically higher ( $P < 0.05$ ) than that at control sites. The total concentrations of PCDD/Fs were higher than available data from some industrialized areas in China (30, 33) but were 1–2 orders of magnitude lower than levels found at Guiyu (5) and some contaminated areas in other countries (32, 34). The PCDD/Fs concentrations were at the same level among different recycling sites in this study. The dominant congeners were 1,2,3,4,6,7,8-HpCDD, OCDD, 1,2,3,4,6,7,8-HpCDF, and OCDF of all the samples; however, 2,3,7,8-TCDF was also prevalent in soils from the simple household recycling workshops. The most toxic congener, 2,3,7,8-TCDD, could be detected in all of the samples at disposal sites.



TABLE 1. Concentrations<sup>a</sup> and Calculated TEQs for PAHs, PCBs, and PCDD/Fs in Soil from E-waste Recycling Sites

chemical	TEF	concentrations at sampling sites										
		large recycling plants					small recycling workshops				control sites	
		A	B	C	D	E	F	G	H	I	J	K
2378TCDD	1	0.3	0.6	0.5	0.4	0.8	0.6	0.4	1.0	N.D.	N.D.	N.D.
12378PeCDD	1	1.2	1.8	1.7	2.0	2.7	2.0	1.1	N.D.	N.D.	N.D.	N.D.
123478HxCDD	0.1	1.0	1.9	1.5	1.4	2.5	1.3	0.7	3.3	N.D.	N.D.	0.3
123678HxCDD	0.1	1.7	3.0	2.5	3.2	4.6	2.4	1.3	5.7	0.4	N.D.	N.D.
123789HxCDD	0.1	2.2	2.5	2.5	3.6	4.6	2.2	1.4	5.3	N.D.	N.D.	N.D.
1234678HpCDD	0.01	21.9	40.2	28.4	22.4	46.1	17.5	14.3	53.1	7.5	7.0	10.4
OCDD	0.0003	236.2	283.3	364.1	192.7	336.0	216.6	197.9	615.1	24.4	10.8	20.3
total PCDDs		264.4	333.3	401.1	225.7	397.1	242.5	217.1	683.5	32.3	17.8	31.0
2378TCDF	0.1	4.3	7.6	8.0	3.1	8.8	17.4	13.2	19.2	0.8	0.9	0.5
12378PeCDF	0.03	3.6	6.5	6.0	7.8	9.2	8.9	4.5	14.7	0.8	1.5	0.0
23478PeCDF	0.3	5.2	7.0	7.8	3.4	13.1	10.6	5.4	22.7	N.D.	N.D.	N.D.
123478HxCDF	0.1	4.6	7.1	8.1	13.0	13.5	9.8	4.3	19.9	0.9	1.4	1.0
123678HxCDF	0.1	4.5	7.0	8.0	7.4	13.3	8.0	3.4	19.1	0.7	1.0	0.9
234678HxCDF	0.1	1.4	2.1	2.1	1.0	3.7	2.0	1.0	5.3	N.D.	N.D.	0.2
123789HxCDF	0.1	5.0	7.2	9.0	2.8	17.4	7.6	3.4	24.8	0.5	N.D.	0.7
1234678HpCDF	0.01	15.4	27.9	32.9	31.2	56.9	25.5	10.2	76.7	2.4	3.4	2.9
1234789HpCDF	0.01	1.7	3.9	3.5	1.7	5.6	2.9	1.2	7.9	N.D.	N.D.	0.4
OCDF	0.0003	9.6	43.9	37.9	12.6	71.9	19.6	7.2	38.7	N.D.	N.D.	6.7
total PCDFs		55.1	120.1	123.2	84.0	213.3	112.3	53.9	249.0	6.1	8.1	13.1
total PCDD/Fs		319.5	453.4	524.3	309.7	610.4	354.8	271.0	932.5	38.4	25.9	44.1
TEQ <sub>PCDD/F</sub>		6.1	9.3	9.6	7.8	15.7	11.6	6.4	20.1	0.5	0.5	0.5
PCB-81	0.0003	33.0	43.0	14.1	68.0	16.4	163.5	84.8	44.1	N.D.	0.1	0.1
PCB-77	0.0001	19.1	14.2	5.5	21.6	5.4	33.1	188.1	150.8	8.9	N.D.	10.2
PCB-123	0.00003	0.9	1.2	0.4	N.D.	N.D.	15.2	7.7	16.2	N.D.	N.D.	N.D.
PCB-118	0.00003	5.3	5.5	2.7	0.5	0.8	77.1	68.2	48.1	1.2	0.1	0.1
PCB-114	0.00003	0.4	0.4	N.D.	0.2	N.D.	N.D.	1.8	6.4	0.5	5.1	N.D.
PCB-105	0.00003	3.4	3.4	1.7	0.4	0.7	50.9	44.7	21.1	0.4	0.3	0.2
PCB-126	0.1	1.4	1.3	1.3	1.0	1.1	2.9	3.3	2.8	N.D.	N.D.	N.D.
PCB-167	0.00003	0.7	0.7	0.6	0.4	0.4	2.4	1.9	2.9	0.5	0.4	0.9
PCB-156	0.00003	1.7	1.5	0.9	0.7	N.D.	N.D.	4.9	2.3	0.6	0.4	0.4
PCB-157	0.00003	1.3	N.D.	N.D.	N.D.	N.D.	2.3	1.3	2.5	N.D.	0.6	0.4
PCB-169	0.03	N.D.	1.4	1.1	1.4	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
PCB-189	0.00003	N.D.	0.8	0.6	0.9	N.D.	N.D.	1.0	N.D.	0.6	0.5	0.5
total PCBs		67.2	73.4	28.9	95.3	24.8	347.4	407.7	297.2	12.8	7.5	12.9
TEQ <sub>PCB</sub>		154.5	190.8	161.7	168.0	118.5	347.6	377.5	311.4	1.0	0.3	1.1
B[a]A	0.0000014	46.3	201.7	170.4	57.6	25.5	25.4	29.8	37.2	3.6	1.3	4.9
Chry	0.0000016	37.6	55.8	129.4	52.0	14.3	16.7	24.0	40.8	5.2	1.4	6.0
B[b]F	0.000004	43.2	112.2	120.4	53.4	25.3	26.3	31.6	41.0	8.1	1.7	7.6
B[k]F	0.00011	26.4	N.D.	71.7	29.6	11.5	12.6	N.D.	27.9	2.5	0.4	2.6
B[a]P	0.0000013	39.9	115.1	182.9	59.9	27.2	27.0	30.6	39.3	5.1	0.4	4.4
I[cd]P	0.000013	30.4	30.6	N.D.	30.4	19.1	20.6	22.3	33.4	1.4	N.D.	1.1
D[ah]A	0.000004	23.3	12.2	12.4	13.8	6.1	6.6	4.3	9.2	3.2	N.D.	2.7
total PAHs		247.1	527.5	687.2	296.7	128.8	135.3	142.6	228.8	29.1	5.2	29.4
TEQ <sub>PAH</sub>		3.7	1.4	9.1	4.2	1.7	1.9	0.6	3.9	0.4	0.1	0.4
TEQ <sub>cal</sub> <sup>b</sup>		164.4	201.5	180.4	180.0	136.0	361.1	384.4	335.3	1.8	0.8	2.0
TEQ <sub>bio</sub>		100.0	194.0	152.0	91.0	140.0	378.0	383.0	381.0	5.3	8.6	2.6

