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Relative Potencies of Individual Chlorinated and Brominated Polycyclic Aromatic Hydrocarbons for Induction of Aryl Hydrocarbon Receptor-Mediated Responses

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Chlorinated and brominated polycyclic aromatic hydrocarbons (ClPAHs and BrPAHs) occur as pollutants in the environment. Nevertheless, there is little information available regarding the toxic effects of ClPAHs and BrPAHs. The potencies of 19 individual ClPAHs and 11 individual BrPAHs to induce aryl hydrocarbon receptor (AhR)-mediated activities (i.e., dioxin-like toxicity), relative to the potency of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), were determined in vitro by use of a recombinant rat hepatoma cell (H4IIE-*luc*) assay. Several ClPAHs elicited AhR-mediated activity; the relative potencies (RePs) of 6-monochlorochrysene, and 7-monochlorobenz[*a*]anthracene were 2.6×10^{-5} and 6.3×10^{-6}, respectively. Among BrPAHs, 7-monobromobenz[*a*]anthracene and 4,7-dibromobenz[*a*]anthracene had the highest RePs, 2.1×10^{-5} and 2.3×10^{-5}, respectively. None of the chlorinated or brominated anthracene or fluorene compounds elicited AhR-mediated activity at the concentrations tested. We developed a structure−activity relationship for AhR-mediated potencies of ClPAHs. The RePs of ClPhe and ClFlu (low-molecular-weight ClPAHs) were directly proportional to the compounds' degrees of chlorination. The RePs of higher-molecular-weight ClPAHs (≥4-rings) were lower than those of the corresponding parent PAHs. The RePs of BrPAHs were higher than the RePs of the corresponding ClPAHs. For instance, 6-BrBaP was more potent than 6-ClBaP and 7-BrBaA was more potent than 7-ClBaA. The RePs determined in this study were applied to literature concentrations of Cl- and Br-PAHs in environmental samples, to calculate dioxin-like toxicities, as toxic equivalents (TEQs). The TEQs of ClPAHs calculated using the concentrations of individual ClPAHs were 4.6 pg-TEQ/g in fly ash, 0.015 fg-TEQ/m^3 in automobile exhaust, and 0.085 fg-TEQ/m^3 in urban air. 6-ClChr accounted for 80% of the total ClPAHs-TEQs in fly ash. This is the first in vitro study to report AhR-mediated activities of Cl- and Br-PAHs relative to the activity of TCDD.

Introduction

Chlorinated polycyclic aromatic hydrocarbons (ClPAHs) are widespread environmental pollutants. ClPAHs are structurally similar to other halogenated hydrocarbons (HAHs) such as polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs). Although the environmental fates and effects of several HAHs have been studied in detail for over 30 years, little is known about the fates or effects of Cl- and Br-PAHs. Polychlorinated naphthalenes (PCNs) are one class of ClPAHs that have been studied and found to be persistent and bioaccumulative, and to induce toxic effects that are mediated through the aryl hydrocarbon receptor (AhR) (*1*−*3*). Relative potencies (RePs) of individual PCN congeners for induction of dioxin-like responses in bioassays have been reported (*4*−*6*). ClPAHs with three to five aromatic rings have been reported to occur in automobile exhaust (road tunnel air) (*7*), sediment (*8*), snow (*9*), and kraft pulp mill wastes (*10, 11*). However, because of the lack of purified, individual ClPAH analytical standards, accurate quantification was not previously possible, and the environmental fates and toxicities of these compounds have not been examined in detail. Recent synthesis and purification of individual ClPAHs in our laboratory made the congener-specific analysis of ClPAHs possible (*12*−*14*). The sources of ClPAHs can be related to various reactions in which chlorine and aromatic precursors exist (e.g., automobile engine combustion, chlor-alkali processes, municipal waste incineration). The occurrence and profiles of 20 ClPAHs and 11 brominated PAHs (BrPAHs) in municipal/hazardous/industrial waste incinerators have been reported in our previous study (*15*).

In terms of the biological effects of Cl- and Br-PAHs, previous studies have reported the mutagenicity of ClPAHs to *Salmonella typhimurium* TA98 and TA100 (*16, 17*). As is true for other HAHs, the major mechanism of action of Cl- and Br-PAHs has been related to their ability to bind to and activate the AhR, which is a cytosolic, ligand-activated transcription receptor (*18*−*20*). The most characterized pathway involves translocation of the activated AhR to the nucleus, where it binds with the AhR nuclear translocator protein (Arnt) to form a heterodimer. Binding of the heterodimer leads to transcriptional modulation of genes that contain a xenobiotic responsive element (*19*). Previously, the AhR-mediated activities of several ClPAHs had been determined using a yeast assay system (YCM3 cell), and the potencies were reported relative to the potency of benzo[*a*]pyrene (BaP) (*21*). Prior to this study, no information on the potencies of Cl- and Br-PAHs relative to that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) was available.

There is interest in assessing the risks and potential adverse effects of dioxin-like compounds, including Cl- and Br-PAHs, but such assessments have been hampered by a lack of analytical standards and toxicological information. In this study, the dioxin-like toxic potencies of individual Cl- and Br-PAHs were determined in an in vitro bioassay utilizing recombinant rat hepatoma (H4IIE-*luc*) cells (*22, 23*). We subsequently applied the RePs of Cl- and Br-PAHs derived in the present study to the concentrations reported for these

