Enzyme Induction and Acute Endocrine Effects in Prepubertal Female Rats Receiving Environmental PCB/PCDF/PCDD Mixtures

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Air, subsurface soil, and superficial dust from a National Priorities List landfill located in southern Illinois were sampled to determine their potential toxicities. The major components of these landfill extracts were polychlorinated biphenyls (PCBs), with significant amounts of polychlorinated dibenzofurans (PCDFs) and small amounts of polychlorinated dibenzodioxins (PCDDs). The 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxic equivalency factor approach has been proposed to estimate the toxic potency of complex mixtures of chlorinated aromatics for environmental risk assessment. However, most components of environmental residues are nonplanar and do not act as aryl hydrocarbon (Ah) receptor agonists, so there is a great risk of not identifying adverse responses that are not dioxinlike. We used a 2-day prepubertal female rat bioassay to examine multiple biological responses, including both dioxinlike and nondioxinlike effects from these landfill extracts. As expected, both types of effects were detected. The soil and dust extracts produced similar dose–response relationships for 7-ethoxyresorufin *O*-deethylase, 7-pentoxyresorufin *O*-dependentylase, 7-benzyloxyresorufin *O*-debenzylase, and 4-nitrophenol UDP-glucuronyltransferase induction; the dose response for the air extract deviated from the other two extracts. Soil, dust, and air extracts effectively reduced serum total thyroxine (T_4) with similar dose–response relationships, despite the significantly different TCDD toxic equivalent (TEQ) values of these three extracts. Both soil (346 mg PCB/kg) and air (175 mg PCB/kg) extracts caused a greater than 30% increase in uterine wet weight. This study suggests that a more comprehensive approach is required to improve current risk assessment of environmental mixtures. TCDD TEQs reflect only a portion of effects and may especially underpredict effects on T_4. *Key words*: cytochrome P450 induction, environmental mixtures, polychlorinated biphenyls, polychlorinated dibenzodioxins, polychlorinated dibenzofurans, thyroxine, toxic equivalency factor, UDP-glucuronyltransferase, uterotropic responses. *Environ Health Perspect* 104:712–722 (1996)

Polychlorinated biphenyls (PCBs) are widespread environmental contaminants with a broad range of biological activities (*1–4*). Worldwide commercial production declined dramatically in the 1970s and essentially ceased by 1990. However, the level of PCB residues has decreased slowly in various environmental and biological samples since the 1980s (*5–8*). Currently, used electrical equipment and leaking disposal sites continue to be anthropogenic sources for PCBs in the environment. In addition, dispersion of PCBs from point sources to global distribution can occur through atmospheric transport and subsequent deposition (*9–12*). Wildlife and humans still continue to be exposed to PCBs through the environment, and only limited data are available on the biological effects of environmental mixtures. The accurate assessment of the potential hazards from environmental PCB exposures remains a challenge for regulatory agencies and environmental toxicologists.

The most accepted approach for assessing the toxicity of environmental PCDD/PCDF/PCB mixtures uses toxic equivalency factors (TEFs). This approach is based on the fact that TCDD and related TCDD-like compounds elicit their toxicity via a common mechanism of action, i.e., the aryl hydrocarbon (Ah) receptor-mediated mechanism. Using this approach, the potencies of mixtures and environmental samples for TCDD-like effects can readily be estimated by calculating their TEF values from the sums of the product of each individual congener and its TEF (*3,4,13*). In developing TEFs, one of the most responsive parameters is induction of the *CYP1A1* gene, most often determined by measuring ethoxyresorufin-*O*-deethylase (EROD) or aryl hydrocarbon hydroxylase (AHH) activity in cultured cells or hepatic microsomes (*3*).

TCDD TEFs were never intended to reflect other toxic actions (*13*), but their use frequently excluded consideration of other actions (*2*). However, most components of environmental PCB residues are nonplanar and do not act as Ah receptor agonists. These chlorobiphenyls (CBs) do not cause TCDD-like effects. For example, the less chlorinated and *ortho*-chlorinated congeners have low affinities for the Ah receptor and are poor inducers of CYP1A1; but these congeners can induce CYP2B and have a profile of hormone and neurotransmitter disruption distinct from the coplanar Ah receptor agonists (*14–17*). In spite of their lower potencies, these congeners are present in far greater amounts than are coplanar CBs (*1,2,18*). Therefore, it is important to evaluate both Ah receptor-dependent and Ah receptor-independent effects of environmental PCB mixtures to better predict the potential hazards of environmental PCB mixtures.

Chemical analysis of all possible compounds in a mixture is costly, time consuming, and usually incomplete. Therefore, short-term bioassays have been suggested to serve as alternative screening methods in assessing the toxicity of complex environmental mixtures (*19,20*). Currently, most of the short-term bioassays proposed for assessing the toxicity of chlorinated aromatic mixtures are *in vitro* assays and only detect Ah receptor-dependent biological effects, such as the induction of cytochrome P4501A in rat H-4-II-E hepatoma cell lines and in chicken embryo primary hepatocytes (*19–21*). Indeed, these *in vitro* bioassays also show a good correlation with dioxin-inducible effects, i.e., *in vivo* cytochrome P4501A1 induction, body weight loss, and thymic atrophy in mammals (*22,23*) and with embryolethality and deformities in bird embryos and chicks (*24*). However, these bioassays cannot detect Ah receptor-independent effects and ignore possible toxicokinetic interactions *in vivo*.

We developed a short-term *in vivo* bioassay in this laboratory by using prepubertal female rats to examine both Ah receptor-dependent and Ah receptor-independent effects of some *ortho*-chlorinated CBs and Aroclor mixtures (*15,17,25,26*). Compared to short-term *in vitro* bioassays, advantages of this short-term *in vivo* bioassay include: 1) examination of both Ah receptor-dependent and Ah receptor-independent effects simultaneously; 2) account-

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ing for possible early toxicokinetic interactions in the whole animal; 3) evaluation of the acute endocrine-disrupting effects (such as depression of thyroid hormone levels), which usually cannot be measured in an *in vitro* bioassay. The intention is to develop a bioassay, such as this female rat integrated endocrine disruption assay (FRIEDA), which can be used as a rapid screening method for potential biological effects of mixtures before deciding whether more intensive and long-term studies are needed. Therefore, similar but distinct environmental mixtures were sought to further test the FRIEDA.

Air, subsurface soil, and superficial dust and debris from the small but highly contaminated Sangamo landfill in southern Illinois (*27*) were sampled to determine potential toxicities of environmental mixtures. The chemical composition of these extracts has been determined, and the chemical profiles of these extracts have been described elsewhere (*28,29*). In a preliminary study (*29*), the extract of this landfill soil caused both Ah receptor-dependent and Ah receptor-independent responses in prepubertal female rats. Therefore, the present study used the short-term bioassay mentioned above, with slight modifications, to examine enzyme induction, thyroid hormone depletion, and uterotropic effects of the Sangamo landfill soil, dust, and air extracts in an attempt to define the net potency of these environmental extracts with different PCB/PCDF/PCDD profiles.

Materials and Methods

Sample collection. The Sangamo landfill has been inactive since 1964, and access was limited when it was placed on the National Priorities List in 1984 (*27*). For the air samples, 18 separate 24-hr high-volume (34 m^3/hr) samples were collected 15 cm above the surface of the landfill between 16 September and 20 October 1992. The vaporized compounds were collected on 45 g XAD-2 resin downstream from a glass fiber particle filter (*30*). Then dust and surface debris were collected from the site by whisk broom; the next 5–10 mm of soil were removed by scraping with trowels, and finally subsurface soil beneath the dust and debris was collected and sieved (no. 10) into a precleaned stainless-steel bucket. In addition, a reference soil sample was collected from a control site located several kilometers south–southeast of the Sangamo landfill.

Extraction and chemical analysis. Detailed procedures of the extraction, cleanup, and chemical analysis have been described elsewhere (*28*). In brief, for air samples, XAD-2 resins were Soxhlet extracted with 300 ml of acetone:hexane (1:1, v:v) for 24 hr and then with 300 ml of dichloromethane for another 24 hr. The individual extracts of each sample were concentrated by rotary evaporation and then combined and solvent was exchanged to hexane (*30*). Each 100-g soil or dust sample was extracted with 200 ml of acetone:hexane (A:H, 1:1). The pooled extracts were dried over sodium sulfate and vacuum-concentrated at 57°C and were exchanged to hexane. Soil and dust extracts were cleaned of oil by Florisil slurry, and all extracts were subsequently cleaned by alumina (3% deactivated) column chromatography. The refined extracts were subdivided and separate aliquots were transferred to other laboratories for chemical analysis.

