RELATIVE POTENCIES OF INDIVIDUAL POLYCHLORINATED NAPTHALENES TO INDUCE DIOXIN-LIKE RESPONSES IN FISH AND MAMMALIAN IN VITRO BIOASSAYS

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Introduction

There is a growing body of evidence which suggests that polychlorinated napthalenes (PCNs) may be widespread in the environment[1,2]. Exposure to PCNs has been linked to chloracne and liver disease in humans[3,4], X-disease in cattle[5], EROD induction and oxidative stress in rats[5,6], EROD induction and embryolethality in chickens[7], and EROD induction and early life stage toxicity in fish[8,9,10]. The profile of biological responses suggests that at least a portion of the toxic responses associated with PCNs may be mediated through an aryl hydrocarbon receptor (AhR)-dependent mechanism of action[11]. As a result, *in vitro* bioassays which measure AhR-dependent reporter gene activation or enzyme induction may be useful tools for characterizing the relative potencies (REPs) of individual PCN congeners and PCN mixtures[11].

This study used *in vitro* EROD assay with PLHC-1 fish hepatoma cells[12] and *in vitro* EROD and luciferase assays with H4IIE-luc recombinant rat hepatoma cells[13] to characterize AhR-dependent, dioxin-like potency of 18 individual PCN congeners and one PCN metabolite, 2,4,-dichloro-1-napthol[14]. REPs, based on luciferase induction in H4IIE-luc cells, have been reported for 20 PCN congeners[11]. This study expands the characterization of ten of these compounds by examining another end-point and cell type. At least one representative congener from each group of PCN isomers (mono- through hepta-chlorinated) was selected for additional characterization. In addition to previously analyzed congeners, this study determined REPs for eight PCN congeners and one PCN metabolite for which REPs were not previously available. The REP estimates reported here contribute to an emerging body of information which will aid determination of the relative contribution of PCNs to the total dioxin-like activity associated with environmental samples.

Methods and Materials

1,4-diCN was obtained 98.3% pure from Accustandard (New Haven, CT, USA). 2-monoCN (99% pure) was obtained from ICN Chemicals (Irvine, CA, USA). 1,2,3,4,5,6,7-heptaCN was synthesized and purified at the Wallenberg Laboratory, Stockholm University, Sweden[15]. PCN congeners 1,2,7-triCN, 2,3,6,7-tetraCN, 1,2,3,6,7-pentaCN, and all the hexaCN congeners tested were obtained >99% pure from Promochem (GmbH, Wesel, Germany). PCN congeners 1,2,4,7-tetraCN, 1,3,5,7-tetracCN, 1,2,4,6,8-pentaCN, 1,2,4,6,7-pentaCN, 1,2,4,5,6-pentaCN, 1,2,3,7,8-pentaCN, 1,2,3,6,8-pentaCN, and 1,2,3,5,7-pentaCN were synthesized in the Nikiforov laboratory, St. Petersburg University, Russia[16,17].

The congeners were >99% pure and supplied in crystalline form. 2,4-OH-CN, a metabolite of some CNs[14], was obtained >95% pure from Ultra Scientific (N. Kingstown, RI, USA). PCN standards were shown to contain less than 100 pg/g 2,3,7,8-substituted polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), or unwanted PCN. At this level the maximum response contributed by PCDD/DF congeners would have been at least 600-fold-below the bioassay detection limits. Concentrations tested in the bioassay varied and were limited by the mass of standard available.

PLHC-1 and H4IIE-luc bioassay methods have been described previously[13,18]. Briefly, cells were seeded into 96-well plates and incubated overnight to allow for cell attachment. Test and control wells were dosed with 2.5 µl of the appropriate sample or solvent control. Six concentrations of each PCN, three replicates per concentration, were analyzed. After 72 h of exposure, EROD or luciferase assays were conducted[18].

Sample responses expressed as mean pmol resorufin/min/mg protein or relative luminescence units were converted to a percentage of the mean maximum response observed for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) standard curves generated on the same day (%-TCDD-max.). This was done to normalize for day-to-day variability in response magnitude and to make response magnitudes comparable among assays. Assumptions of parallelism and equal efficacy (relative to the TCDD standard curve) were evaluated for each PCN dose-response curve[19]. A range of REPs, which accounted for uncertainties due to deviations from parallelism, was calculated for each PCN which induced a magnitude of response sufficient for potency estimation[19].

Results and Discussion

Several individual PCNs, particularly the more chlorinated congeners, were active in the H4IIE assays. Significant activity was observed for most penta- through hepta-CN congeners tested using the H4IIE assays. Magnitudes of induction as great as 97%-TCDD-max. were observed. REPs were ranged from approximately 10^{-3} to 10^{-6}, with the hexachlorinated congeners generally being one to two orders of magnitude more potent than the pentachlorinated congeners. The least active of the penta- through hepta-chlorinated congeners were 1,2,3,5,7- and 1,2,4,6,8-pentaCN. 1,3,5,7-tetraCN produced a weak response (15%-TCDD-max.) in the H4IIE-EROD assay, but was not active in the H4IIE-luc assay. No other tetraCN congeners were active at the concentrations tested. Among the mono- through tri-chlorinated congeners, only 1,4-diCN and 2,4-OH-CN yielded significant responses in one or both of the H4IIE assays. REP estimates for 1,4-diCN and 2,4-OH-CN were around 10^{-8}.

