Effect of *In Vitro* Exposure to Individual and Mixtures of PCBs and Tributyltin on Human Natural (NK) Cell Function

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Introduction

In the natural environment, humans and other animals are exposed to multitude of environmental contaminants. To assess the effects of environmental contaminants, mixtures of the compounds need to be tested as people are often exposed to more than one compound simultaneously. Polychlorinated biphenyls (PCBs) are industrial compounds, which have been identified as contaminants in air, water, soil, sediment, fish, wildlife and humans [1]. Exposure to these compounds results in various harmful effects including reproductive toxicity, immune suppression, birth defects and behavioral changes in animals and humans [2-5]. The primary route of human exposure is through consumption of contaminated food such as dairy products, meat and freshwater fish [6]. Another widespread contaminant is Tributyltin (TBT), which is mainly used in wood preservation, marine antifouling paints, disinfection of circulating industrial cooling waters, slime control in paper mills [7]. TBT is reported to cause serious health problems in lower trophic level organisms in the aquatic food chain [8] and suspected for immunotoxic effects on marine mammals [9]. Human exposure to TBT may come from consumption of TBT contaminated meat, fish products [10-13], and occupational exposure during the manufacture and formulation of TBT compounds, in application and removal of TBT containing paints, and from the use of TBT in wood preservatives [7]. Documented effect of TBT on human health include skin and eye irritation and inflammation of the respiratory track [14,15]. Recent studies have documented that, using *in vitro* tests, several toxic chemicals, particularly estrogenic compounds mixed together had greater effects than the additive effect of each chemical alone [16]. Although the PCBs and TBTs are well known for their individual toxic effects mentioned above, very little is known about their combined (mixtures) effect on immune function such as human natural killer (NK) cell function.

Natural Killer (NK) cells are lymphocytes which are capable of killing tumor cells, virally infected cells and antibody coated cells. NK cells are responsible for limiting the spread of blood-borne metastases as well as limiting the development of primary tumors. They are

defined by the absence of T cell receptor/CD3 complex and the presence of CD56 and/or CD16 on their surface. NK cells are able to lyse the above mentioned target cells without prior sensitization, putting them at the forefront of the lymphocyte defense against tumor cells and virally infected cells [17,18]. The present study describes the effect of *in vitro* exposure to individual and mixtures of PCBs and tributyltin on Human Natural (NK) Cell Function

Material and Methods

Blood Sampling and Isolation of NK Cells: Peripheral blood samples from healthy adult (male and female), volunteer donors were used for this study. Heparin-treated whole blood was applied to Ficoll-Hypaque (1.077 g/ml) and centrifuged at 500 g for 30 min. to remove red blood cells. Mononuclear cells were collected from the Ficoll-Hypaque and washed twice (250 g, 10 min.) with phosphate buffered saline (PBS). In order to remove platelets, the cells were suspended in a small volume (2.5 - 5 ml) of PBS and layered on to 2.5-5 ml of bovine calf serum (BCS) and centrifuged at 200 g for 5 min. The pellet was resuspended in PBS and the process was repeated. The cells were then suspended in complete medium which consisted of RPMI-1640 (Mediatech Cellgro, Fisher Scientific, MO, USA) supplemented with 10% heat-activated BCS, 2 m*M* *L*-glutamine and 50 U penicillin G with 50 µg streptomycin/ml (Sigma). Non adherent cells (30-40% CD16+, 60-70% CD3+) were prepared by incubating the cells in glass Petri dishes (150 X 15 mm) at 37 °C and 5% CO_2 for 1 h.[18].

Chemical Preparation: The PCB congeners, Aroclor mixtures and TBT were purchased from Ultra Scientific, RI, USA and Aldrich Chemical Co. WI, USA respectively. Dimethylsulfoxide (DMSO) was obtained from Sigma Chemical Co. St. Louis, MO, USA). Neat standards were resuspended in DMSO. The chemicals were diluted in gelatin media (0.5% gelatin replaced the calf serum in complete medium) and serial dilutions were prepared to achieve a range of concentrations, so that the final concentration of DMSO did not exceed 0.01%. The ranges of concentrations prepared were 0, 2, 20, 50, 100, 200, 400 nM and 1 µM. Aroclor mixtures were prepared in 0,1.0, 10, 50 and 100 ppm.

