DIFFERENCES IN BIOLOGICAL POTENCY OF LOWER AND HIGHER MOLECULAR MASS POLYCHLORINATED NAPHTHALENES WITH RESPECT TO EFFECTS ON VIABILITY AND APOPTOSIS IN THE MCF-7 BREAST CANCER CELL LINE

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Introduction

Polychlorinated naphthalenes (PCNs) are group of diaromatic compounds composed of two fused benzenes that contain one to eight chlorines per molecule and form 75 possible congeners[1,2]. PCNs were sold as a variety of mixtures (technical formulations), which composed of a lower molecular mass from mono- to trichloronaphthalenes (Halowax 1000 and 1001) up to higher molecular mass octachloronaphthalene as major PCN in Halowax 1051[1]. Toxic episodes because of accidental animal and occupational human poisonings with PCNs took place after exposure to mixtures containing predominately penta- and hexachloronaphthalenes (Halowax 1014)[3]. The Halowax 1014 composed largely of penta- and hexachloronaphthalenes and also some by side impurities produce biological effects largely similar to that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and including induction of the liver cytochrome P450-associated enzymes, ethoxyresorufin-*O*-deethylase (EROD) and aryl hydrocarbon hydroxylase (AHH).[4] The carcinogenicity of PCNs has not been well studied for yet and data relating to PCN cytotoxicity in the context of breast cancer are ambiguous. Kannan et al. examined the dioxin-like and estrogenic activities of environmental pollutants composed of PCNs and polychlorinated biphenyls (PCBs) contained in sediment core from the Tokyo Bay (Japan) and determined the cytotoxicity of extracts toward MCF-7 cells[5]. Unfortunately, to our knowledge, there are no studies on the effect of single PCNs congener on breast cancer.

Materials and methods

MCF-7 human breast cancer cells (ATCC, Manassas, VA, USA; passage No. 151) were routinely cultured in DMEM supplemented with 10% heat-inactivated FBS, 100 IU/ml of penicillin and 100 µg of streptomycin. Cells were seeded into 96-well culture plates at a density of 2.5 x 10^4 cells/well and allowed to attach overnight. After 24 h, the medium was changed and cells were treated with individual PCN congeners at concentrations of 100, 500, 1000, and 10,000 ng/ml. The corresponding molar concentration equivalents were 0.37594, 1.8797, 3.7594 and 37.594 nM for PCN 34, 39, 42, 46 and 48; 0.33, 1.66, 3.33 and 33.29 nM for PCN 52, 53, and 54; 0.30, 1.49, 2.99 and 29.85 nM for PCN 66 and 67; and 0.27, 1.35, 2.71 and 27.06 nM for PCN 70, 71, 73 and 74. Results were compared with those obtained for TCDD administered at concentrations of 0.1, 1.0, 10, and 100 nM. Culture medium was used as a control.

The XTT assay was used to measure the viability of cells. The reduction of XTT in the cultures was determined after a 2-h incubation by measuring the absorbance at 450 nm using an absorbance microplate reader (ELx808; Bio-Tek, Winooski, VT, USA). The concentration-dependent effects of individual PCN congeners on cell apoptosis were measured by detecting DNA fragmentation, determined using a Cellular DNA Fragmentation ELISA kit (Roche Applied Science, Mannheim, Germany).

The activities of caspase-8 and -9 were measured according to the Nicholson et al.[6] method using the fluorescent substrates, Ac-IETD-AMC and Ac-LEHD-AFC, respectively. The amount of fluorescent product was monitored every 30 min for 5 h using a fluorescence microplate reader (FLx800; Bio-Tek Instruments) at an excitation wavelength of 360 nm and an emission wavelength of 460 nm for caspase-8, and at an excitation wavelength of 400 nm and an emission wavelength of 505 nm for caspase-9.

