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Polychlorinated Biphenyls (PCB-153) and (PCB-77) absorption in human liver (HepG2) and kidney (HK2) cells *in vitro*: PCB levels and cell death

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Abstract

An understanding of congener specific cellular absorption of PCBs is important to the study of the organ specific body burden of an individual and to their toxic effects. We have previously demonstrated that single PCB congeners induce cytotoxicity, as evidenced by decreased cellular viability and accelerated apoptotic death. There is very little, if any, information available on the differences in toxicity due to the nature of absorption of PCBs in different cells. To obtain such information human liver (HepG2) cells (in medium with 10% FBS) were exposed to 70 μM of both PCB-153 (non-coplanar hexachlorobiphenyl) and PCB-77 (coplanar tetrachlorobiphenyl), and human kidney (HK2) cells in serum free medium were exposed to 80 and 40 μM of PCB-153 and PCB-77 respectively, according to their LC₅₀ values in these cells. Medium and cells were collected separately at each time interval from 30 minutes to 48 hours, and PCB concentrations were analyzed in both by GC-MS using biphenyl as an internal standard following hexane: acetone (50:50) extraction. We also performed trypan blue exclusion, DNA fragmentation and fluorescence microscopic studies in assessing cell viability and apoptotic cell death. About 40% of PCB-153 (35 μM, 50% of the maximum value) was detected in HepG2 cells within 30 minutes, and it reached its highest concentration at 6 hours (60 μM), concomitant with the PCB depletion in the medium (5 μM). For PCB-77, the highest concentrations within the cells were reached at 3 hours. However, the absorption levels of PCB-153 and PCB-77 in HK2 cells reached their peaks at 3 and 6 hours respectively. Exposure of human liver and kidney cells to PCB-153 and PCB-77 caused accelerated apoptotic cell death in a time-dependent manner. The studies demonstrated that (1) liver cells initiate the absorption of PCBs much faster than kidney cells; however, the concentration reaches its maximum level much earlier in kidney cells; (2) both PCB-153 and PCB-77 induced enhanced apoptotic death in liver and kidney cells; (3) kidney cells are more vulnerable to PCBs based on the results of apoptosis and cellular viability, even with almost similar absorption or tissue burden of PCBs.

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Conflict of Interest Statement

There is no conflict of interest in the present work among the authors.

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Keywords

PCBs; HepG2 Cells; HK2 Cells; Cellular absorption; GC-MS; DNA Fragmentation

1. Introduction

Polychlorinated Biphenyls (PCBs) with their possible 209 congeners are persistent environmental toxicants. Thus commercial production in the United States was stopped in 1977. However, our biosphere still contains approximately 750,000 tons of PCBs, which are responsible for reproductive, neurological, endocrinal and other defects (James, 2001). PCBs, particularly the highly chlorinated congeners, are characterized by high lipophilicity and slow rates of biotransformation. The differences between the individual congeners in their uptake, distribution, biotransformation, and elimination are important controlling factors in the body burden of an individual and in their toxic effects, which are also associated with their particular metabolites along with the parent compound. A major structural factor in determining the toxic properties and potencies in PCBs is the presence of the chlorine atom in the *ortho* position, influencing the ability to adopt co-planar conformation (Safe, 1994). The individual congeners PCB-77 (3,3',4,4'-tertrachlorobiphenyl; a non-*ortho* substituted co-planar congener) and PCB-153 (2,2',4,4',5,5'-hexachlorobiphenyl; a di-*ortho* substituted non-coplanar congener) have different toxicological and chemical properties, representing model substances for the different classes of PCBs. However, the analysis of total PCB concentrations in biological samples gives limited information, whereas congener-specific analysis is more informative, but complicated (Skerfving et al., 1994). It was previously (Kodavanti et al., 1998) reported that, in addition to differential total uptake between tissues, there was a degree of difference in accumulation of PCBs depending up on the number, and the position of chlorine atoms in the molecules.

