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PCB153 reduces Telomerase Activity and Telomere Length in Immortalized Human Skin Keratinocytes (HaCaT) but not in Human Foreskin Keratinocytes (NFK)

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Abstract

Polychlorinated Biphenyls (PCBs), ubiquitous environmental pollutants, are characterized by long term-persistence in the environment, bioaccumulation, and biomagnification in the food chain. Exposure to PCBs may cause various diseases, affecting many cellular processes. Deregulation of the telomerase and the telomere complex leads to several biological disorders. We investigated the hypothesis that PCB153 modulates telomerase activity, telomeres and reactive oxygen species resulting in the deregulation of cell growth. Exponentially growing immortal human skin keratinocytes (HaCaT) and normal human foreskin keratinocytes (NFK) were incubated with PCB153 for 48 and 24 days, respectively, and telomerase activity, telomere length, superoxide level, cell growth, and cell cycle distribution were determined. In HaCaT cells exposure to PCB153 significantly reduced telomerase activity, telomere length, cell growth and increased intracellular superoxide levels from day 6 to day 48, suggesting that superoxide may be one of the factors regulating telomerase activity, telomere length and cell growth compared to untreated control cells. Results with NFK cells showed no shortening of telomere length but reduced cell growth and increased superoxide levels in PCB153-treated cells compared to untreated controls. As expected, basal levels of telomerase activity were almost undetectable, which made a quantitative comparison of treated and control groups impossible. The significant down regulation of telomerase activity and reduction of telomere length by PCB153 in HaCaT cells suggest that any cell type with significant telomerase activity, like stem cells, may be at risk of premature telomere shortening with potential adverse health effects for the affected organism.

Keywords

polychlorinated biphenyls (PCBs); telomere; telomerase; superoxide; cell growth; skin keratinocytes

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Introduction

Polychlorinated Biphenyls (PCBs), first manufactured in 1929, are a group of 209 individual congeners with 1 to 10 chlorine atoms attached to the biphenyl ring (WHO, 1993). These manmade compounds are highly stable and have been used in numerous industrial products, such as diluents, hydraulic fluids, heat transfer fluids, dielectric fluids for capacitors and transformers and many more. Their lipophilic nature and resistance to degradation contribute to their ubiquitous environmental distribution and accumulation in the food chain (Bates *et al.*, 1994); (Fürst *et al.*, 1994). The general population thus is extensively exposed to PCBs through the consumption of contaminated foods, inhalation or skin absorption in work environments (Kogevinas *et al.*, 1997). PCBs are neurotoxic, embryotoxic, immunosuppressant, and are considered to be a probable carcinogen (Silberhorn *et al.*, 1990; ATSDR, 2000; Danis *et al.*, 2006; Yoshizawa *et al.*, 2009). In humans skin lesions like chloracne and hyperkeratosis were the most common adverse health effects in patients exposed to PCBs/PCDFs through contaminated rice oil in the Yusho and Yusheng poisoning episodes in Japan and Taiwan, respectively (Kikuchi, 1984; Tsai *et al.*, 2006). The toxicity of individual PCB congeners varies with their structure (Davis and Safe, 1990). Two major groups of PCBs can be distinguished, namely dioxin-like and non-dioxin-like PCBs. Dioxin-like congeners have few chlorines (1 or none) at the *ortho*-positions; therefore, they can adopt a more coplanar structure and bind to the aryl hydrocarbon receptor (AhR) ((Bandiera *et al.*, 1982). Non dioxin-like PCBs have at least two chlorine substitutions at the *ortho*-positions and are less coplanar due to steric hindrance. They exert their toxic effects via mechanisms independent of the AhR binding (Sandor Sipka, 2008).