Results and discussion

From investigated PCNs tri- and tetraCNs had effect on MCF-7 cells similar to that of TCDD, while PCNs of higher molecular mass were without stimulatory effect and PCN 66 and 74 even exerts inhibitory effect on cell proliferation (Fig.1a). Hood et al. assessed toxicity and potency of PCN 66 and 67 relative to that of TCDD and suggested that despite the fact that PCN 66 and 67 exposure resulted in biochemical and histopathological changes similar to those seen with TCDD, the estimated relative potency values obtained predicted that these compounds would not contribute greatly to the overall human body burden of dioxin-like activity. PCNs 42, 46, 48, 66, 67, and 70 act as inducers while PCNs 52, 53, 73, and 74 as inhibitors of apoptosis (Fig.1b). When

combined with their effects on cytotoxicity, these compounds may lead to tumor progression or exert a protective effect.

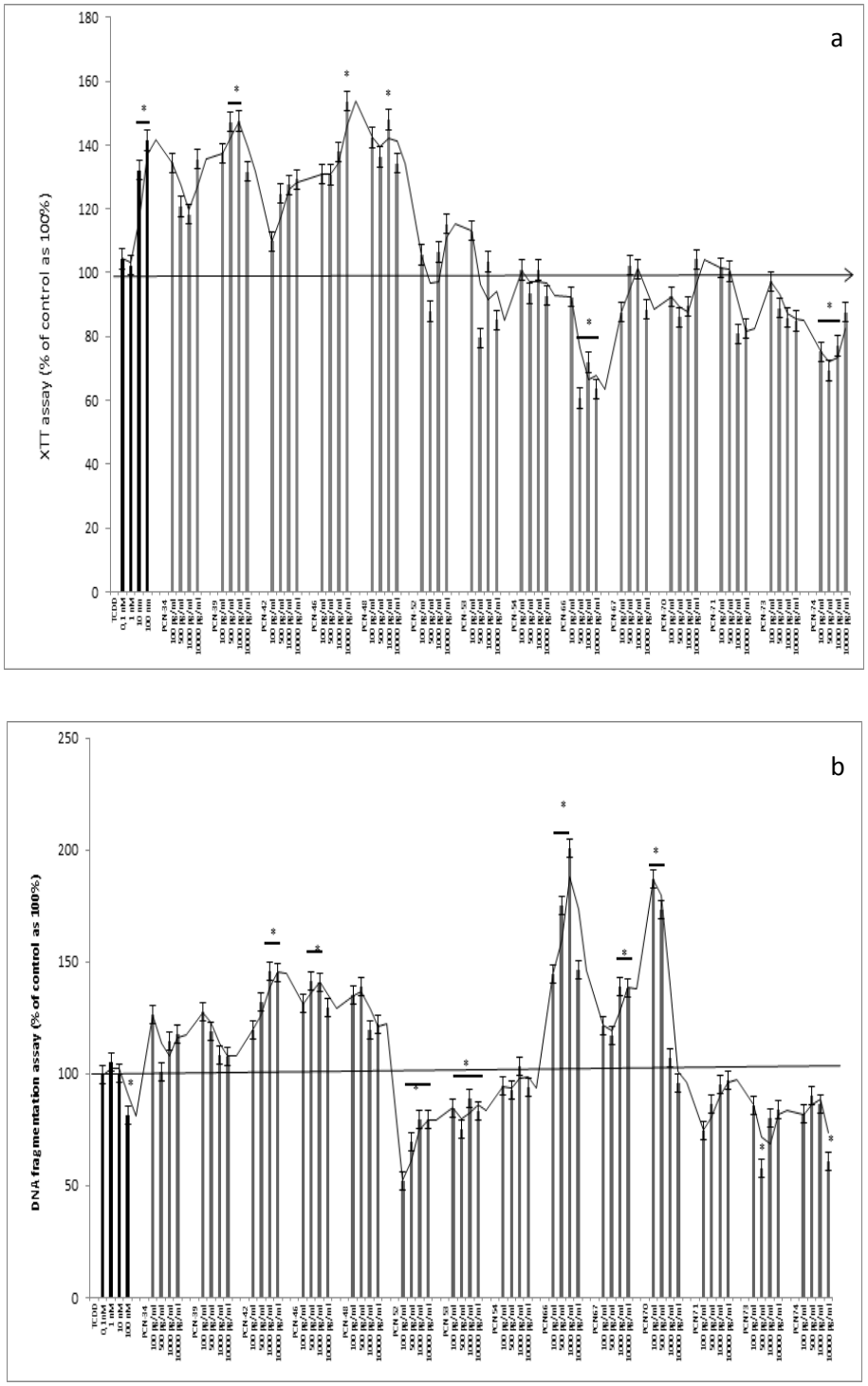

Fig. 1. Concentration-dependent effects of TCDD (0.1, 1.0, 10, and 100 nM respectively) and individual PCN (100, 500, 1000, and 10,000 ng/ml) congeners on a) cell viability determined by the XTT assay and b) apoptosis using DNA fragmentation assay. All means marked with * ($p < 0.05$) are significantly different from control values.

Among of PCN congeners studied, that of tetra- and hexCN homologue groups significantly increased both caspase-9 activity and protein expression. Moreover, hexa- and heptaCNs also increased the intracellular concentration of caspase-8 (Figs. 2 and 3). This could explain the observed inhibitory effect of those congeners on cell viability.

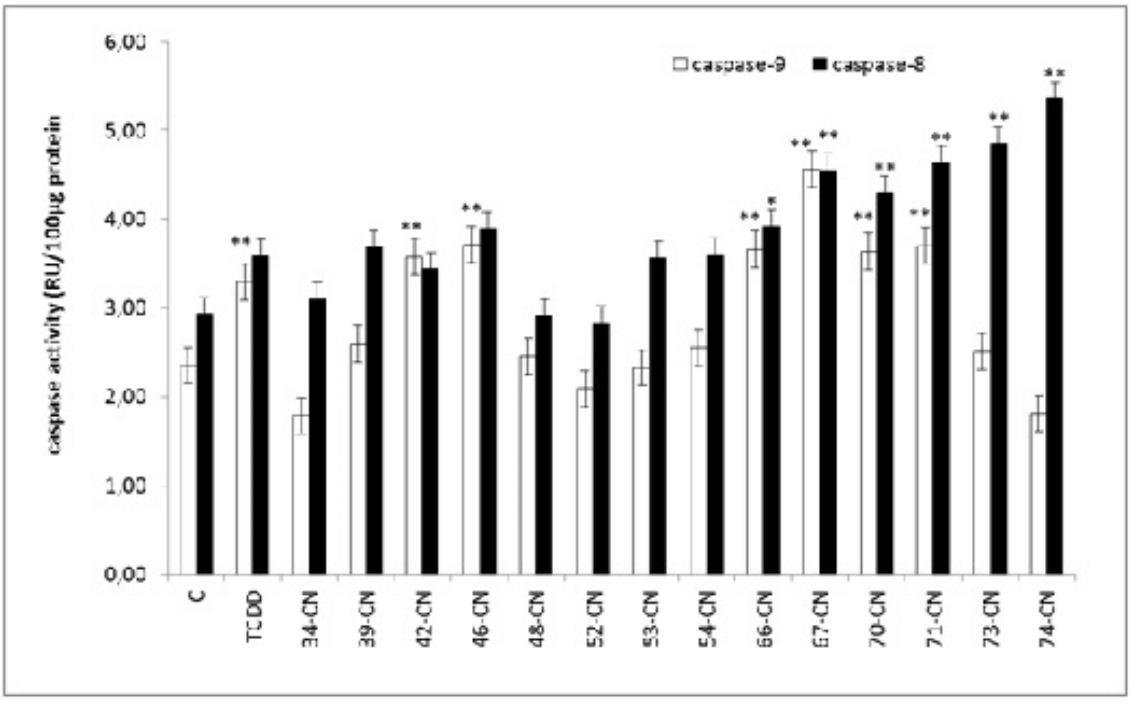

Fig. 2. Effects of TCDD and PCN congeners on caspase-9 and -8 activity All means marked with * ($p<0.05$) or ** ($p<0.01$) are significantly different from control values.