Health effects that have been associated with exposure to PCBs include acne-like skin conditions in adults, neurobehavioral and immunological changes in children (Park et al., 2009). Being an omnivorous mammal, humans are exposed to PCBs primarily via low-level food contamination. When the intake and elimination in humans has reached a steady state, the distribution of lipophilic compounds reaches equilibrium and depends mostly on the cellular transport mechanism. It is found that decrease in PCB congener concentrations were associated with chlorine configuration, which is known to amenable to metabolism (Wolf et al., 1992). The concentrations of PCBs in the human kidney and brain were significantly lower than those in liver and muscle (Bachour et al., 1998). A study on the levels of persistent organochlorine pollutants in human tissues from Belgium found that the total PCB concentrations in the liver were similar to those in three specimens: muscle, kidney, and brain (11.2, 14.4, and 12.7 ng/g tissue wet weight, respectively), where PCB-153 and PCB 180 were the main *ortho*-substituted congeners and each of them accounting for approximately 30% and 25%, respectively, of the total PCB in all samples (Chu et al., 2003), and the PCB congener patterns in different organs from individual subjects were similar. There were no significant differences between the distributions of congeners having different degrees of chlorination. It is well known that the PCB pattern shifts from low-chlorinated congeners to higher chlorinated congeners when the organisms move from lower to higher trophic levels (Kumar et al., 2001). The higher concentrations of PCB-153 and PCB 180 (persistent congeners) and the absence of low chlorinated congeners in individual subjects indicated that the principal source of contamination with PCBs was from diet (food chain/food web), and not from direct exposure (inhalation/contact). The role of PCBs in induction of enhanced apoptotic death has also been well documented (Ghosh et al., 2007; Chen et al., 2006; Howard et al., 2003; Hwang et al., 2001; Lee et al., 2001).

This study is part of an extensive investigation on the possible toxic effects of PCB-77 and PCB-153 in Liver (HepG2) and Kidney (HK2) cells, where some preliminary data has been obtained towards differential gene expression in these tissues (Dutta et al., 2008). We have also demonstrated that CYP1A1 and MT1K are congener specific biomarker genes for liver diseases induced by PCBs. However, little, if any, is known about the toxicity of those PCBs upon immediate absorption upon human cell lines. The PCB levels in human liver and kidney cell lines *in vitro* under the present experimental condition might demonstrate a correlation with PCB accumulations, and thereby can provide knowledge about the most vulnerable sites of human organs. In the present study, we considered: (1) absorption of PCB congeners 77 and 153 in Liver (HepG2) and Kidney (HK2) cells following exposure to PCBs at different time intervals and (2) biological changes after exposure to PCBs (77 & 153) by assessing cell viability and apoptotic cell death, representative cell lines *in vitro*.

2. Materials and Methods

2.1 Chemicals and reagents

Non-planar PCB-153 (2,2',4,4',5,5'-Hexachlorobiphenyl) (Product # RPC-047, CAS # 035065) and co-planar PCB-77 (3,3',4,4'-Tetrachlorinated biphenyl) (Product # RPC-036, CAS # 032598) used here are from Ultra Scientific (North Kingstown, RI). Dimethyl sulfoxide (DMSO) (Sigma, St. Louis, MO) was used for dissolving PCBs. A 50 mM stock solution of the compound was prepared in dimethyl sulfoxide (DMSO) and diluted further, to the working concentration, in the same diluents. The final concentration of DMSO in the culture medium was $\leq 0.1\%$. Hexane, acetone, and HPLC grade water (Fisher, Suwanee, GA) were used for PCB extraction and GC-MS studies. PBS 1x sterilized solution was procured from Quality Biological Inc. (Gaithersburg, MD).

2.2 Cell line and culture conditions

In this study, we selected a metabolically competent human hepatocellular carcinoma cell line (HepG2), which has retained many of the functions of normal liver cells (Knowels et al. 1980) and expresses the activities of several phase I and II xenobiotic metabolizing enzymes (Knasmuller et al. 1998). In particular, HepG2 cells (ATCC # HB 8045, Manassas, VA) were maintained in 1x DMEM medium with low glucose containing 100 units/ml penicillin G, 100 μ g/ml streptomycin medium supplemented with 10% Fetal Bovine Serum (FBS, Invitrogen, heat inactivated) at 37° C in an atmosphere containing 5% CO₂. Human kidney 2 (HK2, ATCC # CRL-2190) is a proximal tubular cell (PTC) line derived from normal kidney. These cells are grown in Keratinocyte-Serum Free Medium with 5 ng/ml recombinant epidermal growth factor, 0.05 mg/ml bovine pituitary extract, 50 U/ml Penicillin, 50 mg/ml Streptomycin, and 2.5 mg/ml Fungizone. All the cell culture media and additives were obtained from GIBCO-BRL, CA.