Specific PCB congener analyses were conducted by two independent laboratories. At the New York State Department of Health Wadsworth Laboratories (NYSDH), the aliquots were analyzed by gas–liquid chromatography (GLC) with an electron capture detector (ECD) (*31*). At the Illinois Hazardous Waste Research and Information Center, Hazardous Materials Laboratory, the samples were diluted and analyzed directly by GLC, but the effluent was split between the ECD and an ion trap MS. The average PCB concentrations from three methods were 43, 21, and 4 mg/ml for soil, dust, and air extracts, respectively, and the variation was only 20%. However, the compositions in Tables 1 and 2 were based on the GLC-MS results that could identify individual PCB congeners not resolved by GLC-ECD. In addition, PCDFs and PCDDs in these three extracts were analyzed by capillary GLC/low-resolution MS at the NYSDH (*28*). The concentrations of PCDDs were 47.3 µg/ml in the soil extract and 11.4 µg/ml in the dust extract, whereas PCDDs were not detected in the air extract. The concentrations of PCDFs were 761.5 µg/ml in the soil extract, 250.3 µg/ml in the dust extract, and 74.1 µg/ml in the air extract. The TCDD toxic equivalence (TEQ) for TCDD-like actions of the extracts were calculated using TCDD TEFs suggested by Ahlborg et al. (*13*) for PCBs and by Safe (*4*) for PCDDs and PCDFs. In the air extract, quantitation of CB 126 (3,3′,4,4′,5-pentachlorobiphenyl) may be unreliable due to its low concentration; therefore, a conservative TEQ estimation was adopted by using the possibly maximal CB 126 concentration (1 µg/ml) in the air extract for the TEQ estimation.

Even though the PCDF content was high relative to most environmental mixtures (*28*), total PCB still accounts for more than 98.3–99.5% of the chlorinated aromatics in

Table 1. Contents of PCDDs, PCDFs, and PCBs in landfill extracts and summations of toxic equivalencies to 2,3,7,8-TCDD

	Concentration (µg/ml)[a]		
Compounds	Soil	Dust	Air
2,3,7,8-TCDD	0	0	0
Tetra-CDDs	2.9	0.8	0
Penta-CDDs	6.3	0.7	0
Hexa-CDDs	10.9	3.3	0
Hepta-CDDs	14.9	3.3	0
Octa-CDD	12.2	3.4	0
Total PCDDs	47.2	11.5	0
Sum PCDD TEQ (µg TCDD/ml extract)	0.52	0.06	0
2,3,7,8-TCDF	46.1	19.3	3.9
Tetra-CDFs	329.9	109.5	18.3
Penta-CDFs	194.8	68.9	0.3
Hexa-CDFs	104.4	35.5	0
Hepta-CDFs	58.8	12.2	0
Octa-CDF	27.6	5.0	0.6
Total PCDFs	761.6	250.4	23.1
Sum PCDF TEQ (µg TCDD/ml extract)	27	8.96	0.39
Mono-CBs	0	0	<1
Di-CBs	407	41	56
Tri-CBs	17820	4010	2095
Tetra-CBs	19361	8069	1799
Penta-CBs	5682	5323	550
Hexa-CBs	2551	2650	102
Hepta-CBs	940	509	3
Octa-CBs	110	52	0
Nona-CBs	8	5	0
Deca-CB	1	1	0
Total PCBs	46888	20660	4606
Sum PCB TEQ (µg TCDD/ml extract)[a]	1.50	1.55	0.11
TEQ/ml of extract (µg TCDD/ml extract)	29.02	10.57	0.50
TEQ/unit of matrix (µg TCDD/g)	14.51	5.29	0.0001
TEQ concentration (µg TCDD/mg PCBs)	0.62	0.51	0.11

Abbreviations: CDD, chlorinated dibenzodioxins; CDF, chlorinated dibenzofurans; TEQ, toxic equivalents.
[a]See Table 2.

Table 2. PCB congeners in the landfill extracts used for calculating TCDD toxic equivalents

IUPAC no.	Chlorine substitution	TEF[a]	Concentration (µg/ml) Soil	Dust	Air
77	3,3′,4,4′	0.0005	212	110	10.5
105	2,3,3′,4,4′	0.0001	369	370	8.4
114	2,3,4,4′,5	0.0005	18	15	0.6
118	2,3,4,4′,5	0.0001	651	726	27
123	2′,3,4,4′,5	0.0001	26	1	0
126	3,3′,4,4′,5	0.1	12	13	1
156	2,3,3′,4,4′,5	0.0005	95	102	0
157	2,3,3′,4,4′,5′	0.0005	25	22	0
169	3,3′,4,4′,5,5′	0.01	<0.001	0	0
170	2,2′,3,3′,4,4′,5	0.0001	140	90	0
180	2,2′,3,4,4′,5,5′	0.00001	312	146	0
189	2,3,3′,4,4′,5,5′	0.0001	4	3	0

[a]Toxic equivalency factors (TEF). TEF values suggested by WHO (*13*).

Table 3. Dose administered and body weight gains in prepubertal female rats treated with landfill-associated extracts containing PCBs, PCDFs, and PCDDs

Group	Dose[a] (g/kg)	n	Actual dose[b] (mg PCB/kg)	TEQ dose (µg TCDD/kg)	% Body weight gain or loss[c]
Control site	1.5	4	0.004 ± 0.001		7.85 ± 1.84
Soil	0	10	0	0	3.55 ± 0.78
	0.1	5	2.44 ± 0.19	1.51 ± 0.12	4.29 ± 1.68
	0.4	5	9.57 ± 0.69	5.93 ± 0.43	5.91 ± 1.32
	1.5	5	32.44 ± 2.59	20.11 ± 1.61	3.88 ± 0.90
	2.6	5	56.54 ± 3.03	35.05 ± 1.88	1.96 ± 1.05
	4.0	5	86.59 ± 6.16	53.69 ± 3.82	2.90 ± 0.56
	16.1	5	345.60 ± 7.71	214.27 ± 4.78	-3.24 ± 1.26*
Dust	0	6	0	0	4.96 ± 1.72
	1.2	5	12.84 ± 0.62	6.55 ± 0.32	6.27 ± 1.95
	3.7	5	38.44 ± 2.27	19.60 ± 1.16	4.54 ± 0.52
	7.5	5	78.33 ± 3.58	39.95 ± 1.83	3.77 ± 1.88
	36.4	5	382.20 ± 13.14	194.92 ± 6.70	1.40 ± 1.63
Air	0	10	0	0	3.67 ± 1.34
	0.005	5	6.19 ± 0.30	0.68 ± 0.03	5.75 ± 2.43
	0.010	6	12.37 ± 0.14	1.36 ± 0.02	7.97 ± 0.95
	0.016	5	19.39 ± 1.50	2.13 ± 0.16	2.67 ± 1.72
	0.032	5	37.95 ± 1.73	4.18 ± 0.19	4.31 ± 1.12
	0.071	3	83.85 ± 0.56	9.22 ± 0.06	6.48 ± 2.40
	0.148	5	175.43 ± 8.92	19.30 ± 0.98	4.18 ± 1.23

TEQ, toxic equivalents.
[a]Matrix equivalent dose expressed as g of matrix/kg of body weight. One ml of soil or dust extract is equal to 2 g of soil or dust and 1 ml of air extract is equal to 3.67 m^3 of air (4.74 kg of air).
[b]The actual dose is [the amount extract administered)/(individual body weight)], mean ± SE.
[c]Body weight gain = [(weight day 23 - weight day 21)/weight day 21] × 100%, mean ± SE.
*Significantly different from controls by Dunnett's *t*-test, $p \leq 0.01$.

