BISPHENOL A LEVELS IN INDOOR DUST, DRINKING WATER AND EFFECT ON HUMAN NATURAL KILLER CELL FUNCTION *IN VITRO*

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Introduction

4,4-dihydroxy-2,2-diphenyl propane ($C_{14}H_{16}O_2$), commonly known as bisphenol A (BPA). It is an organic compound synthesized for the first time in 1891 by condensation of acetone with phenol.[1] Large scale commercial production began in 1957. In 2004, the global annual production rate was > 6 billion pounds[2,3]. BPA is one of the highest production volume chemicals. According to the National Institutes of Health, approximately 940,000 tons of BPA are produced in the US per year.[2]

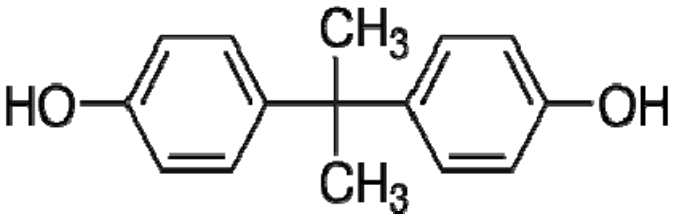

Figure 1. Structure of Bisphenol A. CAS #: 80-05-7.
Bp. 220 °C at 4 mm Hg, Vapor pressure: 0.2 mm Hg at 170°C, Log *K*ow: 3.3 to 3.8.

BPA is used in many plastic consumer products such as toys, water bottles, eye glass lenses, sports safety equipment (eg. Helmets), dental sealants, medical equipment and consumer electronics (CDs. DVDs, cell phones and computers).[3] BPA is a well-known endocrine disruptor. Laboratory animal and human epidemiological studies have shown that BPA can affect reproduction and development and may cause cardiovascular disease, diabetes, prostate cancer, neurobehavioral complications, and liver-enzyme abnormalities.[4]

Exposure to BPA is thought to result primarily from the ingestion of contaminated food. In addition to diet, indoor air and house dust as well as drinking water from plastic water bottles can contribute human exposures to BPA. Ingestion of house dust has been demonstrated to be an important exposure pathway to several organic contaminants in young children.[5] Recent studies have shown that drinking water from bottled water contributes to BPA exposure in adults.[6] BPA is transported to various organs and systems via blood circulation.[7-9] BPA exposure due to dust ingestion and drinking bottled waters in the United States has not been thoroughly evaluated. Further, effect of BPA on blood cells especially cancer fighting natural killer lymphocytes is not known. NK cells are subset of lymphocytes, which are primary defense against tumor cells and virally infected cells. The aim of the present study

was to determine the concentrations of BPA in indoor dust collected from two locations in the Eastern United States and to estimate human exposures through the ingestion of dust. Various brand bottled waters were analyzed for BPA concentrations to evaluate BPA exposure via drinking water from bottled waters. We used high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method to determine BPA in indoor dust samples. Enzyme-linked immunosorbent assay (ELISA) was used to determine BPA concentrations in bottled waters. Chromium release assay was employed to evaluate cytotoxicity of BPA on natural killer lymphocytes with K562 leukemia cells as target cells.

Materials and Methods

Indoor dust samples (n=56) were collected randomly from 22 houses and 6 laboratories in Murray (Kentucky) and Albany (New York) in the spring and summer of 2010 and from 28 houses in Albany in the summer of 2006. Additionally, four clothes drier lint from vents of driers and two dust samples from inside the housings of refrigerators in a subset of homes in Albany in 2010. All samples were stored in a freezer at -20°C until further analysis. The dust samples were passed through a 425 μm mesh sieve and homogenized. Approximately 100 mg of sample was weighed and transferred into a 15-mL polypropylene tube and the sample was spiked with 50 ng d_{16} BPA as an internal standard. Then sample was extracted with 3 mL ethyl acetate by shaking for 30 min using reciprocating shaker. The sample was centrifuged at 4000 rpm for 5 min and aliquot was quantitatively transferred into a new tube. The extraction step was repeated, then extract was concentrated using nitrogen gas to near dryness and 3 mL of methanol was added and the extract was passed through 0.2 μm nylon filter and transferred into a 2 mL sample vial and analyzed for BPA using HPLC-MS-MS.