2.3 In vitro cytotoxicity and cell growth assay

To obtain the LC₅₀, adherent cells (HepG2 and HK2) were exposed to 20–140 μ M PCBs for 24 hours, harvested with trypsin, washed with PBS twice, and collected by centrifugation at 150g for 5 minutes. Viable cell counts were enumerated by trypan blue uptake using 0.2 % trypan blue (Freshney 1987) and compared to control (DMSO only). The experiment was repeated three or more times. The representative data shown in this paper (Figure 3E–H) were reproducible in three independent experiments.

2.4 PCB absorption studies

For experimental purpose, we chose the LC₅₀ concentration, i.e. 70 μ M of PCB-77 and 153 treatments on HepG2 Cells, and 80 μ M and 40 μ M for PCB-153 and PCB-77 treatments for

HK2 cells respectively, as before in our earlier studies (Dutta et al., 2008; Ghosh et al., 2007; Chen et al. 2006). The human liver (HepG2) and kidney (HK2) cells were plated in 100mm tissue culture dishes in their respective media. Cultures were confluent after 4–5 days and all experiments were performed with confluent monolayer. Medium was refreshed prior to the experiment. PCB-153 and PCB-77 (dissolved in DMSO) were added to each plate individually according to their LC₅₀ concentrations where the final concentration of DMSO was $\leq 0.1\%$. The exposures were the same in all the experiments at 2, 4, 6, 12, 24, and 48 hours, where 0 hours exposure served as control. Control cell lines were allowed to grow with DMSO only ($\leq 0.1\%$ of the total medium v/v) to ensure that the changes seen were not due to DMSO.

2.5 Sample preparation

After the stipulated exposure to the respective PCBs, cells were collected from each plate following trypsinization in a 15-ml tube and centrifuged at 600g for five minutes. Medium from the respective plates was also collected and centrifuged to get the clear supernatant medium. Cell pellets were taken in a 10 × 75 mm borosilicate tube and washed twice with 1x PBS. The total cell pellets were then resuspended in 0.5 ml of PBS and pulverized with a Hexane:Acetone (50: 50) solution with 10ng/ml biphenyl as an internal standard. The tubes were then centrifuged at maximum 11,330g at 4°C and allowed to settle for 10 minutes. The supernatant was taken and stored in an amber color tube in dark for Gas chromatography–Mass spectrometry (GC-MS) studies. Similarly, the 0.5 ml of clear medium was treated, centrifuged and stored.

2.6 GC-MS Analysis of PCBs

Gas chromatography–mass spectrometries (GC-MS) were performed using Agilent 6890N gas chromatograph interfaced with an Agilent 5973 *inert* Mass Spectrometer. The interface oven temperature was maintained at 250° C, and the ionizer temperature setting was 230° C, using electron ionization (EI) with electron energy at 70eV. High resolution capillary gas chromatography was conducted with a Supelco fused-silica SPB-5 (30m, 0.32 mm i.d., 0.25 µm film) column (Sigma-Aldrich, St. Louise, MO); oven temperature was programmed from 50° C to 300° C (20° C/min); and helium was used as a carrier gas with head pressure of 9.8 psi. The data was analyzed using HP Chemstation Productivity Software (vD1.00) and NIST 2002 library.

2.7 Quality assurance & calculation of total amount of PCBs

Pure PCB-153 and 77 were dissolved in DMSO and diluted to graded concentrations (1, 5, 10, 25, 50, and 75 µM, with 10 ng/ml Biphenyl as internal control, data not shown). About 1 µL of standard solution was injected into GC-MS system. All of the standard curves have high R² value. The level of biphenyl peak was also monitored showing constant loading during each injection, with retention time at 8.67 minutes, and for PCB-153 and PCB-77 at 13.64 minutes and 11.20 minutes respectively (Figure 1). The total amount of PCBs in the respective medium and in the cell (cytosol) was measured using ratios of PCB/BPH (Area/Area) from the concentration curve obtained earlier.