Table 4. Uterotropic response in prepubertal female rats administered landfill-associated extracts containing PCBs, PCDFs, and PCDDs

			Uterotropic effect[a]		
Group	Dose (mg PCB/kg)	n	Uterine weight (mg)[b]	Uterine weight/ body weight (mg/g)	% of control
Control site		4	29.5 ± 1.7	0.47 ± 0.03	101.9 ± 5.9
17β-Estradiol (20 µg/kg)		4	70.5 ± 6.6**	1.13 ± 0.05**	245.7 ± 10.2
Soil	0	10	29.4 ± 1.1	0.49 ± 0.02	100.0 ± 3.6
	2	5	33.5 ± 1.4	0.56 ± 0.02	114.2 ± 3.5
	10	5	33.5 ± 2.0	0.54 ± 0.02	110.9 ± 3.8
	32	5	32.2 ± 1.5	0.60 ± 0.05*	123.1 ± 10.6
	57	5	33.2 ± 2.5	0.54 ± 0.03	110.7 ± 7.0
	87	5	29.8 ± 1.4	0.55 ± 0.02	113.4 ± 4.4
	346	5	39.3 ± 2.2**	0.64 ± 0.02**	131.2 ± 4.9
Dust	0	6	27.9 ± 0.8	0.43 ± 0.01	100.0 ± 2.2
	13	5	36.5 ± 4.4*	0.58 ± 0.07	136.1 ± 17.5
	38	5	32.6 ± 1.7	0.52 ± 0.04	122.5 ± 9.1
	78	5	35.9 ± 4.1*	0.59 ± 0.09	138.9 ± 2.0
	382	5	33.6 ± 1.3*	0.54 ± 0.03	127.6 ± 7.3
Air	0	10	29.9 ± 1.0	0.46 ± 0.01	100.0 ± 2.6
	6	5	36.0 ± 3.2	0.56 ± 0.03*	122.0 ± 6.0
	12	6	35.7 ± 1.8	0.56 ± 0.03*	120.9 ± 5.7
	19	5	34.6 ± 2.8	0.58 ± 0.07	125.9 ± 14.1
	38	5	33.2 ± 3.4	0.53 ± 0.03	115.2 ± 6.7
	84	3	37.7 ± 1.9	0.59 ± 0.02**	128.2 ± 5.2
	175	5	38.2 ± 2.2*	0.63 ± 0.05**	137.7 ± 11.7

[a]Mean ± SE; uterine weights are wet weights.
[b]Absolute wet weight.
*Significantly different from controls by Dunnett's *t*-test or Student's *t*-test, $p \leq 0.05$.
**Significantly different from controls by Dunnett's *t*-test or Student's *t*-test, $p \leq 0.01$.

the extract (Table 1). Because PCB content is the dominant and most often reported value for similar samples, it was considered useful to present total TEQs relative to the total PCB content. This also provides a useful comparison value for relative Ah receptor-dependent and independent effects compared to relative TEQs for the three extracts.

Animals and dosing. Appropriate dilutions of the extracts were semiquantitatively analyzed by GLC-ECD to permit initiation of toxicity studies before the completion of chemical analysis. Table 3 shows the actual doses of each extract used in this study. Although the intent was to formulate the doses of the three landfill extracts at similar PCB concentrations, the actual doses for each extract deviated from target concentrations because of the variation between initial estimation and the final chemical analysis. In addition, the amount of air extract available was limited.

Sprague-Dawley breeder rats were obtained from Harlan (Indianapolis, Indiana). Pups were culled to 8–10 animals per litter on the day of birth (day 0) and were weaned at 21 days of age. Female pups were injected intraperitoneally with landfill extracts dissolved in 0.1 ml corn oil or corn oil alone between 1300 and 1400 hr on day 21 and day 22. A negative control was included for each litter, along with as many representative dose groups as the number of females would permit. Positive controls were included intermittently and agreed with historical uterotropic responses to 17β-estradiol (*15,17,25,26*) (Table 4). PCB-induced mitogenic activity in the uterus had been confirmed to accompany the weight increase in a previous study (*25*), and increased uterine protein content was also confirmed (*17*).

Necropsy and tissue processing. Rats were decapitated between 0900 and 1100 hr on day 23 and blood was collected immediately after decapitation and allowed to clot. The uterus was excised, trimmed of fat, cut at the cervical os, and weighed to the nearest 0.01 mg. The uterotropic effects were determined by comparing ratios of uterine wet weight in milligrams to body weight in grams to control animals. As soon as the uteri were removed, livers were perfused *in situ* with ice-cold 0.05 M Tris-0.15M KCl (pH 7.4), excised, blotted on tissue paper, and weighed followed by homogenization in 12 ml of the same Tris-KCl buffer. Liver microsomes were then prepared as described in Li et al. (*15*). In addition, thymus and adrenal glands were removed and weighed.

Enzyme assays and thyroid hormone analysis. 7-Ethoxyresorufin (EROD) and 7-pentoxyresorufin (PROD) *O*-dealkylation and 7-benzyloxyresorufin (BROD) *O*-debenzylation were determined by a modifi-

cation of the method of Pohl and Fouts (*32*) as previously described in Li et al. (*15*). UDP glucuronyltransferase (UDPGT) activity in the microsomal suspension was measured using 4-nitrophenol (4-NP) and phenolphthalein (PP) as substrates by a modification method of Watanabe et al. (*33*) as described in Seo et al. (*34*). Microsomal protein was determined by the modification of the Lowry method reported by Guengerich (*35*) using bovine serum albumin as a standard. Serum total T_4 was measured by using a radioimmunoassay (RIA) kit (Coat-A-Count) purchased from Diagnostic Products Corporation (Los Angeles, California). The detection limit of the assay was 0.25 µg/dl. All samples were run in duplicate. T_4 assays were conducted at different times with different RIA kits, and comparison with archived serum samples revealed a significant variation between two sets of RIA kits; therefore, serum T_4 was compared to the control animals of each test replicate.

Data analysis. All data are expressed as means ± SE. Results for serum T_4 were calculated by comparing each T_4 value to its own control in the same litter to reduce the variance between two different sets of RIA kits. Bartlett's test was performed to test for variance homogeneity. In case of heterogeneity of variance ($p \leq 0.05$), rank transformations were used (*36*). A one-way analysis of variance (ANOVA) was performed on homogenous data or transformed data for all the endpoints measured for each extract in this study. If a significant result was found, the Dunnett's *t*-test was used to compare treatment groups versus a control group. The Pearson correlation coefficients (r) for enzyme activities and T_4 levels were calculated from each individual rat collectively for all three extracts. The dose–response relationship of T_4 for each landfill extract was evaluated by linear regression analysis and the difference among three extracts was also determined by ANOVA. In addition, all endpoints measured from control site treatment as well as uterotropic responses from 17β-estradiol treatment were compared to the control group from the soil extract treatment by Student's *t*-test.

Results

Chemical Composition of Extracts

The only pesticide detected was *p,p'*-bis-4-chlorophenyl-1,1 dichloroethene (DDE) at less than 100 µg/ml control soil, 130 µg/ml landfill soil, 170 µg/ml landfill dust, and 10 µg/ml landfill air extracts.

The soil extract contained 46,888 µg/ml total PCBs, 761.6 µg/ml total PCDFs, and 47.2 µg/ml PCDDs. The major PCB isomers present in this extract were tri-CBs (37%) and tetra-CBs (42%) (Fig. 1). The dominant congeners were CB 28 (2,4,4'-trichlorobiphenyl), CB 41 (2,2',3,4-tetrachlorobiphenyl), CB 16 (2,2',3-trichlorobiphenyl), CB 22 (2,3,4'-trichlorobiphenyl), CB 52 (2,2',5,5'-tetrachlorobiphenyl), and CB 18 (2,2',5-trichlorobiphenyl). These six congeners accounted for about 45% of the total PCBs in the soil extract. Both CB 77 (3,3',4,4'-tetrachlorobiphenyl) and CB 126 were present in the soil extract at 212 µg/ml (0.45%) and 12 µg/ml (0.03%), respectively (Table 2), but no 2,3,7,8-TCDD was detected. The major PCDDs in the soil extract were hexa-, hepta-, and octa-CDDs (Fig. 1) and 47% of the penta- to octa-CDDs contained the 2,3,7,8 substitution pattern (*28*). The major PCDFs included tetra-CDFs (49%) and penta-CDFs (26%) (Fig. 1); 2,3,7,8-TCDF was present at 46.37 µg/ml (6%) and other 2,3,7,8-substituted congeners accounted for 23% of total PCDFs (*28*). The relative TCDD TEQ concentra-

