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CAN SOME A LOWER MOLECULAR MASS CHLORONAPHTHALENES BE CONSIDERED AS POTENTIAL NON-GENOMIC CANCER RISK FACTORS?

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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 weight from mono- to trichloronaphthalenes (Halowax 1000 and 1001) to predominately of octachloronaphthalene (Halowax 1051).

Toxic episodes of accidental animal and occupational human poisonings with PCNs took place after exposure to mixtures containing predominately penta- and hexachloronaphthalenes (Halowax 1014)[3].

The higher chlorinated technical PCN mixtures 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 a mixture of pollutants composed of different PCNs and polychlorinated biphenyls (PCBs) in a sediment core collected from the Tokyo Bay (Japan) and determined the cytotoxicity of extracts toward MCF-7 cells[5].

AIM

To our knowledge, there are no studies on the effect of single PCNs congener on breast cancer.
As a end point we evaluated action of single PCN congeners on cell proliferation and apoptosis of MCF-7 brease cancer cells.

MATERIAL AND METHODS

Cell culture

MCF-7 human breast cancer cells (ATCC, Manassas, VA, USA) 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 with TCDD at concentrations of 0.1, 1.0, 10, and 100 nM. Culture medium was used as a control.

XTT cell viability assay

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).

DNA fragmentation assay

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).

Caspase-8 and caspase-9 activity

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.

Caspase-8 and caspase-9 protein levels

Blots were incubated overnight with primary antibodies specific to Specific for caspase-8 (#9502; Cell Signaling Technology, Beverly, MA, USA) and caspase-9 (#9496; Cell Signaling Technology) diluted 1:200. After incubation with the primary antibody, the membranes were washed three times and incubated for 1 h with a horseradish peroxidase (HRP)-conjugated secondary antibody (#7074; Cell Signaling Technology). Immunopositive bands were visualized using Western Blotting Luminol Reagent (Santa Cruz Biotechnology Inc.) and the ChemiDoc XRS+ System (Bio-Rad Laboratories Inc.). The relative levels of protein expression were determined using ImageJ analysis software (US National Institutes of Health, Bethesda, MD, USA). Individual protein levels were normalized to that of β-actin controls, and the ratio of protein to β-actin was normalized to that in the untreated control group (defined as 1).

RESULTS AND DISCUSSION

From investigated PCNs tri-to tetra CN had effects on MCF-7 cells similar to those of TCDD while highly chlorinated PCNs had no stimulatory effect, and in the case PCN 66 and 74, even exerted an inhibitory effect on cell proliferation (Fig.1a). Hood et al. assessed toxicity and potency of PCN 66 and 67 relative to that of TCDD suggested that despite the fact that PCN 66 and 67 exposure resulted in biochemical and histopathologic 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.
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 cell

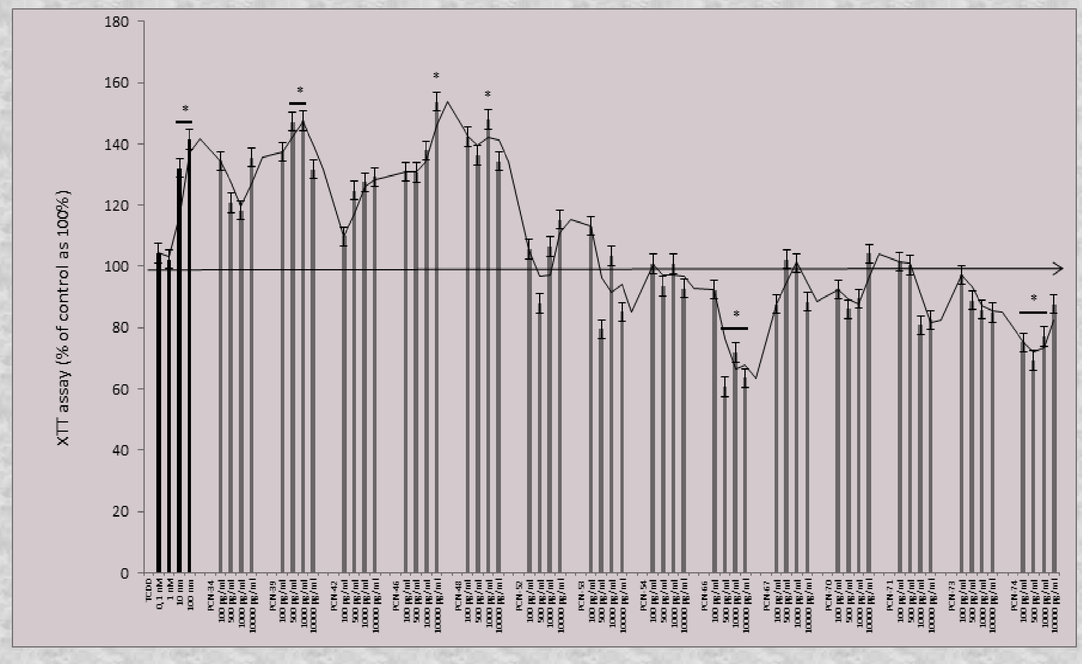

Fig. 1 Concentration-dependent effects of TCDD (0.1, 1.0, 10, and 100 nM respectively) and individual PCN (100, 500, 1000, and 10,000 ng/ml) congeners on a) cell viability determined by the XTT assay ; All means marked with * ($p < 0.05$) are significantly different from control values.