2.8 Detection of cell death

Cell death (apoptotic) was determined by a DNA-fragmentation assay and by a fluorescent detection probe. A simplified and fast protocol for the analysis of DNA-fragmentation during apoptosis was adapted as previously described (Kratzmeier et. al., 1999), after slight modification. Briefly, the liver and kidney cells were grown in 100 mm tissue culture dishes (less than 10⁶ cells) and treated with the same concentration of PCBs used in the absorption study. All the plates containing PCB-treated cells at different time intervals were washed thrice with PBS, and 800 µL of lysis solution (0.2% Triton X, 10mM Tris, 10mM EDTA) was added

to the plates and incubated for 5 minutes at 4° C. The cells were scrapped and transferred into a 2ml microcentrifuge tube. The tubes were then centrifuged at 11,330g for 10 minutes at 4° C, and supernatant was taken in a fresh tube, discarding the pellets. The supernatant containing the DNA was purified with a PCR purification Kit (Qiagen MiniElute PCR Purification Kit; Cat # 28004). For regular DNA precipitation, to each 400 µL of supernatant, 8 µL of 5M NaCl was added and vortexed for 5 seconds. Ethanol (800 µL) was then added, again vortexed for 5 seconds, and the tubes were incubated on ice for 10 minutes. Tubes were then centrifuged at 11,330g for 5 minutes. The supernatant was removed and the ethanol was air dried. The precipitated DNA was re-suspended in 30 µL of DNase-free water. DNA concentration was determined by means of the absorbance at 260 nm, considering that one absorbance unit at 260 nm corresponds to 50 µg/ml of double-stranded DNA. DNA samples (5µg/lane) were electrophoresed in 1.5% agarose gel in buffer containing 89mM Tris (pH 8.0), 89 mM boric acid and 2mM EDTA, and were run for 1.5 hour at 100V along with standard DNA markers. After electrophoresis, the gel was stained in 0.5 µg/ml ethidium bromide solution, and DNA was visualized using ultraviolet lights.

The occurrence of apoptotic death was also analyzed by DNA binding flurochrome Hoechst 33342 dye. Apoptotic cells were identified by the nuclear chromatin condensation and membrane blebbing. Primarily, HepG2 and HK2 cells were grown in a monolayer to 40–50 % confluency in a LAB-TEK chamber slide (2 well ParanoX Slide No. 177429) in their respective medium treated with same concentration of PCB-153 and PCB-77 used in the absorption study (as described previously) with untreated cells as a control. After the treatment schedule (0–24 hours), the cells were washed with 1x PBS to remove the medium. Cells were then fixed with 100% cold methanol for 20 minutes, washed with PBS, and stained with 3M Hoechst 33342 dye in PBS for 15 minutes. Cells were again washed with PBS. Excess PBS was blotted off the slide, and the slide was mounted with antifade mounting medium Vectra Mount (H-5000). Fluorescent nuclei were seen under a microscope using a green filter as described (Ghosh et al., 2007).

2.9 Statistical analysis

As the starting concentrations were different for different types of PCBs, and for different cell types, the data were normalized and percent uptake was calculated based on the maximum cellular concentration value for each cell type and PCBs. For depletion from medium, the initial concentration was used as 100%. All the results were statistically analyzed and the results are expressed as mean $\pm$ SD (GraphPad Prism 5.0). Two way ANOVA with Bonferroni's post test was performed at every time point (as noted in Figure 2A–D) to test the different cellular accumulation of PCBs between liver and kidney, and also to identify significant differences between the PCB-77 and PCB-153. One-Way ANOVA with Tukey's Multiple Comparison test was also carried out for PCB 153 and PCB 77 absorption over time in each cell type and PCB. In all cases $p < 0.001$ was considered as statistically significant level.

3. Results

3.1 PCB absorption

Exposures of human liver (HepG2) and kidney (HK2) cell cultures to PCB-153 and PCB-77 at their respective LC₅₀ levels results in time-dependent accumulation of PCB in the cells. The time course of PCB-153 and PCB-77 absorption within the cell (cytosol), when compared with the medium in HepG2 and HK2 cells are shown in Figure 2. One-Way ANOVA with Tukey's Multiple Comparison test was also carried out for PCB 153 and PCB 77 absorption over time in each cell type. In the human liver (HepG2) cells, PCB-153 started entering into the cells from as early as 30 minutes and resulted in 40% of PCB removal from the medium (Figure 2A/AM₁) resulting in cellular concentration of 35 µM (50% of maximal value, Figure 2A/