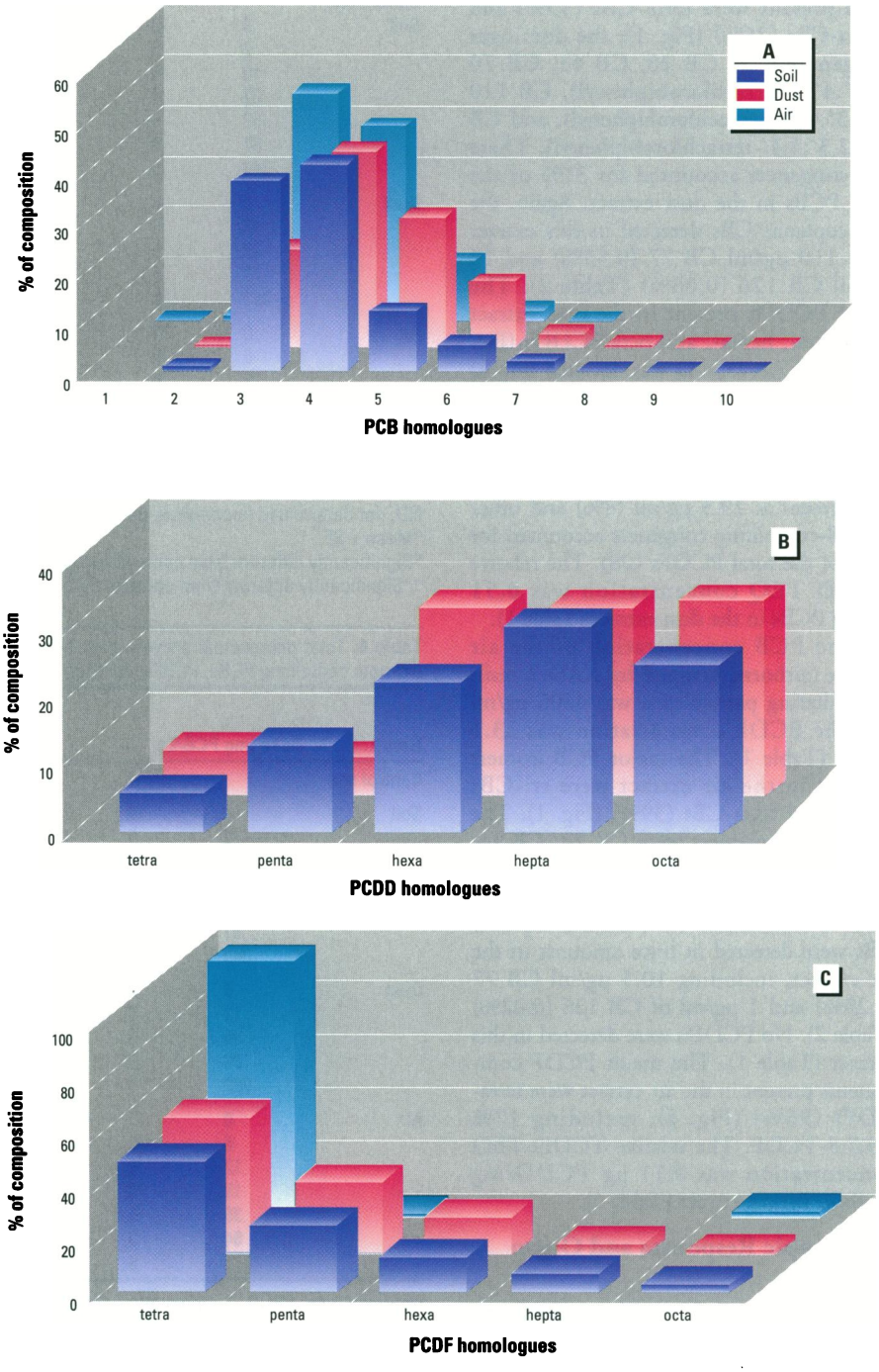

Figure 1. The percent composition of PCB, PCDD, and PCDF homologues in soil, dust, and air extracts.

tion was 0.62 µg TCDD/mg PCBs in the soil extract (Table 1).

The control soil extract contained 0.29 µg/ml total PCBs, 0.96 µg/ml octa-CDF, and no detectable PCDDs (*28*). Traces of polynuclear aromatic hydrocarbons were detected but were not present at concentrations adequate to induce EROD activity.

The PCB concentration of the dust extract was 20,660 µg/ml. The PCDF concentration of the dust extract was 250.4 µg/ml, and the PCDD concentration was 11.5 µg/ml (Table 1). The major PCB isomers present were tetra-CBs (39%) and penta-CBs (26%) (Fig. 1); the dominant congeners were CB 28, CB 41, CB 70 (2,3′,4′,5-tetrachlorobiphenyl), CB 110 (2,3,3′,4′,6-pentachlorobiphenyl), and CB 66 (2,3′,4,4′-tetrachlorobiphenyl). These five congeners accounted for 31% of the total PCBs in the dust extract. Again, the two coplanar CBs detected in this extract were 110 µg/ml CB 77 (0.53%) and 13 µg/ml CB 126 (0.06%) (Table 2). The major PCDDs present in the dust extract were hexa-, hepta-, and octa-PCDDs (Fig. 1); no 2,3,7,8-TCDD was detected but, as with soil, 47% contained the 2,3,7,8-substitution pattern (*28*). The major components of PCDFs included tetra-CDFs (51%) and penta-CDFs (28%) (Fig. 1); 2,3,7,8-TCDF was present at 19.3 µg/ml (8%) and other 2,3,7,8-containing congeners accounted for 19% of the total PCDFs (*28*). The relative TCDD TEQ concentration was 0.51 µg/mg PCBs in the dust extract (Table 1).

The PCB concentration of the air extract (airborne trapped by XAD-2 resin after filtering particulates) was 4606 µg/ml and the PCDF concentration was 23.1 µg/ml (Table 1). The major PCB isomers present in the air extract were tri-CBs (45%) and tetra-CBs (39%) (Fig. 1). The dominant congeners were CB 28, CB 16, CB 52, CB 18, and CB 22. These five congeners accounted for about 47% of the total PCBs in this extract. Two of three coplanar CBs were detected in trace amounts in the air extract, including 10.5 µg/ml CB 77 (0.23%) and 1 µg/ml of CB 126 (0.02%) (Table 2). No PCDDs were detected in this extract (Table 1). The major PCDF components present in the air extract were tetra-CDFs (96%) (Fig. 1), including 17% 2,3,7,8-TCDF. The relative TCDD TEQ concentration was 0.11 µg TCDD/mg PCBs in the air extract (Table 1).

Uterotropic Responses and Organ Weights

Soil extracts caused mild but significant uterotropic responses of 23% and 31% at 32 and 346 mg/kg, respectively (Table 4). Soil extracts also caused significant relative liver weight increases in a dose-dependent manner (Table 5). In addition, there was a decrease in body weight gain at the highest dose, 346 mg PCB/kg (Table 3). Due to the decrease of body weight in the highest dose group, the uterotropic response was also examined by absolute uterine wet weight. The absolute uterine weight in the 346 mg PCB/kg was also significantly higher than controls.