Several brands of bottled water samples were purchased from local supermarket. Solid phase extraction (SPE) techniques were used for each of the samples using OASIS® C18- Hydrophilic Lipophilic Balanced-SPE cartridges obtained from Waters Corporation. (6mL, 0.2g, 30 μm). Cartridges were preconditioned using 7mL of methanol followed by 3mL of deionized water (18 ΩM). Samples were passed through cartridges and vacuum was applied to obtain a flow rate of 2 mL/min. BPA was eluted from the cartridges using approximately 10 mL of a 4:1 ratio of dichloromethane and hexane mixture. Sample extract was evaporated close to dryness using nitrogen gas and then 1mL methanol was added. 100 μL of this solution was used for ELISA to determine BPA concentration in each sample.

Peripheral blood samples (as buffy coats) were purchased from American Red Cross, Portland OR. Donors gave informed consent to the Red Cross. Department of Chemistry, Tennessee State University (TSU) was approved for receipt of blood products by the Institutional Review Board (IRB) of the American Red Cross. Additionally, IRB of TSU has granted approval for the use of buffy coats for research purposes. Natural killer (NK) lymphocytes were isolated using standard procedure.[10] BPA standard was purchased from Spex Certiprep group, U.S.A.). DMSO (dimethylsulfoxide) was purchased from Sigma Chemical Co. Stock solution was prepared by dissolving neat BPA in DMSO. Serial dilutions of the solution were prepared to achieve 10 nM, 100 nM, 500 nM, 1 μM 10 μM and 20 μM. Appropriate DMSO controls were also prepared. Cytotoxicity tests (^{51}Cr Release assay) were performed exposing NK cells with above concentrations of BPA for 1h, 4h, 24h, 48h and upto 5 days. Cell viability was determined by Trypan blue exclusion. Cell numbers and viability were assessed at the beginning and end of the exposure period. NK cells ability to kill cancer cells was measured using ^{51}Cr release assay.

Results and Discussion

BPA Concentrations in Indoor Dust

BPA was found in 95% (n= 56) of the dust samples analyzed from Murray, Kentucky and Albany, New York Table 1). The overall BPA concentrations in the 56 dust samples ranged from <0.5 to 10,200 ng/g. The mean and median BPA concentrations were 843 and 422 ng/g respectively. The highest concentration, 10,200 ng/g was found in the dust sample collected on the surface of an air filer placed at the doors of a laboratory in Albany. The air filter had been collecting dust for <1 yr. Comparison of BPA concentrations of Murray, KY and Albany, NY revealed no significant difference (*F*-test analysis of variance, $P > 0.05$). The man and median concentrations of BPA in dust samples from the Murray Laboratories were greater than the concentrations found in homes, although the difference were not statistically significant (Table 1). The median values for BPA intake by way of the ingestion of dust by adults and toddlers were calculated to be 0.35 and 5.63 ng/kg body weight per day.

Table 1. Concentrations of bisphenol A (ng/g) in indoor dust samples collected from two locations (Murray, KY and Albany, NY) in the eastern United States.

Location	Year(s)	Number of Samples	Mean	Range
Murray, KY	2010	12	837	172-2950
House dust	2010	7	520	172-2130
Laboratory dust	2010	5	1280	445-2950
Albany, NY	2006	28	620	<0.5-1690
House dust	2006	28	620	<0.5-1690
Albany, NY	2010	16	1240	<0.5-102,00
House dust	2010	9	836	135-2320
Laboratory dust	2010	1	10,200	NA
Clothes-drier lint	2010	4	18.7	<0.5-34.8
Refrigerator dust	2010	2	890	550-1410
Overall	2006 and 2010	50	843	<0.5-10,200