AC₁). In liver HepG2, PCB-153 reached its highest concentration after around 6 hours (Figure 2A/AC₄), where cellular and medium concentration was found at 60 μ M and 5 μ M respectively. There were no statistically significant changes in PCB-153 concentration in the cell as well as medium beyond that time point. The results also show that 70% of PCB-77 was removed from the medium by HepG2 cells by 3 hours (Figure 2B/BM₃) along with the increase of PCB-77 in the cytosol (Figure 2B/BC₃). The peak concentration was reached at 18 hours where 90% of PCB was found within the cells (Figure 2B/BC₆) as there was statistically significant difference between 6 and 18 hours (Figure 2B/BC₄ vs. Figure 2B/BC₆).

In kidney (HK2) cells, 30% of PCB-153 (22 μ M) entered the cells within 1.5 hours (Figure 2C/CC₂). This is also corroborated by the depletion of PCB from the medium at the same time (Figure 2C/CM₂). The peak cellular concentration was reached at 3 hours (95%; Figure 2C/CC₃). However, PCB-77 absorption was slower than PCB-153, with only 37% of PCB-77 absorbed at 3 hours (Figure 2D/DC₃). However, once entered, it reached its peak at 6 hours (Figure 2D/DC₄).

The two way ANOVA with Bonferroni's post test showed that there was a significant difference between the cell types and between the PCBs at different time points. Based on these analyses, it can be concluded that PCB-153 uptake was faster in liver cell line cells at early points (up to 1.5 hours, Figure 2A/AC₁ to Figure 2A/AC₂). In contrast, the concentration of PCB 153 in kidney cells reached its maximum by 3 hours (Figure 2C/CC₃) followed by a slight decrease (about 21% decrease; 3 hours vs. 48 hours at $p < 0.05$) may be due to excretion of PCB 153 by kidney cells. In PCB 77, the uptake is more or less similar at early time point in both the cell types (up to 0.5 hours Figure 2B/BC₁ and Figure 2D/DC₁). There was a significant difference between the PCB-77 uptake at 1.5 hours between the HepG2 cells (Figure 2B/BC₂) and Hk2 cells (Figure 2D/DC₂). The significant difference increased up to 3 hours, when the uptake of PCB 77 reached 70% in HepG2 cells (Figure 2B/BC₃), compared to only 37% (Figure 2D/DC₃) in kidney cells. But at the end (6 hours to 48 hours), the accumulations of PCB 77 were same in both the cell types.

3.2 Effect of PCB-153 and 77 exposures on human liver and kidney cell death

To verify the possible involvement of PCB induced cell death (apoptosis), we observed morphological apoptotic features using Hoechst dye fluorescence probe as well as DNA fragmentation studies using agarose gel electrophoresis. Morphological changes in response to the exposure of human liver and kidney cells with PCB-153 and PCB-77 were also examined by fluorescence microscopy after staining with Hoechst dye, which enters into the cells and binds to DNA. Figure 3 shows pyknotic nuclei and chromatin condensation in cells treated with PCB-153 and PCB-77. We have represented our results here in time-dependent manner. When human liver (HepG2) cell cultures were treated with the respective LC₅₀ dose of PCB-153 and PCB-77, the apoptotic nuclei were significant from 12–24 hours of incubation time (Figure 3A). However, treatment of human kidney cells (HK2) with the same PCBs has shown that apoptotic nuclei appear after 6 hours of exposures (Figure 3C).

The cell death due to exposure to PCB-153 and PCB-77 is concentration-dependent in liver and kidney cells, determined by cytotoxicity assay (Figure 3E–H). DNA fragmentation was evident in fluorescence studies in a time-response manner (Figure 3B & 3D). The changes observed in DNA fragmentation started as early as 6 hours for both the PCBs in kidney cells, compared to 24 hours in liver cells, which was corroborated by the results obtained from the Hoechst dye technique. No significant differences in apoptosis induction were noted between untreated cultures and cultures incubated with $\leq 0.1\%$ DMSO during 24 hours, which was used as vehicle for PCBs (data not shown).