For dust extracts, there were no significant differences in body weight gains (Table

Table 5. Relative organ weights in prepubertal female rats administered landfill-associated extracts containing PCBs, PCDFs, and PCDDs

Group	Dose (mg PCB/kg)	*n*	Relative organ weight[a] Liver weight/ body weight (×100)	Adrenal weight/ body weight (mg/g)	Thymus weight/ body weight (mg/g)
Control site		4	4.36 ± 0.01	0.34 ± 0.01	3.99 ± 0.30
Soil	0	10	4.04 ± 0.08	ND	4.33 ± 0.14
	2	5	4.15 ± 0.10		4.15 ± 0.26
	10	5	4.48 ± 0.13*		4.23 ± 0.21
	32	5	4.75 ± 0.11**		4.37 ± 0.34
	57	5	5.06 ± 0.12**		4.43 ± 0.25
	87	5	5.11 ± 0.15**		4.17 ± 0.25
	346	5	6.51 ± 0.11**		4.27 ± 0.39
Dust	0	6	4.05 ± 0.14	0.33 ± 0.03	4.28 ± 0.17
	13	5	4.53 ± 0.16	0.32 ± 0.02	4.53 ± 0.16
	38	5	5.10 ± 0.18**	0.34 ± 0.02	4.45 ± 0.36
	78	5	5.40 ± 0.20**	0.36 ± 0.08	4.27 ± 0.42
	382	5	6.43 ± 0.20**	0.37 ± 0.02	3.40 ± 0.14
Air	0	10	3.91 ± 0.09	0.34 ± 0.01	4.27 ± 0.10
	6	5	4.33 ± 0.14	0.36 ± 0.02	4.82 ± 0.16
	12	6	4.35 ± 0.08	0.35 ± 0.01	3.92 ± 0.19
	19	5	4.45 ± 0.16	0.35 ± 0.01	4.13 ± 0.24
	38	5	4.68 ± 0.11**	0.37 ± 0.01	4.55 ± 0.33
	84	3	4.65 ± 0.14**	0.34 ± 0.02	4.63 ± 0.30
	175	5	5.57 ± 0.18**	0.40 ± 0.01	4.14 ± 0.23

ND, not determined (incomplete data).
[a]Mean ± SE.
*Significantly different from controls by Dunnett's *t*-test, $p \leq 0.05$.
**Significantly different from controls by Dunnett's *t*-test, $p < 0.01$.

Table 6. Total microsomal enzyme activities in prepubertal female rats administered landfill-associated extracts containing PCBs, PCDFs, and PCDDs

Group	Dose (mg PCB/kg)	*n*	Total microsomal P450 enzyme activities[a] (pmol/min/liver) EROD	PROD	BROD
Control site		4	651 ± 127	68 ± 8	142 ± 20
Soil	0	10	799 ± 181	55 ± 8	194 ± 42
	2	5	4146 ± 3551	239 ± 149	185 ± 101
	10	5	60895 ± 6487**	283 ± 14**	1119 ± 92**
	32	5	107895 ± 14772**	388 ± 43**	1712 ± 288**
	57	5	215685 ± 12429**	535 ± 26**	2310 ± 42**
	87	5	166133 ± 21080**	510 ± 44**	2674 ± 161**
	346	5	317009 ± 34381**	955 ± 55**	9281 ± 230**
Dust	0	6	1002 ± 244	57 ± 14	249 ± 49
	13	5	85542 ± 30263**	278 ± 17**	1488 ± 129**
	38	5	130024 ± 13327**	472 ± 13**	2178 ± 214**
	78	5	162340 ± 14725**	613 ± 45**	2327 ± 334**
	382	5	284322 ± 19266**	793 ± 39**	8484 ± 580**
Air	0	10	1114 ± 317	71 ± 14	260 ± 45
	6	5	7920 ± 2011**	134 ± 16	337 ± 71
	12	6	9874 ± 2407**	229 ± 37*	768 ± 140*
	19	5	27557 ± 3022**	292 ± 32**	881 ± 200**
	38	5	58489 ± 4269**	418 ± 52**	1591 ± 245**
	84	3	115883 ± 13339**	306 ± 22**	4821 ± 221**
	175	5	228313 ± 30069**	875 ± 10**	10497 ± 603**

Abbreviations: EROD, 7-ethoxyresorufin-*O*-deethylase; PROD, 7-pentoxyresorufin-*O*-dependentylase; BROD, 7-benzyloxyresorufin-*O*-debenzylase.
[a]Mean ± SE.
*Significantly different from controls by Dunnett's *t*-test, $p \leq 0.05$.
**Significantly different from controls by Dunnett's *t*-test, $p \leq 0.01$.

3), thymus weights, or adrenal gland weights (Table 5). Although absolute wet uterine weights were significantly higher than controls, differences in relative weights were variable and not statistically significant (Table 4). Relative liver weights increased in a dose-dependent manner to 159% controls in the highest dose group (Table 5).

For air extracts, there were no differences in body weight gain during the 2-day treatment (Table 3). Mild but significant increases in relative uterine wet weights were observed at the lower doses (21–22%) and at the highest doses (28–38%) (Table 4). Relative liver weights increased significantly in a dose-dependent manner to 142% of controls at 175 mg PCB/kg (Table 5). On the other hand, there were no marked changes in relative thymus weights or adrenal gland weights at any dose (Table 5).

Serum Total T_4 Levels

Decreases in serum T_4 were plotted against both TCDD TEQs (Fig. 2A) and total PCB (Fig. 2B). The relative potency of the air extract was similar to soil and dust based on total, but greater than soil and dust when based on TCDD TEQs (Fig. 2).

At 2 mg PCB/kg, serum total T_4 was not affected by soil extracts. The serum total T_4 started to decline significantly from 62% of control values at 32 mg PCB/kg to less than 15% of control values at 346 mg PCB/kg ($r = 0.989$, $p<0.001$; Fig. 2).

Serum total T_4 decreased from 94% of control at 10 mg PCB/kg to 15% of control levels at 382 mg PCB/kg from dust extracts ($r = 0.985$, $p<0.001$; Fig. 2). For air extracts, serum total T_4 increased significantly at 12 mg PCB/kg, then declined precipitously in a dose-dependent manner to less than 25% of control values at the highest dose (175 mg PCB/kg; $r = 0.892$, $p<0.001$; Fig. 2). There were no significant differences among the slopes of the three extracts by ANOVA.

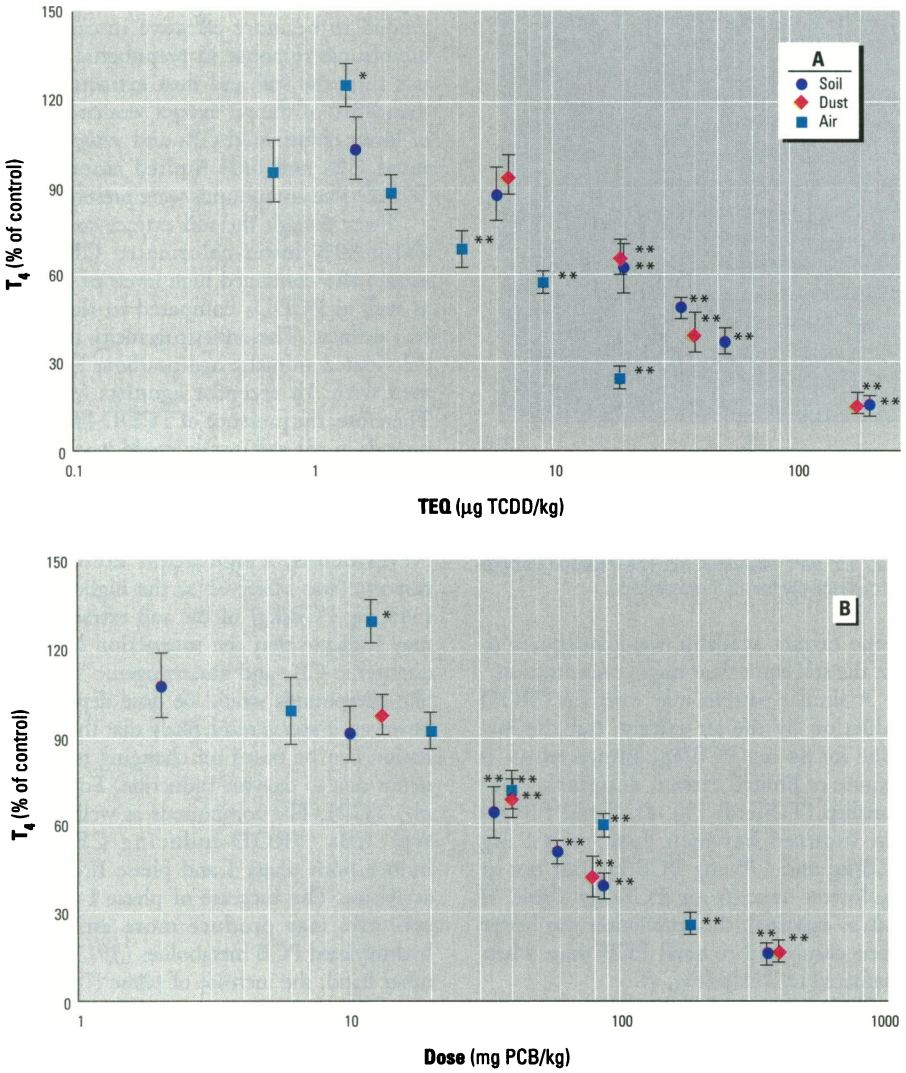

Figure 2. Relative serum total T_4 in prepubertal female rats dosed with landfill extracts. (A) Relative serum total T_4 versus TEQ (µg TCDD/kg; $r = 0.845$); and (B) relative serum total T_4 versus PCB (mg PCB/kg; $r = 0.854$). Each symbol represents the mean of a dose group, bars represent SE, and asterisks indicate a significant difference from control values. The Pearson correlation coefficient (r) was calculated from each individual rat collectively for all three extracts.