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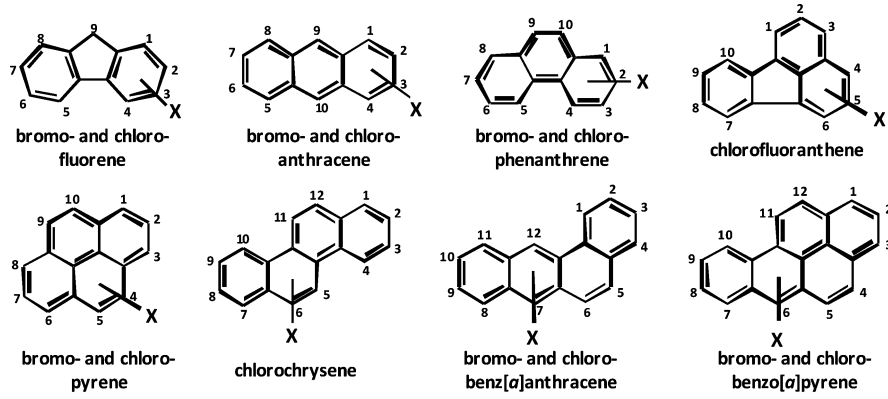

FIGURE 1. Chemical structures of brominated and chlorinated polycyclic aromatic hydrocarbons assayed in this study. X indicates bromine or chlorine atom.

compounds in environmental samples (*7, 14, 15*), in order to calculate dioxin-like toxic equivalents (TEQs).

Materials and Methods

Chemicals. Nineteen individual ClPAHs, representing mono- through trichloro PAHs, were tested for their individual abilities to induce AhR-mediated activity. The compounds studied included chlorofluorene (ClFle), chlorophenanthrene (ClPhe), chloroanthracene (ClAnt), chlorofluoranthene (ClFlu), chloropyrene (ClPyr), chlorochrysene (ClChr), chlorobenz[*a*]anthracene (ClBaA), and chlorobenzo[*a*]pyrene (ClBaP). In addition, 11 individual BrPAHs representing mono- and di-bromoPAHs were tested, including bromofluorene (BrFle), bromophenanthrene (BrPhe), bromoanthracene (BrAnt), bromopyrene (BrPyr), bromobenz[*a*]anthracene (BrBaA), and bromobenzo[*a*]pyrene (BrBaP). Chemical structures and abbreviations of individual ClPAH and BrPAH analyzed in this study are shown in Figure 1 and Table 1. Standards of 2-ClAnt, 9-ClAnt, and 9,10-BrAnt were purchased from Aldrich (St. Louis, MO). Standards of 9-BrAnt, 9-BrPhe, and 7-BrBaA were purchased from Tokyo Chemical Industry (Tokyo, Japan). 9-ClPhe was obtained from Acros Organics (Geel, Belgium). The remaining ClPAHs were synthesized by the authors at the University of Shizuoka following published procedures (*12, 13*). For BrPAHs, the bromination was performed with *N*-bromosuccinimide in place of *N*-chlorosuccinimide, which was used in the synthesis of ClPAHs. The crude products were purified by high-performance liquid chromatography (HPLC). The purities of the synthesized standards of Cl- and Br-PAHs were >95% (confirmed by gas chromatography−mass spectrometric analysis; GC/MS).

Cell Culture and Bioassay. H4IIE-*luc* cells are rat hepatoma cells that are stably transfected with the luciferase gene under the control of dioxin-responsive elements (*23*). Culture conditions for H4IIE-*luc* cells have been described in detail previously (*22*). In brief, H4IIE-*luc* cells were cultured in 100-mm disposable Petri plates and incubated at 37 °C in a humidified 95:5 air: CO_2 atmosphere. Cells for the bioassay were plated into the 60 interior wells of 96-well culture plates (250 μL/well) at a density of approximately 18 000 cells/well. Cells were incubated overnight prior to dosing. Test wells were dosed with 2.5 μL of the standard solutions of individual Cl- and Br-PAHs prepared in isooctane. Luciferase activity was measured 3 days after dosing. For the screening purpose, all test compounds were prepared with two concentrations of 3.3 and 30 μg/mL and tested. Selected compounds were further determined to obtain full-dose−response curves where dosed at six concentrations ranging from 0.12 to 30 μg/mL at 3-fold dilutions (0.12, 0.37, 1.1, 3.3, 10, and 30 μg/mL). Control wells were dosed with 2.5 μL of isooctane. A minimum of three control wells and three blank wells were tested on each plate. Samples were also tested using three replicate wells.

Data Analysis. When determining ReP values, two assumptions were made. Thus to ensure accurate values for the ReP, these assumptions were tested. First, it was assumed that the efficacy (maximum response achieved) is equal for the compound of interest and that of the reference chemical, which was TCDD, in this study. The efficacy of some of the test compounds was not the same as that of TCDD. The second assumption was that the slopes of the log-transformed dose−response relationships are equivalent. That is to say that the slope of the dose−response relationship of the chemical for which a ReP value is being determined must be parallel to that of the reference chemical (TCDD). If the slopes are equal, then the values of the ReP determined by use of the EC (effect concentration) values between 20 and 80% will all be the same. However, if the ReP values estimated from EC_{20} and EC_{80} are significantly different, this assumption has been violated and the most appropriate estimate of the ReP can be determined by use of EC values nearer to the point of departure of the dose−response relationship from the ordinate. The assumption of parallel slopes was tested by calculating RePs at multiple levels from 20 to 80%-TCDD-max. including 50%-TCDD-max (EC_{50}). ReP_{20} and ReP_{80} values are reported as an estimate of the uncertainty in the relative potency estimate due to deviations from parallelism between the standard and sample curves (*26*). The theoretical basis of these assumptions has been described previously (*27*). The greater the variation among these estimates of the ReP values, the greater the violation of the assumption of parallelism. In cases where the observed maximum response for the sample was less than 80%-TCDD-max., extrapolation beyond the range of the empirical results was used to estimate ReP_i at Y_i greater than the observed maximum. This was done in order to make the ReP_{20-80} ranges comparable from sample to sample. Responses, expressed in mean relative luminance units (RLUs) averaged for three replicate wells, were converted to the percentage of the maximum response observed for TCDD (%-TCDD-max.) standard curves generated on the same day. Potencies of samples relative to that of TCDD were estimated. Responses were defined as significant at a threshold of three times (3×) the standard deviation (expressed in % standard max.) of the mean solvent control response (0% standard max.). Further details regarding the derivation of ReP values have been described in our earlier publications (*22, 24−26*). Concentrations of TEQs in selected environmental samples were calculated by multiplying ReP values of individual Cl- and Br-PAHs and the concentrations reported in the previous studies (*7, 14, 15*).