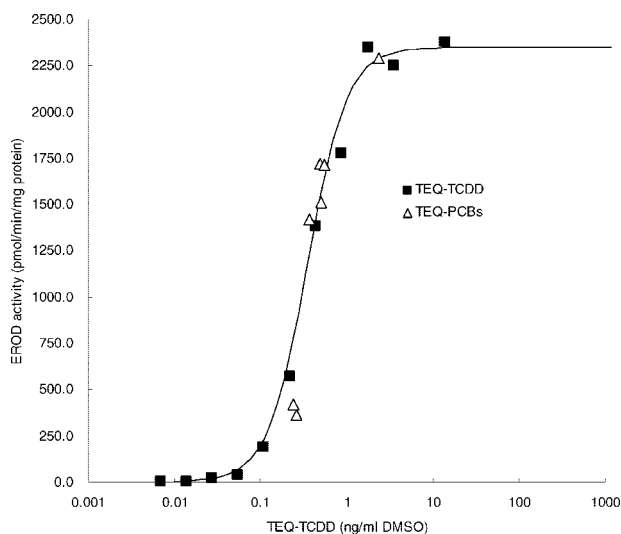
<sup>a</sup> Concentration is expressed as ng/g dw for PAHs and PCBs and as pg/g dw for PCDD/Fs and TEQs. <sup>b</sup> TEQ<sub>cal</sub> is the sum of TEQ<sub>PCB</sub>, TEQ<sub>PCDD/F</sub>, and TEQ<sub>PAH</sub>.

The concentrations of seven dioxin-like PAHs varied from 128.8 to 687.2 ng/g dw with a mean value of 299.3 ng/g dw at recycling sites, which was significantly higher ( $P < 0.05$ ) than that of control samples. In comparison, the levels of PAHs were consistent with those observed in Guiyu (7) and published data from other industrialized areas (35). There were no statistical differences on the levels of the seven identified PAHs between small workshops and large plants.