Microsomal Enzyme Activities

The total hepatic P450 enzyme activities versus PCB concentrations were calculated (Table 6), and the specific enzyme activities versus TEQ and PCB concentrations for EROD and PROD are shown in Figures 3 and 4, respectively. Sangamo landfill soil extracts induced all three P450 activities in a dose-dependent manner (Table 6). Specific EROD was increased 4-fold at 2 mg PCB/kg and 173-fold at 346 mg PCB/kg (Fig. 3), whereas total EROD was induced 5-fold at 2 mg PCB/kg and 397-fold at 346 mg PCB/kg (Table 6). Both PROD and BROD were significantly increased from 10 mg PCB/kg to 346 mg PCB/kg, but not at the lowest dose (2 mg PCB/kg) (Table 6). At 346 mg PCB/kg, total PROD and BROD were increased 17-fold and 48-fold, respectively (Table 6). The total UDPGT activity versus PCB concentration was calculated for Table 7. Total 4-nitrophenol UDPGT activities were significantly induced from 10 mg PCB/kg to 346 mg PCB/kg. Specific phenolphthalein UDPGT was slightly increased in all soil extract-dosed groups, whereas total phenolphthalein UDPGT was significantly induced from 10 mg PCB/kg to 346 mg PCB/kg due to increased liver weights in these dose groups (Table 7).

The control site soil extract from the non-PCB-containing landfill was only tested at a single dose with the landfill soils. It did not induce P450 enzyme activities (Table 6) or UDPGT activities (Table 7) compared to the landfill soil controls.

For dust extracts, all three P450 enzyme activities were induced in a dose-dependent manner (Table 6). Total EROD activity was increased about 86-fold at 13 mg PCB/kg and 284-fold at 382 mg PCB/kg (Table 6). Total PROD was increased about 5-fold at 13 mg PCB/kg and 14-fold at 382 mg PCB/kg, and total BROD was increased 6-fold at 13 mg PCB/kg and 34-fold at 382 mg PCB/kg (Table 6). The dose response for specific EROD and PROD activities were similar to those for the soil extract (Figs. 3 and 4). Total 4-nitrophenol UDPGT activities were significantly induced at 38, 78, and 382 mg PCB/kg (Table 7). Like the soil extract, specific phenolphthalein UDPGT was only slightly increased in all dust extract-dosed groups, but total phenolphthalein UDPGT was significantly increased at 38, 78, and 382 mg

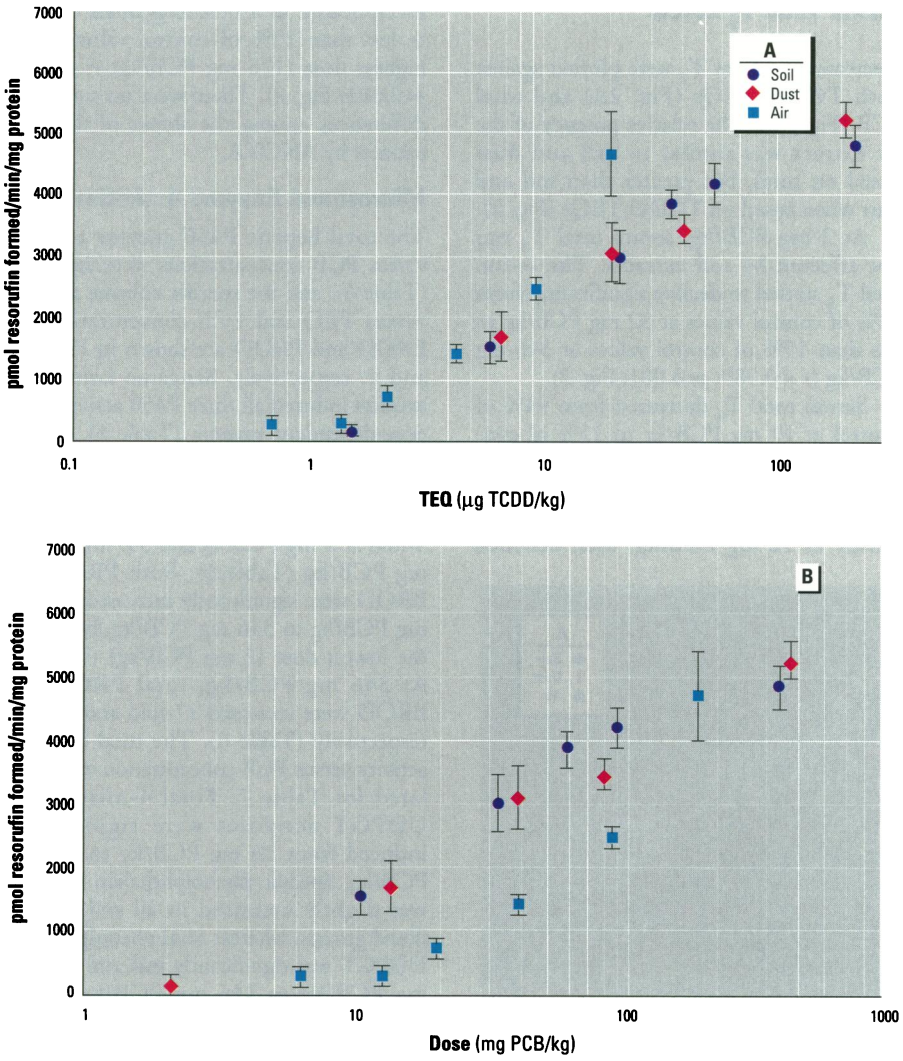

Figure 3. The specific EROD activities versus doses expressed as (A) TEQ concentrations (µg TCDD/kg; r = 0.902); and (B) as PCB concentrations (mg/kg; r = 0.867) in prepubertal female rats administered landfill extracts. Each symbol represents the mean of a dose group and bars represent SE. The Pearson correlation coefficient (r) was calculated from each individual rat collectively for all three extracts.

PCB/kg because of increased liver weights at these doses (Table 7).

Like the other two extracts, all three P450 enzyme activities were induced by air extracts. Total EROD activity was significantly increased about 7-fold at 6 mg PCB/kg and continued to increase to 205-fold at 175 mg PCB/kg (Table 6). Induction of EROD by the air extract was less than that by the soil and dust extracts at lower PCB concentrations; however, when normalized to TEQs for all three extracts, specific EROD activity was similar for all three extracts (Fig. 3). Nevertheless, the pattern of dose response for EROD induction by the air extract appears to be markedly different from induction by the soil extract: at the higher doses, induction by air has an upward inflection, whereas that by soil is trending toward a plateau (Fig. 3). Unfortunately, the amount of airborne extract available was inadequate to test the soil equivalent high concentration.

A similar pattern may exist for PROD induction by the air extract, but the low value for 84 mg PCB/kg, limited to n = 3 because of limited extract, distorts the relationship (Table 6; Fig. 4). Total PROD was induced between doses of 12 mg PCB/kg and 175 mg PCB/kg, but not at the lowest dose (6 mg PCB/kg; Table 6) and, as expected, the correlation was better when compared to total PCB than when compared to TEQs (Fig. 4).

BROD was induced in a dose-dependent manner by the air extract from 6 mg PCB/kg to 175 mg PCB/kg and to a greater extent than by the other two extracts (Table 6). Total 4-nitrophenol UDPGT activities were significantly induced at 12, 38, 84, and 175 mg PCB/kg. Like the other two extracts, specific phenophthalein UDPGT activities were not significantly induced at any dose, but total phenophthalein UDPGT was significantly increased at 38 and 84 mg PCB/kg (Table 7).