Results

ClPAHs. Fifteen of the nineteen ClPAHs screened initially (two concentrations tested) elicited some AhR-mediated luciferase activity, but their responses varied from 0 to 94%-TCDD-max. (Table 1). ClFle, ClAnt, monochlorophenan-

TABLE 1. Results of Initial Screening of Chlorinated PAHs (ClPAHs) and Brominated PAHs (BrPAHs) Relative to the Potency of 2,3,7,8-Tetrachlodizenzo-*p*-dioxin (TCDD) in the H4IIE-*luc* in Vitro Bioassay

compound	abbreviation	no. of aromatic rings	%-TCDD-max.[a] mean	%-TCDD-max.[a] SD	significant level[b]	significant activity
ClPAHs						
9-monochlorofluorene	9-ClFle	3	0.9	0.3	0.6	yes
2-monochloroanthracene	2-ClAnt	3	1.3	1.1	0.6	yes
9-monochloroanthracene	9-ClAnt	3	0.9	0.1	0.6	yes
9,10-dichloroanthracene	9,10-Cl_2Ant	3	0.8	0.7	2.5	no
9-monochlorophenanthrene	9-ClPhe	3	2.0	0.6	2.5	no
1,9-dichlorophenanthrene	1,9-Cl_2Phe	3	5.0	1.8	2.5	yes
3,9-dichlorophenanthrene	3,9-Cl_2Phe	3	25[c]	7.2	2.3	yes
9,10-dichlorophenanthrene	9,10-Cl_2Phe	3	8.6	1.0	2.3	yes
3,9,10-trichlorophenanthrene	3,9,10-Cl_3Phe	3	59[c]	10	2.3	yes
3-monochlorofluoranthene	3-ClFlu	4	1.0	0.8	2.2	no
8-monochlorofluoranthene	8-ClFlu	4	2.0	1.0	2.2	no
3,4-dichlorofluoranthene	3,4-Cl_2Flu	4	18[c]	3.8	2.2	yes
3,8-dichlorofluoranthene	3,8-Cl_2Flu	4	39[c]	28	3.3	yes
1-monochloropyrene	1-ClPyr	4	6.2	1.0	3.3	yes
6-chlorochrysene	6-ClChr	4	80[c]	7.2	3.3	yes
6,12-dichlorochrysene	6,12-Cl_2Chr	4	17[c]	4.5	2.8	yes
7-chlorobenz[*a*]anthracene	7-ClBaA	4	71[c]	6.5	2.8	yes
7,12-dichlorobenz[*a*]anthracene	7,12-Cl_2BaA	4	14[c]	2.3	2.8	yes
6-monochlorobenzo[*a*]pyrene	6-ClBaP	5	25[c]	5.1	1.9	yes
BrPAHs						
2-monobromofluorene	2-BrFle	3	0.7	0.4	1.9	no
9-monobromoanthracene	9-BrPhe	3	<0.0	0.3	1.9	no
9,10-dibromoanthracene	9,10-Br_2Ant	3	0.6	1.0	3.2	no
9-monobromophenanthrene	9-BrAnt	3	<0.0	0.4	3.2	no
1-monobromopyrene	1-BrPyr	4	0.1	0.3	3.2	no
7-monobromobenz[*a*]anthracene	7-BrBaA	4	84[c]	17	3.4	yes
4,7-dibromobenz[*a*]anthracene	4,7-Br_2BaA	4	94[c]	8.8	3.4	yes
5,7-dibromobenz[*a*]anthracene	5,7-Br_2BaA	4	45[c]	6.0	3.4	yes
7,11-dibromobenz[*a*]anthracene	7,11-Br_2BaA	4	27[c]	6.6	0.4	yes
7,12-dibromobenz[*a*]anthracene	7,12-Br_2BaA	4	5.6	0.5	0.4	yes
6-monobromobenzo[*a*]pyrene	6-BrBaP	5	60[c]	6.7	0.4	yes

[a] Maximum response observed expressed as a percentage of the mean maximum response observed for the TCDD standard (%-TCDD-max.). [b] Significant level is defined as three times the standard deviation (expressed in % standard max.) of the mean solvent control response (0% standard max.) on plate by plate basis. [c] Indicates the response close to or above 20%-TCDD-max.; those compounds were further determined for full-dose response relationship to obtain assay-specific RePs (see Table 2).

threne, and monochlorofluoranthene were found to be inactive in the initial screening. Nine ClPAHs, with responses close to or above 20%-TCDD-max., were further selected to determine a full-dose response relationship in order to calculate corresponding ReP values (Table 2 and Figure 2). In the dose−response analysis, 6-ClChr and 7-ClBaA each elicited a significant response, with respective potencies relative to that of TCDD of 2.6×10^{-5} and 6.3×10^{-6}. Dichlorophenanthrene and dichlorofluoranthene elicited relatively low responses. The RePs of lower-molecular-weight ClPAHs increased with increasing chlorination of the compound (Figure 3). For example, among the four ClPhe congeners tested, the ranges of ReP_{20-80} decreased in the order 3,9,10-Cl_3Phe >3,9-Cl_2Phe >1,9-Cl_2Phe >9-ClPhe. Among the four ClFlu congeners tested, the ranges of ReP_{20-80} decreased in the order 3,8-Cl_2Flu > 3,4-Cl_2Flu > 8-ClFlu = 3-ClFlu. Alternatively, dichloro-Chr and dichloro-BaA both showed little response, while monochloro-Chr and monochloro-BaA elicited significant responses in our bioassay. Furthermore, the RePs of monochloroChr/BaA were 3−10 times greater than the RePs of the corresponding parent PAHs in the H4IIE-*luc* bioassay (*26*). The magnitude of the ReP_{20-80} range for 6-ClBaP (five-ringed PAH), a high-molecular-weight ClPAH, was smaller than that of the range for parent PAH, BaP (1.6×10^{-6}) (Figure 3). This result suggests that the AhR activities of ClPAHs are dependent on the spatial dimensions of the molecule.