**TEQ<sub>cal</sub> from Chemical Analysis.** From chemical data, the TEQ<sub>cal</sub> at recycling sites ranged from 136.0 to 384.4 pg TEQ/g dw (Table 1), and the mean value was 157 times higher than that of the control sites ( $P < 0.05$ ). Therefore, all the crop land soil samples close to the e-waste recycling sites could be judged as severely polluted by dioxin-like compounds in comparison to the agricultural soil guidelines of 4–10 pg TEQ/g dw from Canada, New Zealand, and Sweden (5). The TEQ<sub>cal</sub> values were significantly higher for samples from simple household recycling workshops than those from large-

scale recycling plants ( $P < 0.05$ ). By chemical analysis, it was observed that the contributions to TEQ<sub>cal</sub> by three kinds of dioxin-like chemicals decreased in the following order: PCBs (118.5–377.5 pg TEQ/g dw, among which are the non-*ortho* PCBs, 118.4–373.5 pg TEQ/g dw, and mono-*ortho* PCBs, 0.1–4.4 pg TEQ/g dw) > PCDD/Fs (6.1–20.1 pg TEQ/g dw) > PAHs (0.6–9.1 pg TEQ/g dw). Therefore, it appeared that the TEQ<sub>cal</sub> could be attributed primarily to dioxin-like PCBs in the soil near the e-waste recycling sites.

The contribution of halogenated aromatic hydrocarbons to the TEQ<sub>cal</sub> was greater than 90%, while PCBs and PCDD/Fs contributed 87.2–98.2% and 1.7–11.6%, respectively. The contribution of PAHs could be considered negligible. However, B[k]F accounted for more than 73% of the TEQs from PAHs throughout the soil samples at recycling sites except for sites B and G. PCB126 contributed more than 61% of the TEQ<sub>PCB</sub>, since it has the highest TEF among dioxin-like PCBs. PCB81 and PCB77 contributed the second greatest proportion



**FIGURE 2.** Response of *in vitro* assay with H4IIE rat hepatoma cells to 2,3,7,8-TCDD standards and soil extracts. Dose is expressed as ng TEQ/mL media present in the test solution for TCDD standards or for dioxin-like PCBs. Response is expressed as EROD activity.

to the  $TEQ_{PCB}$  because of their highest levels in the soil samples. Among different kinds of dioxins, PCDFs accounted for 55.0–84.3% of the  $TEQ_{PCDD/F}$ . The congener 2,3,4,7,8-PeCDF contributed 12.9–33.9% to the  $TEQ_{PCDD/F}$ .

It should be noted that the brominated analogues such as polybrominated diphenyl ethers (PBDEs) and polybrominated dibenzo-*p*-dioxins/furans (PBDD/Fs) have not been studied in this work. Though PBDEs, which were widely used as flame retardants in e-products, do not have AhR agonist properties by themselves, PBDD/Fs occur as trace contaminants in commercial PBDEs and can be produced during open burning of e-waste containing PBDEs (8). Both *in vitro* and *in vivo* studies have shown that PBDD/Fs are potent AhR agonists and cause dioxin-like effects (8). Therefore, these compounds should be further studied in the e-waste recycling area. In addition, the  $TEQ_{cal}$  in this study is useful for characterizing contamination by dioxin-like chemicals in soil. However, factors such as fate, transport, and bio-availability should be specifically considered, when an assessment of the potential toxic potency of the soil is to be done.

**$TEQ_{cal}$  and  $TEQ_{bio}$ .** There was a significant dose-dependent relationship between  $TEQ_{cal}$  and EROD activity of the soil raw extracts from e-waste recycling sites ( $R = 0.77$ ,  $P < 0.05$ ,  $n = 8$ ). A nearly linear dose–effect relationship existed between TEQs for dioxin-like PCBs and EROD activity ( $R = 0.77$ ,  $P < 0.05$ ,  $n = 8$ ). Furthermore, the  $TEQ_{PCB}$  value calculated from the TEFs and concentrations was in the linear part of the calibration curve for standard solutions (Figure 2), which confirmed that PCBs could be stressors for the observed potency.

The combination of instrumental analysis and *in vitro* bioassay was useful to assess soil quality and characterize the causative agents or potential toxic compounds present. Some investigators have found that the raw organic extracts from sediments had high potency in inducing EROD activity; however, the attempts to attribute the potency to typical AhR agonists had often failed (25–27). At this time, the research is very limited on identifying and quantifying the ecological stressors in soils containing multiple contaminants by combining chemical analysis with bioassays. In the present work,  $TEQ_{bio}$  was significantly correlated with  $TEQ_{cal}$  (Figure S2, Supporting Information) and  $TEQ_{PCB}$  (Figure S3, Supporting Information). The lack of correlations between  $TEQ_{bio}$

and  $TEQ_{PCDD/F}$  or  $TEQ_{PAH}$  reflects the dominance of the PCBs. In view of the potential uncertainty in the TEQ estimates, our results suggested that, in certain complex circumstances, the known AhR agonists could account for the potency observed in bioassays.