Discussion

Acute Endocrine Effects

The estrogenicity of polychlorinated aromatic hydrocarbons is not mediated via the Ah receptor and therefore is not a TCDD-like effect. Estrogenicity, as measured by the uterotropic response, is characteristic of some lower chlorinated CBs such as CB 18 (*26*) and of some nonplanar *ortho*-substituted CBs such as CB 47 (*17*), CB 52 (*25*), and CB 153 (*15*). Coplanar, dioxin-like compounds tend to be antiestrogenic (*37,38*) and can decrease the response to estrogens (*25*). Therefore, it is not surprising that the air extract with a low TEQ tended to be more effective in causing a uterotropic response in prepubertal female rats than the soil and dust extracts. More than 85% of the air extract was composed of lower-chlorinated CBs and *ortho*-substituted CBs and only limited amounts of TCDD-like compounds were present.

Even though the soil extract contained about 80% lower-chlorinated CBs, this extract also contained high levels of PCDFs as well as PCDDs compared to the other two extracts. The antiestrogenicity of polychlorinated aromatic hydrocarbons is associated with Ah receptor agonists (*37,38*). Therefore, the presence of TCDD-like compounds in the soil extract could have antagonized the weak estrogenic effects of those lower-chlorinated PCBs. This may explain the lack of estrogenicity in the soil extract. Nevertheless, a significant uterotropic response was observed at the highest dose (345 mg PCB/kg) of the soil extract. This may indicate that the interaction between estrogenic CBs and antiestrogenic TCDD-like compounds would be dose dependent; however, it seems more likely that the explanation can be based on changing toxicokinetics due to enzyme induction. For example, TCDD-like compounds as well as PB-type CBs (PROD-inducing CBs) can induce both phase I and phase II enzyme activities. The increase of phase I enzyme activities may produce more estrogenic hydroxylated PCB metabolites (*39*). On the other hand, the increase of phase II enzyme activities can enhance the elimination of these estrogenic hydroxylated PCB metabolites. Therefore, a balance between bioactivation and inactivation processes can influence the estrogenic activity and estrogenic/antiestrogenic balance of a mixture. However, total phase II UDPGT induction was about equal to PROD induc-

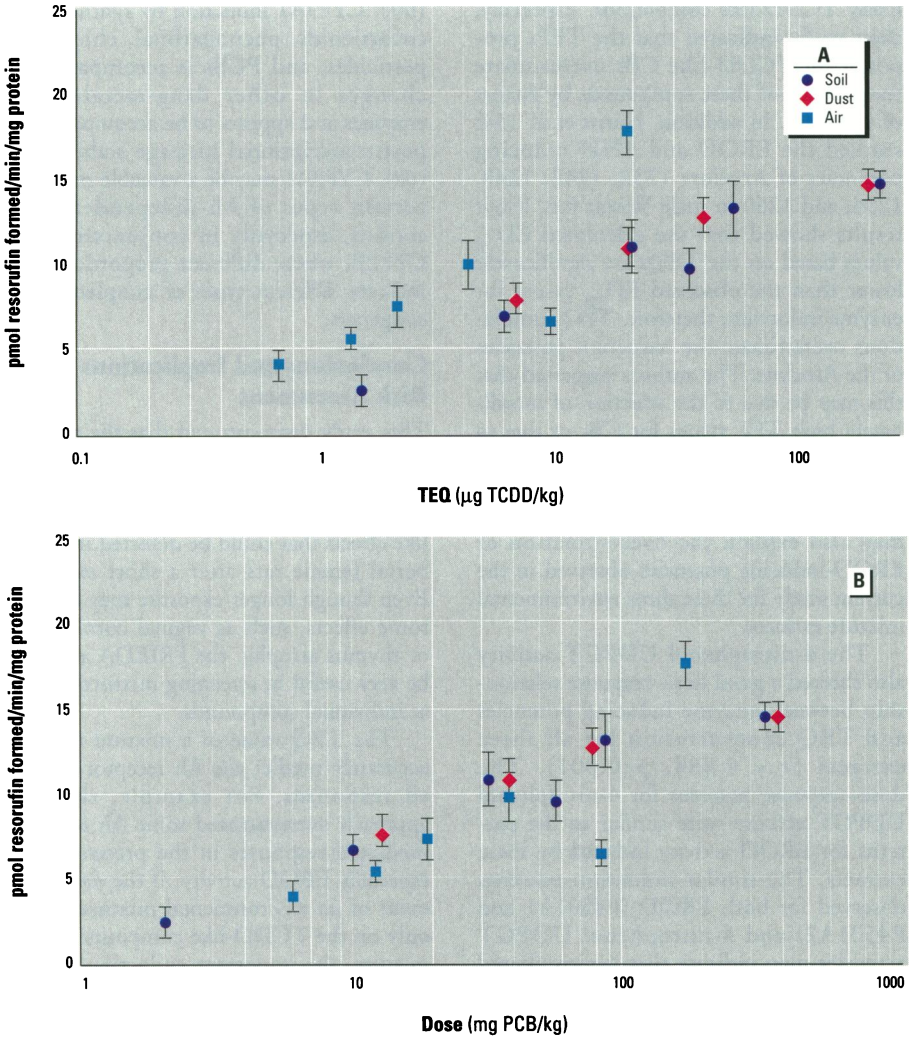

Figure 4. The specific PROD activities versus doses expressed as (A) TEQ concentrations (µg TCDD/kg; r = 0.744; and (B) as PCB concentrations (mg/kg; r = 0.806) in prepubertal female rats administered landfill extracts. Each symbol represents the mean of a dose group and bars represent SE. The Pearson correlation coefficient (r) was calculated from each individual rat collectively for all three extracts.

Table 7. Total microsomal UDPGT activities in prepubertal female rats administered landfill-associated extracts containing PCBs, PCDFs, and PCDDs

Group	Dose (mg PCB/kg)	n	Total microsomal UDPGT activities (nmol/min/liver)[a] 4-NP	PP
Control site		4	651 ± 80	427 ± 55
Soil	0	10	501 ± 74	242 ± 36
	2	5	687 ± 133	316 ± 25
	10	5	1827 ± 119**	486 ± 37
	32	5	2533 ± 286**	493 ± 46
	57	5	4459 ± 253**	584 ± 34**
	87	5	4162 ± 481**	524 ± 96
	346	5	7873 ± 355**	664 ± 59
Dust	0	6	757 ± 146	267 ± 85
	13	5	1819 ± 227	432 ± 56
	38	5	3641 ± 394**	609 ± 63*
	78	5	4186 ± 298**	659 ± 67**
	382	5	8139 ± 416**	543 ± 86*
Air	0	10	617 ± 95	312 ± 52
	6	5	959 ± 71*	375 ± 33
	12	6	1274 ± 84**	340 ± 36
	19	5	1344 ± 168**	513 ± 75*
	38	5	2305 ± 190**	591 ± 34**
	84	3	3029 ± 696**	577 ± 61*
	175	5	5152 ± 631**	464 ± 34

Abbreviations: UDPGT, UDP-glucuronyltransferase; 4-NP, 4-nitrophenol; PP, phenolphthalein.
[a]Mean ± SE.
*Significantly different from controls by Dunnett's t-test, $p \leq 0.05$.
**Significantly different from controls by Dunnett's t-test, $p \leq 0.01$.

tion, lower than BROD induction, and much less than the degree of induction of EROD activity.

A more likely explanation can be based on the fact that coplanar CB 77 and some mono-*ortho* CBs are substrates for the highly-induced EROD, while *ortho*-nonplanar CBs are substrates for the less profoundly induced PROD (*40*). At the higher doses of the soil extract, the disproportionate increase in EROD activity would be expected to reduce the effective *in vivo* levels of antiestrogenic non-*ortho* and mono-*ortho* coplanar compounds, permitting the expression of estrogenicity by noncoplanar compounds. In humans exposed to higher levels of PCBs (i.e., chloracne patients), the more responsive CYP1A induction results in lower residues of coplanar (CBs 37, 77, and 126) and mono-*ortho* (CBs 28, 70, 105, 118, and 156) congeners than in humans exposed to ambient PCBs (*41*). In the same comparison, weakly estrogenic CBs 18, 47, 52, and 153 are found at higher levels in the chloracne patients, as would be expected. In summary, balance between bioactivation and inactivation and more rapid metabolism of coplanar antiestrogens may explain the significant uterotropic response at the highest dose (346 mg PCB/kg) of the soil extract. The possible mechanisms involved need to be further investigated in order to predict the possible estrogenicity and/or antiestrogenicity of environmental mixtures during chronic exposure.