BrPAHs. Six of the eleven BrPAHs screened for AhR-mediated luminescence were active, and the remaining compounds were inactive (Table 1). Representative dose−response curves for some of the active BrPAHs are shown in Figure 2. Among BrPAHs, 4,7-Br_2BaA elicited the highest dioxin-like activity, with a ReP of 2.3×10^{-5}, followed by 7-BrBaA (2.1×10^{-5}) (Table 2). BrAnt, BrPhe, and BrPyr did not elicit significant activity. The RePs were quite similar between mono-BrBaA and di-BrBaA and were 1 order magnitude greater than the RePs of parent PAH (i.e., BaA) (*26*). No apparent structure−activity relationship could be discerned for BrPAHs.

Discussion

Little is known about the environmental fates or toxicities of Cl- and Br-PAHs as compared to PCDD/Fs, PCBs, PAHs, and PCNs. In this study, RePs were determined, for the first time, for individual mono- through tri-Cl- and Br-PAHs, by means of the H4IIE-*luc* cell assay. Several Cl- and Br-PAHs were found to be AhR-active, as determined by their ability to induce luciferase activity through an AhR-mediated mechanism. The RePs of the most potent Cl- and Br-PAHs were 100 000-fold lower than the ReP of TCDD and were in the range of 2.0×10^{-5} to 3.0×10^{-5}. In comparison, the RePs of some hexa- and heptachlorinated naphthalene (CN) congeners in the H4IIE-*luc* bioassay were 10- to 100-fold

TABLE 2. Maximum Observed Response, Slope and Efficacy, and Relative Potencies (RePs) of Chlorinated PAHs (ClPAHs) and Brominated PAHs (BrPAHs) Relative to the Potency of 2,3,7,8-TCDD in the H4IIE-*luc* in Vitro Bioassay

	%-TCDD-max.[a]		condition[b]		relative potency estimates	
compound	screening data	dose−response data	equal efficacy	equal slope	ReP[c]	ReP_{20-80}[d]
ClPAHs						
3,9-dichlorophenanthrene	25	19	no	no	NQ	2.3×10^{-7} to 3.9×10^{-12}
3,9,10-trichlorophenanthrene	59	48	no	yes	NQ	7.5×10^{-6} to 3.0×10^{-6}
3,4-dichlorofluoranthene	18	10	no	no	NQ	1.8×10^{-8} to 4.7×10^{-16}
3,8-dichlorofluoranthene	39	46	no	yes	NQ	6.2×10^{-6} to 1.9×10^{-6}
6-chlorochrysene	80	61	yes	yes	2.6×10^{-5}	2.2×10^{-5} to 3.1×10^{-5}
6,12-dichlorochrysene	17	14	no	no	NQ	1.1×10^{-7} to 5.4×10^{-13}
7-chlorobenz[*a*]anthracene	71	62	yes	yes	6.3×10^{-6}	6.5×10^{-6} to 6.2×10^{-6}
7,12-dichlorobenz[*a*]anthracene	14	6	no	no	NQ	$1.9-10^{-10}$ to 8.9×10^{-24}
6-monochlorobenzo[*a*]pyrene	25	24	no	no	NQ	1.4×10^{-6} to $2.2-10^{-8}$
BrPAHs						
7-monobromobenz[*a*]anthracene	84	63	yes	yes	2.1×10^{-5}	1.5×10^{-5} to 3.0×10^{-5}
4,7-dibromobenz[*a*]anthracene	94	68	yes	yes	2.3×10^{-5}	1.5×10^{-5} to 3.6×10^{-5}
5,7-dibromobenz[*a*]anthracene	45	35	no	yes	NQ	1.8×10^{-6} to 5.8×10^{-7}
7,11-dibromobenz[*a*]anthracene	26	28	no	no	NQ	2.1×10^{-6} to 6.6×10^{-8}
6-monobromobenzo[*a*]pyrene	60	34	no	no	NQ	3.7×10^{-6} to 5.7×10^{-7}

[a] Maximum response observed in initial screening (at 2 concentrations) and full-dose−response (6 dilution series); expressed as %-TCDD-max. [b] Condition for equal efficacy and equal slope assumption between samples and TCDD in full-dose−response curves [c] ReP: Single point estimate of ReP made for a response of 50%-TCDD-max. (EC-50). NQ indicates dose−response relationship was insufficient to estimate (because of the violation of either equal efficacy or equal slope). [d] ReP_{20-80}: RePs reported as the range of estimates generated from multiple points over a response range from 20 to 80%-TCDD-max. Extrapolation was used for samples which yielded maximum response less than 80%-TCDD-max.