Cell Viability and Cytotoxicity: Cell viability was determined by trypan blue exclusion. Prior to assay, the NK cells were separated by centrifugation from complete medium (RPMI 1640) and transferred to gelatin media. Assays were conducted as short-term (1 hr, 24 h) and long-term (3 and 6 days) basis. Cell numbers and viability were assessed at the beginning and end of the assays. Cell numbers and their viability did not vary among experimental conditions. NK cytotoxicity was measured using a ^{51}Cr release assay [18]. The target cell in all cytotoxicity assay was the NK-susceptible K562 (human chronic myelogenous leukemia) cell line. An appropriate number of K562 cells were incubated with ^{51}Cr in 1 ml of BCS for 1.5 hr at 37 °C in air/CO_2 (19:1). The cells were then washed twice with gelatin medium. NK (effector) cells ($5.0x10^5$/100 µL for 50:1 ratio) were added to the wells of round-bottom microtitre plates. The effectors were diluted to 25:1 ratio ($2.5x10^5$/100 µL), 12.5:1 ratio ($1.25x10^5$/100 µL) and 6.25:1 ratio ($6.25x10^5$/100 µL); each ratio was tested in triplicate. Targets were added ($1x10^4$/100µL) to each well, and the plate was centrifuged at 600 rpm for 3 min. and incubated for 2 hr at 37 °C (air/CO_2 ,19:1). After incubation 0.1 ml aliquot of the supernatant was collected and counted for radioactivity for 60 sec in a CAPTUS-500 Model SWC (CAPINTEC, Inc. NJ, USA) gamma radiation counter. Specific lysis was

calculated as follows: 100x[(test c.p.m - spontaneous c.p.m.)/maximum c.p.m.- spontaneous c.p.m.)]. Maximum release was produced by adding 100 µL of 10% Triton X-100.

Results and Discussion

PCBs and TBT have been suspected for increased susceptibility of animals to infectious diseases [19]. This increased susceptibility to infections may be due to immunosuppressive impact of one or more compounds. Recent studies have shown that, in some *in vitro* tests, several toxic chemicals, particularly estrogenic compounds mixed together had greater effects (300 to 1600 times) than the additive effect of each chemical alone [16]. PCBs and TBT are widespread environmental contaminants and bioaccumulate in human and other animals. Individual and combined *in vitro* effect of PCBs and TBT on human NK cells function was studied and the results are summarized in Table 1.

Table 1. Effect of individual and mixtures of PCBs and TBT on human NK cells *in vitro*.

Compound	Treatment Concentration	Exposure Period	Result
3,3',4,4'-T_4CB	0, 2,20,100,200 nM	1 hr, 24 hr, 3 days	No Inhibition
3,3',4,4',5-P_5CB	0, 2,20,100,200 nM, 1µM	1 hr, 24 hr, 3 days	No Inhibition
3,3',4,4',5,5'-H_6CB	0, 2,20,100,200 nM	1 hr, 24 hr, 3 days	No Inhibition
T_4CB+ P_5CB+ H_6CB	0, 50, 200 nM, 1µM	3 days	No Inhibition
2,3',4,4',5- P_5CB	0, 200, 400 nM	3 days	No Inhibition
2,2',4,4',5,5'- H_6CB	0, 100,200,400 nM	3 days	No Inhibition
Aroclor 1016	50 ppm	3 days	**47% Inhibition**
Aroclor 1016	50 ppm	24 hr	No Inhibition
Aroclor 1254	100 ppm	3 days	**27% Inhibition**
TBT	50 nM	3 days	**18% Inhibition**
TBT	50 nM	24 hr	No Inhibition
TBT + P_5CB	50 nM + 100 nM	3 days	**17% Inhibition**
TBT + Aroclor 1016	50 nM + 50 ppm	24 hr	**No Inhibition**

The results revealed that dioxin-like PCB congeners, individually or as mixtures and other congeners (mono-*ortho* and di-*ortho* PCBs) under the *in vitro* experimental conditions did not suppress NK cell function. However, Aroclor mixtures (1016 and 1254) exhibited inhibition of NK cell function at higher exposure concentration and longer period (Table 1). TBT affects NK cell function drastically even at lower concentrations (<100 nM) in 3 day period. Comparison of individual and mixtures of Dioxin-like PCB congener (PCB-126) and TBT did not show elevated inhibition. Similarly, TBT and Aroclor 1016 did not alter NK cell function *in vitro*. The results give credence to infer that mechanism of action of PCBs and TBT are different and TBT and PCB combination did not influence the degree of effect on NK cell function under *in vitro* experimental conditions.

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