4. Discussion

The present studies clearly show that there is a correlation between cellular PCB concentration and cell death. Chronic exposures to PCBs over 48 hour resulted into a growing level of bioaccumulation. Humans, sea mammals and some animals at the highest trophic levels, have the highest concentration of PCBs (Mossner and Ballschmide, 1997; Fisher, 1999). The overall biological and toxic effects of PCBs, as deduced from animal studies, are rather complex, and they are not confined to one organ system. Our earlier study on the PCB-153 induced chronic exposure in HepG2 cells have shown significant alterations in the different apoptotic (e.g., Bcl-2, Bak, Caspase 3) and tumor suppressor (e.g. p21, p53 and c-Myc) proteins in the cellular model (Ghosh et al., 2007), corroborating the complexity of PCB exposures.

We chose human liver (HepG2) and kidney (HK2) cells *in vitro*, as the liver is mainly responsible for the uptake, storage, and disposal of nutrients (protein, carbohydrates and fat), drugs, and toxins physiologically. In contrast, the kidney mainly regulates the body's fluid volume, mineral composition and acidity by regulating excretion and re-absorption of water and inorganic electrolytes. The HepG2 liver cell line was chosen since the liver is one of the target sites of PCB accumulation due to its lipophilicity, and plays a role in the oxidative metabolism in this organ. Above all, several authors have used this cell line as a model system for the study of inhibition of cell proliferation and cell death *in vitro* (Michalakakis et al., 2007), for the role of genes involved in transcriptional and translational processes (Dutta et al., 2008; Ghosh et al., 2007; Castaneda et al., 2006; De et al., 2010), for apoptosis studies (Ho et al., 2007), and for the study of genotoxic effects (An et al., 2006). These cells have also been selected to see the efficacies of antioxidant potential of many compounds (Goya et al., 2007). They have also been used as artificial livers in hepatic failure dogs (Wang et al., 2005). HK-2 is a proximal tubular cell (PTC) line derived from a normal adult male kidney (Ryan et al., 1994). Out of the total 209 possible congeners, we have used *ortho*-substituted PCB-153 (non-coplanar) and non-*ortho*-substituted PCB-77 (coplanar) in order to compare the biological effects depending upon their specificity in chemical structures. This is in contrast to conventional studies using commercial PCB mixture, resulting in limited information on congener specificity (Skerfving et al., 1994).

In the present study, it is observed that the initial absorption of both PCBs is faster in the liver cells than in kidney cells. However, peak concentrations were reached much earlier in kidney cells. The differing rates of uptake of the two cell types may be due to differences in the lipid composition of liver and kidney cell membranes, and rates of the two congeners dissolving in them. The cellular concentration is more prominent in PCB-153, which could be due to congener specificity. In a direct comparison of ^{14}C -CB 153 and ^{14}C -CB 169 (3,3', 4,4', 5,5'-hexachlorobiphenyl) organ concentration in rats, the concentration of ^{14}C -CB 153 was found to be 4-9 fold higher than that of ^{14}C -CB 169 in the brain, however, the opposite was found for the liver and kidneys (Saghir et al., 2000). Non-coplanar PCB-153 is absorbed more quickly in our study, particularly in liver cells (0-90 minutes), which may be due to quicker penetration through the cell membrane, whereas coplanar PCB-77 absorption is slower. Study on the cell membranes showed that hepatic cell membrane lipid peroxidation increased and membrane order (steady-state fluorescence anisotropy values) decreased with (coplanar) PCB 126 exposure (Katynski et al., 2004). Studies also indicated that the uptake and transport of PCB-77 might be disturbed by a similar mechanism (peroxidation) of cell membrane lipid and membrane disorder (Fadhel et al., 2002). Thus coplanar PCBs may reduce their cellular uptake by induction of lipid peroxidation in the liver. It is also noteworthy to mention that the bio-availability and the effects of many chemicals on cellular processes are governed by their ability to enter the cell, which is in turn a function of the composition of the cell's external environment. It has been shown that serum alters the uptake of halogenated compounds into cells (Hestermann et al., 2000). Thus cellular uptake may differ in different media. One solution

to this potential problem is to treat different cells in a single medium. Serum-free medium is the best candidate, as variations in composition among the chemically defined basal media should have a negligible effect on bioavailability. It should be pointed out that our experiments were performed using different media for the two cell lines, i.e. medium with 10% FBS for the liver cell line and serum-free medium for the kidney cells. Thus the presence or absence of FBS may also have contributed to the difference in PCB-uptake kinetics and efficiency which therefore may not solely due to the difference in cell lines.