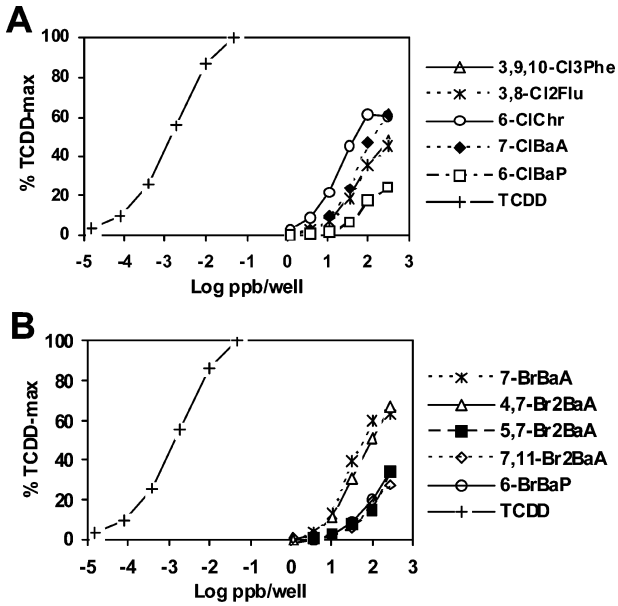

FIGURE 2. Dose−response curves for chlorinated PAHs (A) and brominated PAHs (B) in H4IIE-*luc* cell bioassay. Doses are shown along the *x*-axis, expressed as log ppb (ng/mL) present in each test well. Response (*y*-axis) is expressed as percentage of the maximum response that was observed for the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin standard (% TCDD-max.).

greater (i.e., 1.0×10^{-3} to 4.0×10^{-4}) than the RePs found for the ClPAHs (Table 3) (*4*). The mammalian toxic equivalency factors (TEFs) of mono-ortho PCBs were reported to range from 1.0×10^{-5} to 5.0×10^{-4} (*28*). The TEFs of most toxic non-ortho coplanar PCBs have been reported to be in the range of 1.0×10^{-4} to 1×10^{-1} (*28*). The RePs of several Cl- and Br-PAHs are of the same order of magnitude as the RePs of mono-ortho PCBs. Mono- and dichloro-CN congeners exhibited AhR-mediated activities 3 to 4 orders of magnitude lower than the activities of hexa- and hepta-CN congeners (*5*). The RePs of three-ring ClPAHs, such as ClPhe, increased with increasing degree of chlorination (Figure 3); this trend is similar to the pattern reported for PCNs (*29*). We analyzed only less highly chlorinated and brominated congeners (i.e., mono- through trisubstituted congeners); we could not test highly chlorinated or brominated PAHs, because of the lack of analytical standards. Previous studies have suggested that highly chlorinated congeners are more potent than less highly chlorinated ones (*21, 29*). Further studies are needed to evaluate the environmental occurrence and toxicities of highly chlorinated and/or brominated PAHs.

The pattern of RePs for Cl- and Br-PAHs indicates that the position of the chlorine or bromine atom on the PAH molecule is an important determinant of AhR-mediated activity. For instance, a shift of the position of a chlorine (or bromine) on 3,4-Cl_2Flu to 3,8-Cl_2Flu or on 7,12-Br_2BaA to 4,7-Br_2BaA, or the addition of a single chlorine on 9,10-Cl_2Phe to 3,9,10-Cl_3Phe, increased the AhR-mediated activity significantly (Table 2). Nevertheless, addition of a chlorine on 6-ClChr to form 6,12-Cl_2Chr diminished the AhR activity. AhR-mediated activities of individual ClPAHs in a yeast assay (YCM3 cells) have been reported (*14*); in that study, the RePs were calculated relative to that for BaP. In the yeast bioassay, 3,8-Cl_2Flu and 6-ClChr were the most potent AhR ligands, with activities that were respectively 2.0 and 5.7 times greater than that of BaP. Similarly, in our study, 6-ClChr and 3,8-Cl_2Flu were found to be the two most potent ClPAHs. The yeast bioassay also showed an interesting structure−activity relationship for ClPAHs: the individual AhR activity was strongly dependent on the spatial dimensions of the molecule. Further, the AhR activity for low-molecular-weight ClPAHs (e.g., ClPhe and ClFlu), was found to increase with the number of chlorine substitutions, and for high-molecular-weight ClPAHs (e.g., ClBaA and ClBaP), the AhR activity decreased with increasing number of chlorine substitutions on the PAH molecule. This trend, which was echoed by the trend seen in our study, could be related to the optimal structural dimension of the ligand, for binding to AhR (*21*).

The RePs of BrPAHs are greater than those of the corresponding ClPAHs. For example, 6-BrBaP is more potent than 6-ClBaP and 7-BrBaA is more potent than 7-ClBaA. A similar trend was reported previously for PCNs and polybrominated naphthalenes (*4*). The LD_{50} for 2,3,6,7-tetrabromonaphthalene, in guinea pigs, was 88-fold lower than that for TCDD, whereas the LD_{50} was not reached for the

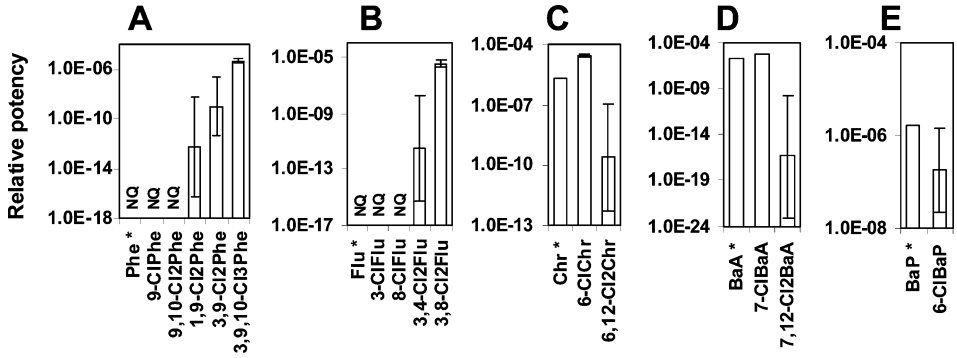

FIGURE 3. Relative potencies (RePs) of chlorinated PAHs and corresponding parent PAHs: chlorophenanthrene (A), chlorofluoranthene (B), chlorochrysene (C), chlorobenz[*a*]anthracene (D), and chlorobenzo[*a*]pyrene (E). Asterisk indicates H4IIE-*luc* bioassay system data from Villeneuve et al. (*26*). ReP values of ClPAHs were represented by the range ReP_{20-80}. NQ indicates that the relative potencies were not quantifiable, because of the lack of activity.