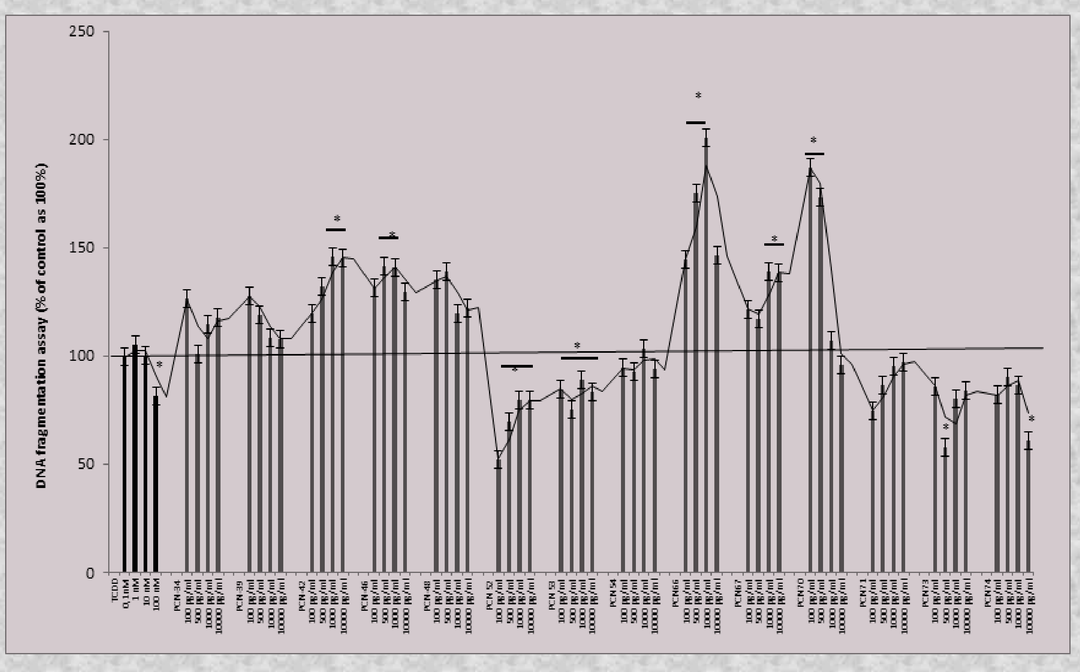

Fig. 2. Concentration-dependent effects of TCDD (0.1, 1.0, 10, and 100 nM respectively) and individual PCN (100, 500, 1000, and 10,000 ng/ml) congeners on apoptosis using DNA fragmentation assay. All means marked with * ($p < 0.05$) are significantly different from control values.

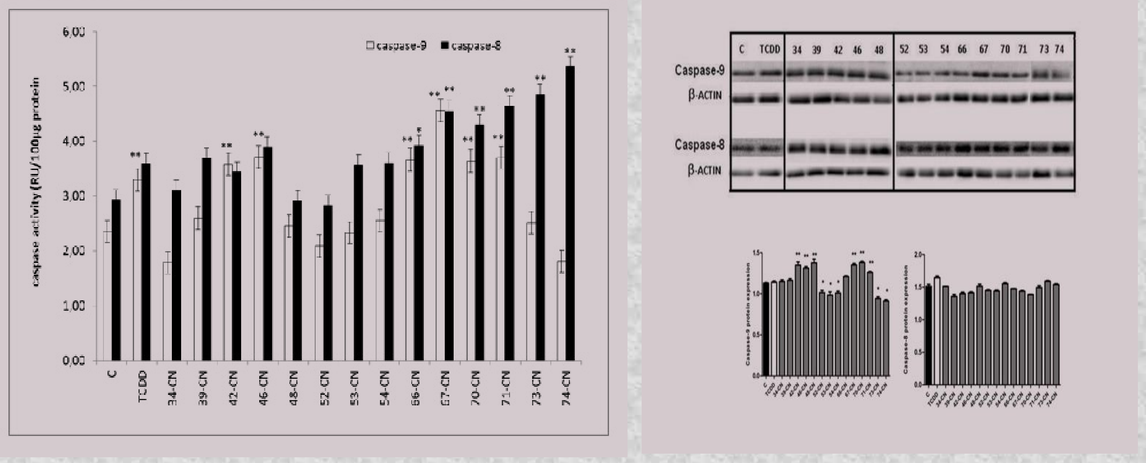

Fig.3. Effects of TCDD and PCN congeners on A) caspase-8 and -9 activity and B) caspase-8 and -9 protein expression. Densitometric analysis show caspase-9 and -8 expression normalized to β-actin. Representative Western blots from three experiments are shown. All means marked with * ($p<0.05$) or ** ($p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

- These results clearly revealed opposite actions of lower- and higher-chlorinated naphthalenes on cell proliferation and apoptosis.
- Because they simultaneously increase cell proliferation and suppress apoptotic processes, lower-chlorinated PCNs should be considered potential cancer risk factors.
- Establish the toxicological significance of this nongenomic pathway of ligand-activated AhR in mediating the toxic actions of PCNs is needed.

References:

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