The present study also demonstrates that PCB-153 and PCB-77 significantly induce loss of cell viability in human liver and kidney cell cultures in a concentration and time-dependent manner. Under our experimental conditions, the loss of cell viability due to the exposure of human liver and kidney cell cultures to PCBs has been correlated to the induction of apoptosis as also evident in our earlier study (Ghosh et al., 2007). Two different pathways are reported in apoptosis by these two PCBs (Howard et al., 2003). In liver, it is mainly through mitochondrial apoptosis in the case of PCB-153 compared to the nuclear apoptotic pathway for PCB-77 (Dutta et al., 2008). In the kidney, apoptosis is linked to the death-receptor in both these PCBs, and caspase plays an important role (Chen et al., 2006). Our earlier studies and other research into the mechanism of toxicity of PCBs have been focused on PCB congeners (Dutta et al., 2008; Ghosh et al., 2007; Chen et al. 2006; Perez-Reyes et al., 2001; Slim et al., 2000; Vezina et al., 2004). However, in the present study, tissue-specificity plays a more dominant role. When exposed, the kidney cells are more susceptible to PCB toxicity in spite of their initial slower absorption. The extent of apoptosis is greater in HK2 cells compared to HepG2 cells, as it is initiated well within 6 hours of incubation. This was further corroborated through fluorescence microscopy and DNA fragmentation studies. It needs to be examined whether the different apoptosis pathways or other factors are important for this higher sensitivity of kidney cells.

In conclusion, the present study clearly shows that under our culture conditions (1) HepG2 liver cells initiate the absorption of PCBs much faster than HK2 kidney cells; however, the concentration reaches its maximum level much earlier in kidney cells, (2) PCB-153 and PCB-77 induce enhanced apoptotic death in these liver and kidney cells; and (3) the kidney cells are more vulnerable to PCBs based on the results of apoptosis and cellular viability, even with almost similar absorption or tissue burden of PCBs.

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FIGURE 1.

The Gas Chromatography Mass Spectra (GC-MS) snap shots of PCB-153 and PCB-77 from treated HepG2 cells taken after 12 Hours. Identification of PCB-153 (A), PCB-77 (B), and biphenyl peaks from NIST Mass Spectrum database 2002. The Retention time for biphenyl was 8.67 minutes, 13.64minutes for PCB-153, and 11.20 minutes for PCB-77.

FIGURE 2.

Uptake of PCB-153 and PCB-77 in HepG2 (A, B), and in HK2 (C, D) cells. Cells were exposed to PCBs according to their respective LC_{50} and culture conditions, and were sampled at the indicated time points to determine the amount of PCBs associated with the cells. The maximum concentration was considered 100% which for depletion from the medium was the initial concentration. Points are mean $\pm$ SD of triplicate experimental results. The ordinate represents the percent of the total amount of PCBs recovered that was associated with the cells and medium. * is used to indicate a significant difference within HepG2, # within HK2 cells, @ within PCB-153 and \$ within PCB-77 at different time points (ANOVA, $p < 0.05$). $\uparrow$ or $\downarrow$ is used to denote the peak concentration.

FIGURE 3.

The works presented here comprise several different experiments done separately. Fluorescence Microscopy (A, C) and DNA fragmentation (B, D) studies following exposures to PCB-153 and PCB-77 on liver and kidney cells: nuclear morphological changes of apoptosis in human liver (A) and kidney (C) cells after exposures to the respective LC₅₀ of PCB-153 and PCB-77 for 0, 6, 12, and 24 hours, followed by fixation and staining with Hoechst 33342 dye. Some of the condensed nuclei are indicated by arrows. The representative ladder pattern of apoptotic DNA fragmentation in exposed human liver (B) and kidney (D) from the corresponding treatment group of A & C. The DNA samples (5 μ g/lane) and DNA markers (L) were electrophoresed on 1.5% agarose gel and ethidium bromide stained. Cytotoxicity of

PCB-153 and PCB-77 in liver (**E, F**) and kidney cells (**G, H**) by Trypan blue after 24 hours of exposures compared to the control.