TABLE 3. Relative Potencies (RePs) of Chlorinated PAHs (ClPAHs) and Brominated PAHs (BrPAHs), Compared with the RePs Reported for Polychlorinated Naphthalenes (PCNs) and PAHs

ClPAH[a]		BrPAH[a]		PCN[b]		PAH[c]	
6-ClChr	2.6×10^{-5}	7-BrBaA	2.1×10^{-5}	1,2,3,6,7-PeCN	1.7×10^{-4}	benzo[*a*]anthracene	1.9×10^{-6}
7-ClBaA	6.3×10^{-6}	4,7-Br_2BaA	2.3×10^{-5}	1,2,3,4,6,7-HxCN	4.0×10^{-3}	benzo[*a*]pyrene	1.6×10^{-6}
				1,2,3,5,6,7-HxCN	1.0×10^{-3}	benzo[*b*]fluoranthene	5.1×10^{-6}
				1,2,3,5,6,8-HxCN	1.5×10^{-4}	benzo[*k*]fluoranthene	1.4×10^{-4}
				1,2,3,6,7,8-HxCN	5.9×10^{-4}	chrysene	2.3×10^{-6}
				1,2,3,4,5,6,7-HpCN	1.0×10^{-3}		

[a] This study. [b] Data from Blankenship et al. (*4*). [c] Data from Villeneuve et al. (*26*).

TABLE 4. Toxic Equivalents (TEQs) Estimated for Individual Chlorinated PAHs (ClPAHs) and Brominated PAHs (BrPAHs) in Various Previously Published Environmental Samples

	incineration ash[a] [pg-TEQ/g]	automobile exhaust[b] [fg-TEQ/m^3]	urban air[c] [fg-TEQ/m^3]
ClPAH			
6-ClChr	3.6	NA[d]	0.049
7-ClBaA	0.98	0.015	0.035
total in ClPAH	4.6	0.015	0.085
BrPAH			
7-BrBaA	0.51	NA[d]	NA[d]
4,7-Br_2BaA	0.007	NA[d]	NA[d]
total in BrPAH	0.52		

[a] Data from Horii et al. (*15*). [b] Data from Nilsson et al. (*7*); ClPAH concentrations used are in road tunnel air. [c] Data from Ohura et al. (*14*). [d] NA, not available.

corresponding 2,3,6,7-tetrachloronaphthalene congener (*4*). The difference in toxic potency between chlorinated and brominated congeners is thought to be due to the distance between the lateral halogens in the naphthalene molecule. This difference in lateral-halogen distance between ClPAH and BrPAH would explain why the potencies of BrPAHs are higher than those of the corresponding ClPAHs in our bioassay.

TEQs for Cl- and Br-PAHs in Environmental Samples. The RePs determined for Cl- and Br-PAHs in this study were used to estimate the TEQs contributed by these compounds in environmental samples cited in the literature (Table 4). It should be noted that RePs of several other Cl- and Br-PAHs that are present in environmental samples, are not available. The concentrations of total ClPAHs ranged from 1.1×10^1 to 3.3×10^2 pg/m^3 in urban air from Shizuoka, Japan (*12*–*14*), and from $<6 \times 10^{-2}$ to 7.0×10^3 ng/g in fly ash from South Korea (*15*); a value of 1.3×10^2 pg/ m^3 was found in automobile exhaust (road tunnel air) (*7*). The TEQs of ClPAH that we calculated using the mean concentrations of individual ClPAHs were 8.5×10^{-2} fg-TEQ/m^3 in urban air, 4.6 pg-TEQ/g in fly ash, and 1.5×10^{-2} fg-TEQ/m^3 in automobile exhaust. The calculated ClPAHs-TEQ concentration found for automobile exhaust was 6 times less than that in urban air; the number of ClPAHs quantified in the automobile exhaust study (*7*) was smaller. 6-ClChr accounted for 80% of the total ClPAHs-TEQs in fly ash. The TEQs of BrPAHs were estimated to be 5.2×10^{-1} pg-TEQ/g, values 10 times less than that for ClPAHs in the fly ash samples. Mean TEQ concentrations for PCDD/Fs and coplanar PCBs in ambient air ($n = 740$, 2007) and in fly ash from industrial solid waste incinerators ($n = 21$, 2001−2005) were 41 fg-TEQ/m^3 (*30*) and 4.8 ng-TEQ/g (*31*), respectively. On the basis of this comparison, ClPAHs in urban air and fly ash accounted for 0.5 and 0.1% of the total TEQs, respectively. However, this comparison is crude and does not involve same matrixes for which both PCDD/F and ClPAH values have been reported. The contributions of Cl- and Br-PAHs to total TEQs, relative to the contributions from PCDD/Fs, PCBs, and PCNs in various environmental matrixes are still unknown. Contribution of ClPAHs to TEQs in environmental samples collected near an electronic waste recycling facility in China was similar to or higher than that by PCDD/Fs (*32*).

The contributions of Cl- and Br-PAHs to the total dioxin-like activities in complex environmental mixtures need to be assessed.