Individual PCBs have different physical/chemical characteristics which influence their accumulation, uptake and metabolism in the environment and in organisms, giving rise to marked differences in PCB congener composition between the commercial PCB mixtures (Aroclor) and biological extracts. In most biological extracts PCB138 (2,2',3,4,4',5-hexachlorobiphenyl), PCB153 (2,2',4,4',5,5-hexachlorobiphenyl), and PCB180 (2,2',3,4,4',5,5-heptachlorobiphenyl) are the dominating components ((Safe, 1994) (Cogliano, 1998). Of the various congeners, PCB153 appears to be a predominant PCB congener present in human and fish tissues (Chao *et al.*, 2004; Hayward *et al.*, 2007). PCB153 is persistent in the environment and its half-life may exceed 100 years in marine sediments ((Jönsson *et al.*, 2002). PCB153 is a di-ortho-substituted PCB congener that does not activate the AhR and exhibits no dioxin-like activity. It was shown to cause several adverse biological effects, including DNA damage and promotion of tumorigenesis (Wei *et al.*, 2009); (Tharappel *et al.*, 2002). One of the main mechanisms by which PCBs may exert their toxic effect is by increasing oxidative stress ((Tharappel *et al.*, 2002) and PCB153 has been shown to increase oxidative stress in human breast epithelial cells (Venkatesha *et al.*, 2008).

Telomeres are specialized structures of tandem arrays of G-rich repeats (TTAGGG in humans) present at the end of chromosomes ((Moyzis *et al.*, 1988). Telomeres function to protect the double-stranded DNA ends from degradation, fusion, recombination ((McClintock, 1941). Telomerase, an enzyme complex, contains a reverse transcriptase component (in human it is called hTERT) and RNA component (in human it is called hTR). It helps in adding TTAGGG to the telomeric ends to compensate the progressive loss of telomeric sequences during normal DNA replication (Greider and Blackburn, 1987). Both telomeres and telomerase play a vital role in a variety of biological activities in the body including but not limited to maintenance of cellular genome integrity and nuclear architecture, expression of growth-promoting genes, and cellular proliferation (Smith *et al.*, 2003). Telomere DNA is highly sensitive to damage by oxidative stress (Henle *et al.*, 1999); (Oikawa and Kawanishi, 1999). The telomerase hTERT subunit is prevented from adding nucleotides to telomeric DNA via translocation of hTERT from the nucleus to mitochondria

by oxidative stress (Haendeler *et al.*, 2003). Dysfunctional telomeres and telomerase leads to various diseases like ageing and cancer. Although telomeres and telomerase play a vital role in many diseases, very little research has been done so far concerning the effect of environmental contaminants on telomeres/telomerase and to our knowledge no research work has been done on the influence of the most prevalent PCB congener in our bodies, PCB153. Considering the ubiquitous exposure to PCBs through food and inhalation and skin exposure (Ludewig *et al.*, 2008), this is an astonishing gap in our knowledge. Therefore this study is exploring the effect of PCB153 on telomerase activity, telomere length, and cell growth changes in immortalized human skin keratinocytes (HaCaT) and primary human foreskin keratinocytes (NFK).

Materials and Methods

Materials

Dulbecco's Modified Eagle Medium (DMEM), Keratinocyte Serum Free Medium, Epidermal Growth Factor (EGF), Bovine Pituitary Extract (BPE), Fetal Bovine Serum (FBS), Pencillin-Streptomycin (P/S), 0.25% trypsin with EDTA, and Phosphate Buffered Saline (PBS) were obtained from Invitrogen (Carlsbad, CA, USA). Dimethyl sulfoxide (DMSO), resazurin and propidium iodide were purchased from Sigma (St. Louis, MO, USA). Colcemide was bought from Alexis Biochemicals (San Diego, CA, USA). PCR reagents and kits were obtained from Qiagen (Valencia, CA, USA). PCB153 (2,2',4,4',5,5'-hexachlorobiphenyl) was synthesized and characterized as described previously (Schramm *et al.*, 1985). The purity of PCB153 was greater than 99%, as assayed by gas-chromatography.

Cell culture and PCB exposure regimen

The human keratinocyte (HaCaT) cell line was obtained from Dr. C. Svenson, University of Iowa and maintained in DMEM with 10% FBS and 1% P/S in a humidified incubator at 37°C with 5% CO₂. Human Normal Foreskin Keratinocytes (NFK) were obtained from Dr. Aloysius Klingelhutz, University of Iowa and maintained in Keratinocyte Serum Free Medium with 0.16 ng/ml EGF, 25 µg/ml BPE and 1% P/S in a humidified incubator at 37°C with 5% CO₂. These cell types were chosen since skin is a major route of exposure and excretion for PCBs (ATSDR, 2000). HaCaT is a cell line obtained from spontaneously immortalized skin keratinocytes from normal skin (Boukamp *et al.*, 1988). They have already been used for telomere length studies (Zhang *et al.*, 2003b; Jacobus *et al.*, 2008a) and are positive for telomerase activity (Harle-Bachor and Boukamp, 1996b). NFK (Normal Foreskin Keratinocytes) were chosen to compare the effects observed in HaCaT cells with those in normal skin cells. NFK have only negligible telomerase activity.