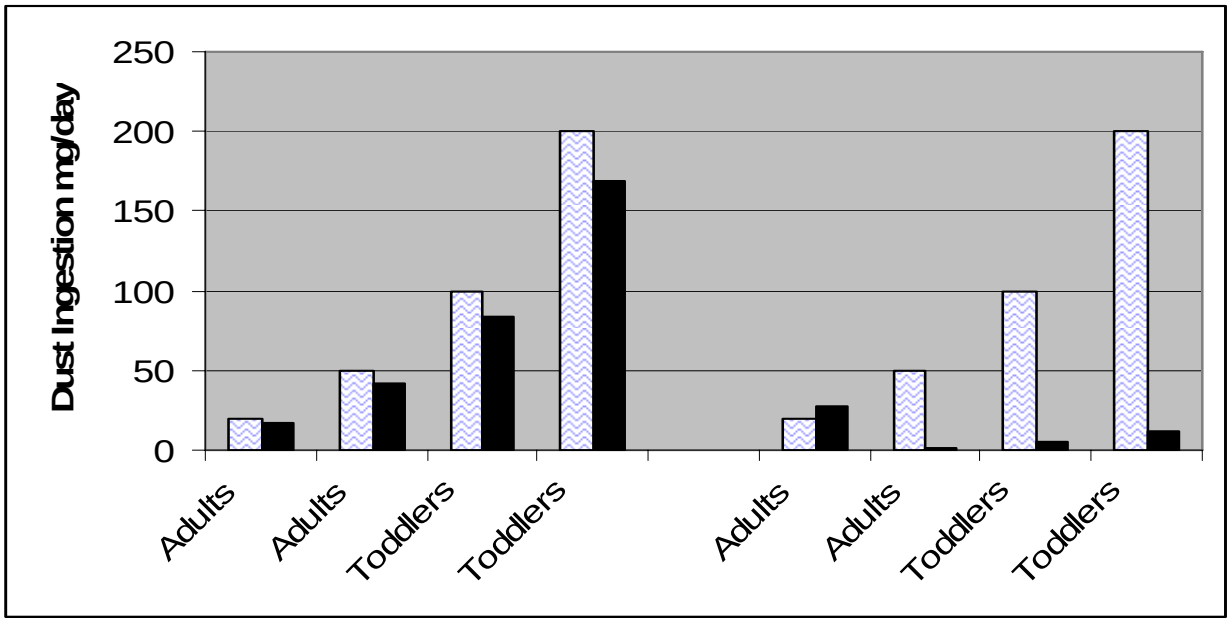

Figure 1. Calculated levels of (ng/day and ng/kg/day) of mean, by way of house-dust ingestion to bisphenol A by adults and toddlers from Murray, KY and Albany, NY in the United States.

These estimated exposure doses of BPA through dust ingestion are of the same order of magnitude as the recently reported low concentrations that induced health effects in laboratory animal studies. The contribution of dust to total human BPA intake was estimated to be <1%, however, suggesting that dietary intake is the predominant source of exposure in humans.

BPA Concentrations in Bottled Waters

Figure 2 shows BPA concentrations found in several brands of bottled waters. BPA concentrations ranged from 0.5 to 430 ng/L. Except one of the bottled waters, other samples had mean BPA concentrations were below 100 ng/L.