Photodegradation of ClPAHs and their parent PAHs was investigated using chemical model systems (*33*). Higher molecular weight ClPAHs (e.g., 7-ClBaA and 6-ClBaP) are more stable than are the corresponding parent compounds. Furthermore, half-lives of ClFlu increased with increasing chlorination (3,8-Cl2Flu; 198 h, 3-ClFlu; 158 h, Flu; 22 h). Previous studies (*4*, *5*) have shown that the more highly substituted ClPAHs are more potent AhR-ligands and are stable in the environment. Information regarding concentrations of highly substituted chlorinated PAHs in environmental matrixes is limited. Previous studies suggested the existence of highly chlorinated PAHs (more than three chlorine atoms) in fly ash samples when such samples were analyzed by GC/MS using M, M^{2+}, and M^{4+} molecular ions (*15*), and in road tunnel air (*8*). Therefore, it is important to investigate the occurrence and profiles of all ClPAHs, including the highly substituted ones, in various environmental and biological matrixes.

In summary, the potencies of several Cl- and Br-PAHs relative to the potency of TCDD were determined for the first time using the in vitro H4IIE-*luc* bioassay. Several ClPAHs and BrPAHs are found to be potent ligands of AhR; they elicit dioxin-like activity with potencies comparable to those of several mono-ortho PCB congeners. Determination of the relative contributions of Cl- and Br-PAHs to total TEQs in complex environmental mixtures would help to delineate the significance of halogenated PAHs as environmental pollutants of concern.

Literature Cited

(1) Falandysz, J. Polychlorinated naphthalenes: an environmental update. *Environ. Pollut.* **1998**, *101*, 77–90.

(2) Kannan, K.; Yamashita, N.; Imagawa, T.; Decoen, W.; Khim, J. S.; Day, R. M.; Summer, C. L.; Giesy, J. P. Polychlorinated naphthalenes and polychlorinated biphenyls in fishes from Michigan waters including the Great Lakes. *Environ. Sci. Technol.* **2000**, *34*, 566–572.

(3) Horii, Y.; Falandysz, J.; Hanari, N.; Rostkowski, P.; Puzyn, T.; Okada, M.; Amano, K.; Naya, T.; Taniyasu, S.; Yamashita, N. Concentrations and fluxes of chloronaphthalenes in sediment from Lake Kitaura in Japan in past 15 centuries. *J. Environ. Sci. Health A* **2004**, *39*, 587–609.

(4) Blankenship, A. L.; Kannan, K.; Villalobos, S. A.; Villeneuve, D. L.; Falandysz, J.; Imagawa, T.; Jakobsson, E.; Giesy, J. P. Relative potencies of individual polychlorinated naphthalenes and halowax mixtures to induce Ah receptor-mediated responses. *Environ. Sci. Technol.* **2000**, *34*, 3153–3158.

(5) Villeneuve, D. L.; Kannan, K.; Khim, J. S.; Falandysz, J.; Nikiforov, V. A.; Blankenship, A. L.; Giesy, J. P. Relative potencies of individual polychlorinated naphthalenes to induce dioxin-like responses in fish and mammalian in vitro bioassays. *Arch. Environ. Contam. Toxicol.* **2000**, *39*, 273–281.

(6) Kannan, K.; Villeneuve, D. L.; Yamashita, N.; Imagawa, T.; Hashimoto, S.; Miyazaki, A.; Giesy, J. P. Vertical profiles of dioxin-like and estrogenic activities associated with a sediment core from Tokyo Bay, Japan. *Environ. Sci. Technol.* **2000**, *34*, 3568–3573.

(7) Nilsson, U. L.; Oestman, C. E. Chlorinated polycyclic aromatic hydrocarbons: method of analysis and their occurrence in urban air. *Environ. Sci. Technol.* **1993**, *27*, 1826–1831.

(8) Ishaq, R.; Naf, C.; Zebuhr, Y.; Broman, D.; Jarnberg, U. PCBs, PCNs, PCDD/Fs, PAHs and Cl-PAHs in air and water particulate samples-patterns and variations. *Chemosphere* **2003**, *50*, 1131–1150.

(9) Haglund, P.; Alsberg, T.; Bergman, A.; Jansson, B. Analysis of halogenated polycyclic aromatic hydrocarbons in urban air, snow and automobile exhaust. *Chemosphere* **1987**, *16*, 2441–2450.

(10) Koistinen, J.; Paasivirta, J.; Nevalainen, T.; Lahtipera, M. Chlorinated fluorenes and alkylfluorenes in bleached kraft pulp and pulp mill discharges. *Chemosphere* **1994**, *28*, 2139–2150.

(11) Koistinen, J.; Paasivirta, J.; Nevalainen, T.; Lahtipera, M. Chlorophenanthrenes, alkylchlorophenanthrenes and alkylchloronaphthalenes in kraft pulp mill products and discharges. *Chemosphere* **1994**, *28*, 1261–1277.

(12) Kitazawa, A.; Amagai, T.; Ohura, T. Temporal trends and relationships of particulate chlorinated polycyclic aromatic hydrocarbons and their parent compounds in urban air. *Environ. Sci. Technol.* **2006**, *40*, 4592–4598.

(13) Ohura, T.; Kitazawa, A.; Amagai, T.; Makino, M. Occurrence, profiles, and photostabilities of chlorinated polycyclic aromatic hydrocarbons associated with particulates in urban air. *Environ. Sci. Technol.* **2005**, *39*, 85–91.

(14) Ohura, T.; Fujima, S.; Amagai, T.; Shinomiya, M. Chlorinated polycyclic aromatic hydrocarbons in the atmosphere: Seasonal levels, gas-particle partitioning, and origin. *Environ. Sci. Technol.* **2008**, *42*, 3296–3302.