Most PCBs appear to depress serum total T_4 (*42–44*). Both TCDD-like CBs and non-TCDD-like CBs can depress rat serum T_4 (*34,44–46*). In fact, there are multiple mechanisms by which PCBs can affect thyroid hormone homeostasis, and both TCDD and PCB effects on T_4 are species, stage, and time dependent. PCBs could directly affect thyroid hormone synthesis or release in thyroid glands (*43,47*). In addition, PCBs can indirectly influence thyroid function via either enhanced thyroid hormone metabolism by UDPGT induction and increased bile flow (*48–50*) or decreased plasma T_4 levels through enhanced metabolism and excretion after displacement of T_4 from its carrier protein by hydroxylated PCB metabolites (*51,52*). In this study, all three extracts effectively depressed serum T_4 levels to similar extents in immature female rats, even though the PCB congener composition in these extracts were varied and there was a more than five-fold difference in their TEQ values based on µg TCDD/mg PCB. This indicates that both TCDD-like compounds (such as PCDFs and coplanar CBs) and *ortho*-substituted CBs (such as PROD-inducing CBs) can effectively depress serum T_4. A recent model suggests that Ah receptor-mediated T_4 depletion by TCDD is monodimensional, depending mainly on UDPGT induction (*53*). However, different combinations of mechanisms that influence thyroid hormone homeostasis must be considered for different PCB mixtures. If only the effect of

TCDD-like compounds in a mixture, such as the TEQ value of a mixture, is considered, the effect of a PCB mixture on thyroid hormone homeostasis may be underestimated (Fig. 2).

Thymic atrophy, an Ah receptor-mediated response, was not observed for any of the three extracts in this study. Because of the significant P4501A1 induction, a sensitive Ah receptor-mediated response observed for all three extracts, the lack of thymic atrophy in this study was probably due to the relatively short exposure time (44 hr). Nevertheless, Harris et al. (*54*) examined thymic atrophy in immature Wistar rats treated with Aroclors 1232, 1242, 1248, 1254, and 1260 (10, 40, 160, 480, and 2000 mg/kg) measured 14 days after treatment. Thymic atrophy was not observed in any of the dose groups. This may indicate that thymic atrophy is not a sensitive indicator in this short-term bioassay for exposure to Ah receptor agonists, at least when present in a mixture.

Enzyme Induction

EROD activity is one of the most sensitive indicators of exposures to Ah receptor agonists (*3,4*). All three extracts significantly induced EROD activities in prepubertal female rats in a dose-dependent manner. As shown in Figure 3, there was a good dose-response relationship for EROD-specific activity when concentrations of the three extracts were expressed as TEQ concentrations (r = 0.902, p<0.001), but the lower TEQ air extract was clearly less potent at lower concentrations when plotted against total PCB (Fig. 3). In a previous study using the same bioassay conditions (*55*), EROD activity was 1142 pmol/min/mg protein at 1.6 µg CB 126/kg (TEQ = 0.16 µg TCDD/kg) and 4315 pmol/min/mg protein at 65.5 µg CB 126/kg (TEQ = 6.55 µg TCDD/kg). In the present study, EROD activity was only 1500 pmol/min/ mg protein at 10 mg PCB/kg (TEQ = 6.35 µg TCDD/kg) in the soil extract-treated group and 1705 pmol/min/mg protein at 13 mg PCB/kg (TEQ = 7.45 µg TCDD/kg) for the dust extract-treated group. Thus, the EROD induction caused by these extracts was lower than expected based on their TEQ values; therefore, calculated TEQs for these extracts would overestimate their EROD inducing potencies. Conversely, EROD activity alone would underestimate the TEQs.

De Vito et al. (*56*) compared the ability of various PCBs, PCDFs, and TCDD to induce EROD activity in female B6C3F$_1$ mice after 4 weeks of treatment. Their results showed that the present TEFs do not reliably predict induction potency for many TCDD-like compounds. Especially, their study indicated that the TEFs proposed for TCDD-like CBs overestimate the potency of these compounds by factors of 10–1000. In addition, Harris et al. (*54*) studied the EROD and AHH inducing potencies of Aroclors 1232, 1242, 1248, 1254, and 1260 in male Wistar rats. Their results showed that the calculated ED_{50} values based on the TEQs are significantly lower than the observed ED_{50} values for enzyme induction; therefore, TEQ summations overestimate the induction potencies of the Aroclors. The authors suggested that this may be due to the selection of inordinately high TEF values for CBs or due to the possible antagonistic interactions between the coplanar and mono-*ortho* coplanar CBs and other CBs. These factors may also explain the overestimation of EROD-inducing potencies observed in the current study for these three environmental mixture extracts.

The 4-nitrophenol UDPGT activity also showed a good dose–response relationship between enzyme inducing potencies and TEQ concentration for all three extracts (r = 0.884, p<0.001). The dose–response patterns for 4-nitrophenol UDPGT activity were similar to the patterns for EROD activity induced by these extracts. The similar induction patterns observed for both EROD (P4501A1 and P4501A2) and 4-nitrophenol UDPGT activities may indicate that 4-nitrophenol UDPGT induction can also be used for an indicator of Ah receptor-mediated responses, even though 4-nitrophenol is a substrate for several UDPGTs. However, the degree of 4-nitrophenol UDPGT induction was much less than EROD induction in these extracts. The mild induction of phenolphthalein UDPGT was only apparent if total liver activity was considered.

Neither PROD nor BROD activities are induced by TCDD-like compounds in rats. The air extract was a more potent inducer of PROD and BROD activities than soil or dust extracts at the same TEQ dose level (Fig. 4; Table 6). Even though the patterns of PROD and BROD induction were more similar for the three extracts when expressed as PCB concentrations, BROD activity was still more highly induced by the air extract (Table 6). BROD activity can be regarded as a measurement of CYP2B and CYP3A induction (*57,58*), whereas PROD activity is more specific as a measurement of CYP2B induction. The prototype inducer for CYP3A1 is pregnenolone 16α-carbonitrile (*59*); however, nonplanar PCBs also induce CYP3A1, and the structure–activity relationships are different *in vivo* than *in vitro* (*60*). CYP3A1 induction by synthetic glucocorticoids, phenobarbital, chlorinated pesticides, and PCBs is accompanied by changes in other drug-metabolizing enzymes and appears to be accompanied by posttranscriptional message stabilization (*61*). CYP3A1 may be a valuable marker of certain types of Ah-independent PCB actions, especially in conjunction with CYP2B, where different proportions may indicate different types of nonplanar PCB congeners.

Conclusions and Implications for Risk Assessment

This study demonstrated that the environmental mixtures containing mainly PCBs with significant proportions of PCDFs caused both TCDD-like and non-TCDD-like effects that could be detected in prepubertal female rats after a short exposure. Even though longer exposure may enhance some effects, such as vaginal cornification or thymus atrophy, the FRIEDA assay can be very useful in screening mixtures as well as individual compounds.

The TEQ value of a mixture may not accurately predict the Ah receptor-mediated responses. For example, the TEF approach overestimated some Ah receptor-mediated responses in the present study, especially EROD activity. If the risk assessment of an environmental mixture focuses only on the TCDD-like compounds in the mixture, the important endocrine-disrupting effects of a mixture could be underestimated. For example, total serum T_4 was effectively depressed by the three extracts at similar PCB levels despite large differences (sixfold) among the relative TEQs of the three extracts when expressed as micrograms TCDD per milligram PCB in the present study. Therefore, the thyroid hormone depression by air extract would be underestimated based on its low TEQ compared to the other two extracts based on the TEF approach.

In summary, the different matrices from the same environmental source not only can vary widely in their congener compositions, but also differ significantly in their net biological effects. Furthermore, it is important to note that humans and wildlife are exposed to profiles of PCBs/PCDFs/PCDDs not reflected by profiles in food or human tissues. Transient exposure to airborne PCBs, for example, would superimpose a higher proportion of lower-chlorinated and readily metabolized congeners onto existing residues; thus, effects due to these congeners and/or their metabolites might be manifest at a later developmental stage when evidence of exposure (i.e., residues of parent CB) would no longer be apparent.

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