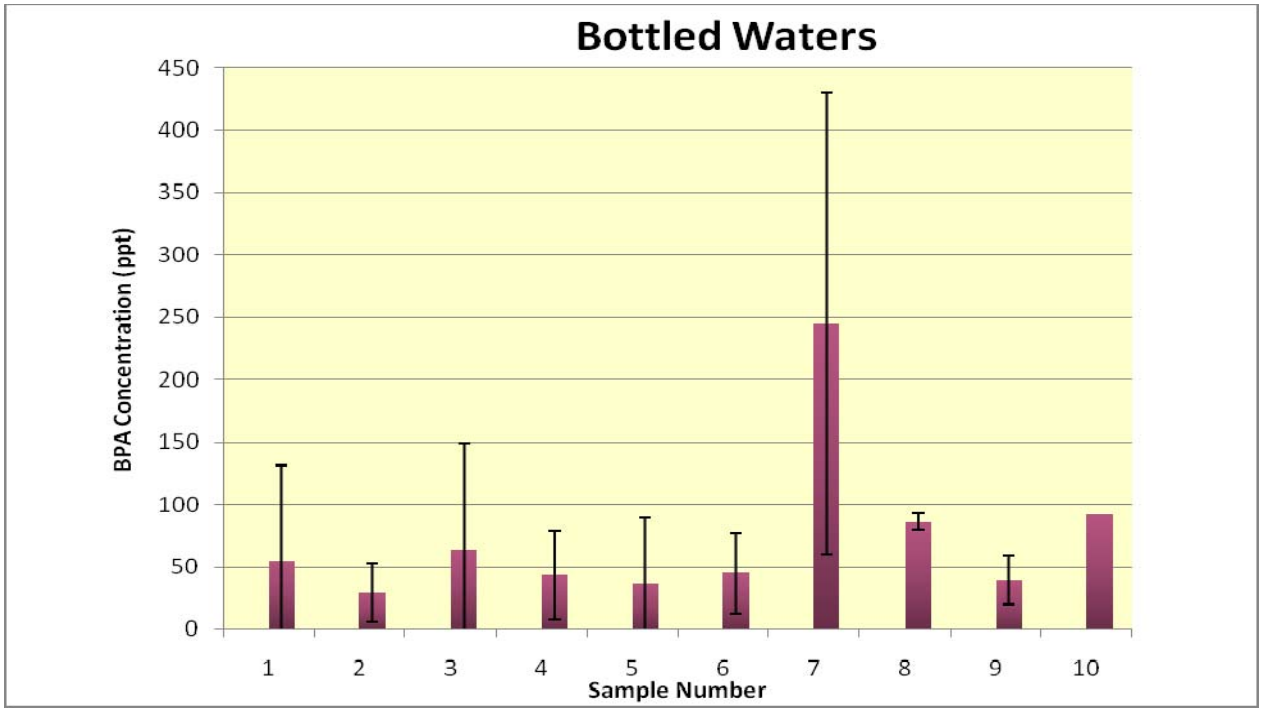

Figure 2. Bisphenol A concentrations in various brands of bottled water samples purchased from local markets in Murray, Kentucky, USA.

Reported BPA concentrations in drinking water in Guangzhou, China was 2.3 to 317 ng/L. BPA concentrations in natural waters such as Kentucky Lake and Clarks River, KY ranged from 60-140 ng/L and 80-290 ng/L respectively. A person weighing 50 kg (110 lbs) would need to consume over 1,390 L of bottled water to surpass the current EPA (United States Environmental Protection Agency) daily safety limit of BPA. Earlier studies have documented levels of BPA in blood samples.

Table 2. shows BPA levels reported in human blood samples.

Sample Type	BPA Concentration	Reference
Maternal Blood	0.21-0.79ng/L	7
Umbilical Cord Blood	0.45-0.76ng/L	7
Blood Serum	0.22-0.87ng/L	7
Blood Serum	0.32ng/ml (*n*=5, RSD 5.0%)	8
Unspiked Human Sera	0.32-0.92ng/ml	9

Since BPA is present blood samples, it can be surmised that BPA may have some effect on functions of blood cells. I n this study, we examined whether, BPA affect human natural killer cell's cancer-fighting ability using K562

Leukemia cancer cells in vitro. Table 3 shows various concentrations and exposure period and effects on NK cell's function.

Table 3. Effect of bisphenol A on human NK cell's ability to kill K562 leukemia cells.

Exposure Concentration	**Hours**	**Effects**
10 nM	1h, 24h, 5 days	No effect
100 nM	1h, 24h, 5 days	No effect
500 nM	1h, 24h, 5 days	No effect
1μM	1h, 24h, 5 days	No effect
10 μM	1h, 24h, 5 days	No effect
20 μM	1h, 24h, 5 days	No effect

The results revealed that bisphenol A did not affect human natural killer cell function up to 20 μM l exposure concentrations up to 5-days exposure period under laboratory conditions. Although BPA is a suspected carcinogen, it is not inhibiting NK cells ability to kill cancer cells.

References

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