(15) Horii, Y.; Ok, G.; Ohura, T.; Kannan, K. Occurrence and profiles of chlorinated and brominated polycyclic aromatic hydrocarbons in waste incinerators. *Environ. Sci. Technol.* **2008**, *42*, 1904–1909.

(16) Colmsjo, A.; Rannug, A.; Rannug, U. Some chloro derivatives of polynuclear aromatic hydrocarbons are potent mutagens in *Salmonella typhimurium*. *Mutat. Res.: Genet. Toxicol. Test.* **1984**, *135*, 21–29.

(17) Lofroth, G.; Nilsson, L.; Agurell, E.; Sugiyama, T. Salmonella/microsome mutagenicity of monochloro derivatives of some di-, tri- and tetracyclic aromatic hydrocarbons. *Mutat. Res.: Genet. Toxicol. Test.* **1985**, *155*, 91–94.

(18) Blankenship, A.; Matsumura, F. 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced activation of a protein tyrosine kinase, pp60(src), in murine hepatic cytosol using a cell-free system. *Mol. Pharmacol.* **1997**, *52*, 667–675.

(19) Poland, A.; Knutson, J. C. 2,3,7,8-tetrachlorodibenzo-para-dioxin and related halogenated aromatic-hydrocarbons - examination of the mechanism of toxicity. *Annu. Rev. Pharmacol.* **1982**, *22*, 517–554.

(20) Blankenship, A.; Matsumura, F. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) causes an Ah receptor-dependent and ARNT-independent increase in membrane levels and activity of p60(Src). *Environ. Toxicol. Pharm.* **1997**, *3*, 211–220.

(21) Ohura, T.; Morita, M.; Makino, M.; Amagai, T.; Shimoi, K. Aryl hydrocarbon receptor-mediated effects of chlorinated polycyclic aromatic hydrocarbons. *Chem. Res. Toxicol.* **2007**, *20*, 1237–1241.

(22) Khim, J. S.; Villeneuve, D. L.; Kannan, K.; Lee, K. T.; Snyder, S. A.; Koh, C. H.; Giesy, J. P. Alkylphenols, polycyclic aromatic hydrocarbons, and organochlorines in sediment from Lake Shihwa, Korea: Instrumental and bioanalytical characterization. *Environ. Toxicol. Chem.* **1999**, *18*, 2424–2432.

(23) Sanderson, J. T.; Aarts, J.; Brouwer, A.; Froese, K. L.; Denison, M. S.; Giesy, J. P. Comparison of Ah receptor-mediated luciferase and ethoxyresorufin-O-deethylase induction in H4IIE cells: Implications for their use as bioanalytical tools for the detection of polyhalogenated aromatic hydrocarbons. *Toxicol. Appl. Pharmacol.* **1996**, *137*, 316–325.

(24) Khim, J. S.; Kannan, K.; Villeneuve, D. L.; Koh, C. H.; Giesy, J. P. Characterization and distribution of trace organic contaminants in sediment from Masan Bay, Korea. 1. Instrumental analysis. *Environ. Sci. Technol.* **1999**, *33*, 4199–4205.

(25) Villeneuve, D. L.; Khim, J. S.; Kannan, K.; Giesy, J. P. In vitro response of fish and mammalian cells to complex mixtures of polychlorinated naphthalenes, polychlorinated biphenyls, and polycyclic aromatic hydrocarbons. *Aquat. Toxicol.* **2001**, *54*, 125–141.

(26) Villeneuve, D. L.; Khim, J. S.; Kannan, K.; Giesy, J. P. Relative potencies of individual polycyclic aromatic hydrocarbons to induce dioxinlike and estrogenic responses in three cell lines. *Environ. Toxicol.* **2002**, *17*, 128–137.

(27) Villeneuve, D. L.; Blankenship, A. L.; Giesy, J. P. Derivation and application of relative potency estimates based on in vitro bioassay results. *Environ. Toxicol. Chem.* **2000**, *19*, 2835–2843.

(28) Van den Berg, M.; Birnbaum, L. S.; Denison, M.; De Vito, M.; Farland, W.; Feeley, M.; Fiedler, H.; Hakansson, H.; Hanberg, A.; Haws, L.; Rose, M.; Safe, S.; Schrenk, D.; Tohyama, C.; Tritscher, A.; Tuomisto, J.; Tysklind, M.; Walker, N.; Peterson, R. E. The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicol. Sci.* **2006**, *93*, 223–241.

(29) Olivero-Verbel, J.; Vivas-Reyes, R.; Pacheco-Londono, L.; Johnson-Restrepo, B.; Kannan, K. Discriminant analysis for activation of the aryl hydrocarbon receptor by polychlorinated naphthalenes. *J. Mol. Struct. (THEOCHEM)* **2004**, *678*, 157–161.

(30) Ministry of the Environment, Japan. Report of environmental monitoring on dioxins in 2007; Tokyo, Japan, 2007. http://www.env.go.jp/air/report/h20−06/. (In Japanese; accessed Dec 2008).
(31) Ohtsuka, N.; Hosono, S.; Nojiri, K.; Minomo, K. Estimation of TEQs originated from four kinds of dioxins-sources using four indicator isomers. *J. Environ. Chem* **2007**, *17*, 377–386 (in Japanese).
(32) Ma, J.; Horii, Y.; Cheng, J.; Wang, W.; Wu, Q.; Ohura, T.; Kannan, K. Chlorinated and parent polycyclic aromatic hydrocarbons in environmental samples from an electronic waste recycling facility and a chemical industrial complex in China. *Environ. Sci. Technol.* **2009**, *43*, 643–649.
(33) Ohura, T.; Amagai, T.; Makino, M. Behavior and prediction of photochemical degradation of chlorinated polycyclic aromatic hydrocarbons in cyclohexane. *Chemosphere* **2008**, *70*, 2110–2117.

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