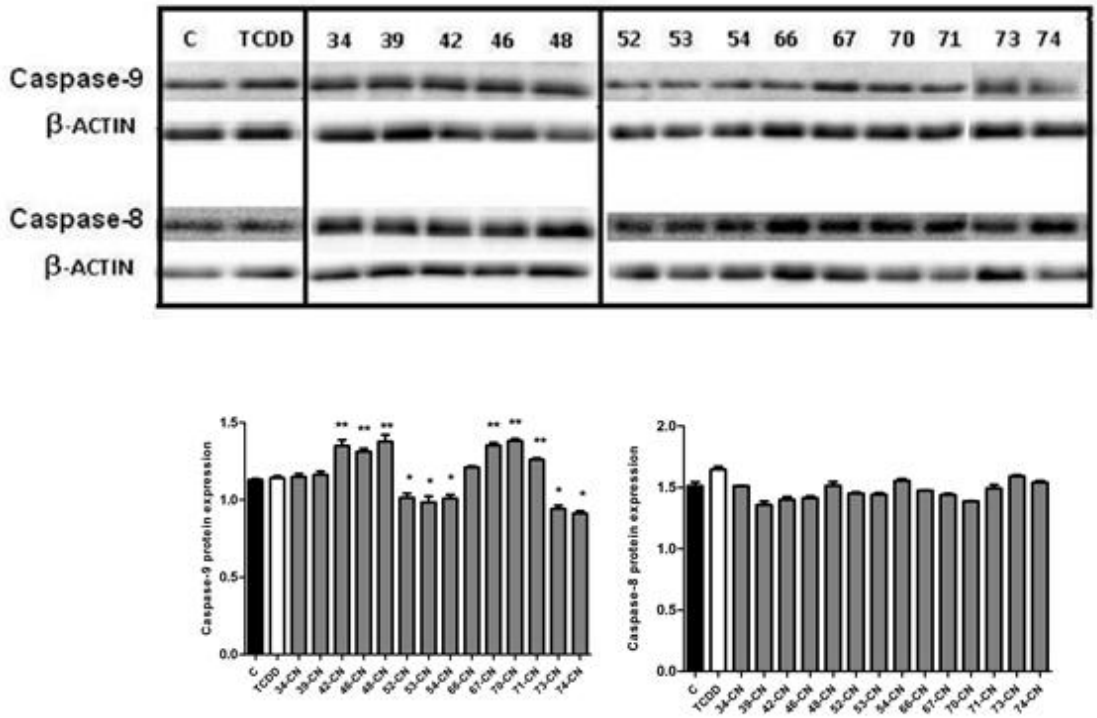

Fig. 3. Effects of TCDD and PCNs on caspase-8 and -9 protein expression. Densitometric analysis displays caspase-9 and -8 expression normalized to β-actin. Representative Western blots from three experiments are shown. All means marked with * ($p<0.05$) or ** ($p<0.01$) are significantly different from control values.

In conclusion, to the best of our knowledge, this is the first report showing the effect of a wide range of PCN congeners on MCF-7 breast cancer cells. These results clearly revealed opposite actions of lower- and higher-molecular mass chloronaphthalenes on cell proliferation and apoptosis. Because they simultaneously increase cell proliferation and suppress apoptotic processes, some of lower molecular mass chloronaphthalenes should be considered as potential cancer risk factors. Establishing of a toxicological significance of this non-genomic pathway of ligand-activated AhR in mediating the toxic actions of PCNs is needed.

References:

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