**Sources for Dioxin-Like Chemicals.** A total of 36 PCBs and 16 PAHs were further determined, and the profiles of PCBs, PCDD/Fs, and PAHs homologues were characterized in detail in order to identify the sources of these dioxin-like chemicals. In general, very similar profiles of PCB congeners were found in the eight soil samples from recycling sites (Table 1; Figure S4, Supporting Information). Moreover, the concentrations of six indicator PCBs and 12 dioxin-like PCBs correlated very well with the total concentration of 36 PCBs (Figures S5 and S6, Supporting Information). Taking these findings into consideration, it could be confirmed that the profile was fairly constant at different e-waste recycling sites and that the PCBs must have come from similar sources. The fingerprints of PCB congeners in the soil samples were accordant with those of Aroclor1242 and Aroclor1254 type mixtures (Figure S7, Supporting Information). This finding indicated that the main sources of PCBs were commercial PCBs preparations leaking from dismantled electric power capacitors or transformers in these sites (6). However, PCBs were also suspected of being discharged from the combustion of electric wires and printed circuit boards, because the PCB levels were statistically higher ( $P < 0.05$ ) at the simple household recycling workshop sites, where illegal open burning was used, in contrast to those from large-scale recycling plants. A study carried out in Taiwan, which was a former e-waste disposal center in China, also found that incineration of waste electric wires and magnetic cards for metal reclamation could result in soil PCBs contamination (30).

Though PCDD/Fs were not the primary contributor to the TEQs in this study due to the high contribution of dioxin-like PCBs, the high contamination of PCDD/Fs in the e-waste recycling area must also be considered for risk management. Most industrial mixtures of PCBs contain PCDD/Fs, and the level of PCDD/Fs could increase sharply during the combustion of electrical devices containing PCBs (36). Therefore, commercial PCBs are suspected of being a source of PCDD/Fs in the study areas where electric power capacitors and transformers were crudely recycled (often incinerated). However, some features of the PCDD/Fs composition that were expected for indicating the industrial mixture of PCBs, as the contamination sources were not observed in the present study. The levels of PCDFs were lower than those of PCDDs in all of the sampling sites, and tetra-, penta-, and hexa-CDFs were not prevailing, which indicated that there must be other main sources in this area. The dominant 2,3,7,8-substituted PCDD/Fs congeners were OCDD, 1,2,3,4,6,7,8-HpCDD, 1,2,3,4,6,7,8-HpCDF, and OCDF, matching the fingerprint of PCDD/Fs contamination from combustion (37). Therefore, the open burning of PVC-coated copper cables and plastic wastes and atmospheric deposition were also considered as sources of PCDD/Fs in this area.

PAHs only contributed 0.1–5.0% to the  $TEQ_{cal}$ . However, this does not necessarily reflect a low risk of PAHs in this area. In contrast to PCDD/Fs and PCBs, PAHs are less potent CYP1A1 inducers. Nevertheless, they can be normally biotransformed by CYP1A1 into genotoxic metabolites, which subsequently interacted with DNA to initiate carcinogenesis. Furthermore, the 16 PAHs found were predominated by 3–5-ring compounds ranging from 267.0 to 1277.6 ng/g dw with a mean of 576.4 ng/g dw, which was high when compared with other cities in China (7, 35). Many researchers have reported that isomer ratios are useful indicators of PAH sources (12). The results of the present study (Table S1, Supporting Information) suggested a predominantly petro-

genic origin for PAHs in the soil from large-scale recycling plants, but a pyrolytic origin for those from the simple household recycling workshops that carried out e-waste open burning.

## Acknowledgments

This work was supported by the National Natural Science Foundation of China (20607019 and 40590392) and Zhejiang Province Bureau of Science and Technology (2007C23037). We gratefully acknowledge Professor Yongming Luo and Professor Yongguan Zhu from the Chinese Academy of Sciences for their valuable comments on this study. Special acknowledgment is also given to Ruiyang Xiao, Qinghui Huang, Qiulin Zhang, and other members in our group for their great assistance during the research.

## Supporting Information Available

PAH diagnostic ratios in soil from Taizhou; photos of e-waste recycling sites in Taizhou; relationships between TEQ<sub>cal</sub> and TEQ<sub>bio</sub>; TEQ<sub>PCB</sub> and TEQ<sub>bio</sub>, concentrations of indicator PCBs and total PCBs, and concentrations of coplanar PCBs and total PCBs; the composition of PCB homologues; and PCB fingerprints. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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ES071162Z