PCNs generally did not elicit significant activity in the PLHC-1 bioassay. Only 1,4- and 2,4-OH-CN elicited significant activity in the PLHC-1 bioassay at the concentrations tested in this study. In both cases, the magnitude of response was less than 20%-TCDD-max. Although the PLHC-1 fish hepatoma bioassay was consistently less responsive to individual PCNs than the H4IIE rat hepatoma bioassay, it was also approximately 10-fold less sensitive to TCDD. Due to the difference in sensitivity to the TCDD standard, PLHC-1-based REP estimates (expressed as <X) were consistently greater than corresponding H4IIE-based REPs. Thus there was not sufficient evidence to support a hypothesis that REPs and/or structure activity relationships for individual PCNs were different between fish or fish cells and mammals or mammalian cells, despite the differences in responsiveness observed.

Table 1. Maximum concentrations of individual PCN congeners tested in PLHC-1, H4IIE-EROD, and H4IIE-luc *in vitro* bioassays, relative potency (REP) estimates[a,b], and observed maximum responses[c].

PCN Congener	Max. Conc. (nM in well)	PLHC-1		H4IIE-EROD		H4IIE-luc	
		REP[a,b]	obs max[c]	REP[a,b]	obs max[c]	REP[a,b]	obs. max.[c]
2	$1.2x10^{5}$	$<7.1x10^{-7}$	0	$<1.1x10^{-7}$	0	—	—
1,4	$5.1x10^{5}$	$<1.6x10^{-7}$	11	$2.6x10^{-8}$-$3.6x10^{-10}$	33	$1.2x10^{-7}$-$1.0x10^{-8}$	47
2,7	$5.1x10^{4}$	$<1.6x10^{-6}$	0	$<2.6x10^{-7}$	1	$<4.2x10^{-7}$	3
2,4-OH	$1.1x10^{5}$	$<7.4x10^{-7}$	19	$5.5x10^{-8}$-$7.6x10^{-9}$	29	$<1.9x10^{-7}$	2
1,2,7	$2.2x10^{4}$	$<3.8x10^{-6}$	0	$<6.1x10^{-7}$	-1	—	—
2,3,6,7	$3.8x10^{1}$	$<2.2x10^{-3}$	-1	$<3.5x10^{-4}$	3	—	—
1,2,4,7	$3.8x10^{4}$	$<2.2x10^{-6}$	0	$<3.5x10^{-7}$	3	$<5.7x10^{-7}$	4
1,3,5,7	$3.8x10^{3}$	$<2.2x10^{-5}$	0	$<3.5x10^{-6}$	15	$<5.7x10^{-6}$	-3
1,2,3,6,7	$3.3x10^{1}$	$<2.5x10^{-3}$	1	$2.7x10^{-4}$-$2.2x10^{-5}$	35	$<6.4x10^{-4}$	2
1,2,3,5,7	$3.3x10^{3}$	$<2.5x10^{-5}$	0	$<3.9x10^{-6}$	3	—	—
1,2,3,7,8	$3.3x10^{3}$	$<2.5x10^{-5}$	0	$3.9x10^{-5}$-$1.3x10^{-5}$	61	$4.6x10^{-5}$-$4.5x10^{-5}$	66
1,2,4,5,6	$3.3x10^{4}$	$<2.5x10^{-6}$	0	$7.8x10^{-6}$-$3.1x10^{-7}$	97	$8.1x10^{-6}$-$1.5x10^{-6}$	56
1,2,4,6,7	$3.3x10^{4}$	$<2.5x10^{-6}$	0	$<3.9x10^{-7}$	37	$2.6x10^{-5}$-$3.6x10^{-7}$	60
1,2,4,6,8	$3.3x10^{4}$	$<2.5x10^{-6}$	0	$<3.9x10^{-7}$	30	—	—
1,2,3,4,6,7	$3.0x10^{2}$	$<2.8x10^{-4}$	0	$1.5x10^{-3}$-$2.6x10^{-4}$	68	$2.2x10^{-3}$-$3.0x10^{-3}$	80
1,2,3,5,6,7	$3.0x10^{3}$	$<2.8x10^{-5}$	0	$3.1x10^{-4}$-$2.7x10^{-4}$	44	—	—
1,2,3,5,6,8	$3.0x10^{1}$	$<2.8x10^{-3}$	-1	$<4.4x10^{-4}$	20	—	—
1,2,3,6,7,8	$3.0x10^{2}$	$<2.8x10^{-4}$	0	$1.7x10^{-3}$-$2.6x10^{-3}$	89	$2.2x10^{-2}$-$4.5x10^{-3}$	91
1,2,3,4,5,6,7	$2.7x10^{1}$	$<3.1x10^{-3}$	0	$3.8x10^{-4}$-$5.6x10^{-4}$	84	$1.3x10^{-3}$-$3.8x10^{-4}$	67

[a] REPs reported as the range of REP estimates generated from multiple point estimates over a response range from 20-80%-TCDD-max. ($REP_{20\text{-}80}$-range). Extrapolation was used for samples which yielded maximum responses less than 80%-TCDD-max. All REP estimates were based on molar concentrations.

[b] REPs < x were calculated by dividing the maximum concentration tested by the EC-50 of the TCDD standard.

[c] Maximum response observed expressed as a percentage of the mean maximum response observed for the TCDD standard (%-TCDD-max.). Maximum response was not necessarily achieved at the maximum concentration tested.

Consideration of PCNs in risk assessments concerned with dioxin-like effects may be important. Two recent studies, for example, found that PCNs accounted for up to 50% of the total TCDD equivalents (TEQs) present in fish from the Detroit River[2] and sediments and biota collected near the site of a former chlor-alkali plant[20]. REPs determined for penta- through hepta-CNs are similar to REPs or toxic equivalency factors (TEFs) reported for some PCB congeners[21]. Furthermore, recent studies suggest very widespread distribution in the environment[1,2]. Assay-specific REPs may serve as a reasonable basis for the formulation of consensus values that may be applied for risk assessments until a greater database of *in vivo* relative potencies are available for PCNs.

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