The cytotoxicity of PCB153 was determined by seeding 30,000 HaCaT or NFK cells per well in 24-well tissue culture plates and exposing them to 2–20 µM PCB153 for six days with a change of medium containing fresh compound on the third day. Control cultures received solvent (DMSO, 0.05% final concentration) alone. After six days the medium was removed and cells were incubated with resazurin (5µM, dissolved in fresh medium) for two hours and the fluorescence intensity at 535 nm excitation and 590 nm emission wavelength in each well was measured using a microplate reader (TECAN, Seestrasse, Switzerland). The fluorescence intensity correlates with the number of living cells in the well, since cells metabolize nonfluorescent resazurin to the fluorescing resorufin. Based on these results a concentration of 5 µM (HaCaT) and 2 µM (NFK) was selected for all following experiments.

Experiments with HaCaT cells were performed by seeding 100,000 cells in 10 cm diameter Petri dishes and exposing them continuously to 5µM PCB153 for up to 48 days. Solvent

controls received DMSO (0.05% final concentration) only. Media with test compound or solvent were changed every three days and cells were trypsinized, counted, and re-seeded at low density (100,000 per plate) in medium with test compound every sixth day. The remaining cells on days 6, 18, 30, 42 and 48 were used to determine telomerase activity, telomere length, and cell growth. In addition, cell viability and cell cycle distribution were measured on days 6, 30 and 48. Cell morphology was monitored throughout the exposure.

Similarly 100,000 NFK cells were seeded and exposed continuously to solvent only (0.05% DMSO) or 2 μ M PCB153 for 24 days. Media with test compound or solvent were changed every other day. Cells were trypsinized, counted, and re-seeded at low density (100,000 per plate) in medium with fresh test compound every sixth day. Cells from day 6, 18 and 24 were used to determine telomerase activity, telomere length, and cell growth. Cell viability and cell cycle distribution were measured on days 6 and 24.

Exposure periods of up to 48 days were used to mimic the continuous and chronic human exposure to PCBs in daily life. The concentration of 5 μ M PCB153 for HaCaT and 2 μ M for NFK appears excessively high, but is justifiable considering that PCB levels in the blood of individuals living in Anniston, Alabama, vary from 0.003 to 6.5 μ M (Hansen *et al.*, 2003) and tissue levels of PCBs are usually by several magnitudes higher than blood levels.

Analysis of telomerase activity

Telomerase activity was measured following the method of Wege and coworkers (Wege *et al.*, 2002; Senthilkumar *et al.*, 2011). After trypsinization and centrifugation of cells, cell pellets were resuspended in cell lysis buffer at 1000 cells/ μ l and incubated on ice for 30 minutes. To compensate for the low telomerase activity in NFK cells a 10x higher number of cells was used in the NFK telomerase protocol than in the HaCaT protocol. Aliquots of cell lysate were centrifuged at 16,000g for 20 minutes at 4°C and the supernatant was used for qPCR. The SYBR Green based qPCR reaction was performed using a Telomerase Primer (TS) and Anchored Return Primer (ACX) (Kim and Wu, 1997), cell lysate supernatant, and Eppendorf MasterMix SYBR ROX (Fisher Scientific, PA, USA). SYBR green fluorescence was measured in an Eppendorf RealPlex Thermal Cycler (Eppendorf, Hamburg, Germany) and the Ct (threshold cycle value) was determined from the semi-log amplification plots and compared with the standard curves generated from serial dilutions of cell extracts. Each sample was analyzed in duplicate. Telomerase activity was expressed as the percentage relative to the control sample.

Determination of telomere length

DNA was isolated from trypsinized cells using the DNA blood mini kit from Qiagen (Valencia, CA, USA) following the manufacturer's protocol. PCR reactions were performed as described previously (Senthilkumar *et al.*, 2011) following the method of Cawthon (Cawthon, 2002). For each PCR reaction 12 ng of DNA with telomere primer 1 (270 nM) and telomere primer 2 (900 nM) for telomere (T) PCR or 36B4u (300 nM) and 36B4d (500 nM) for single copy gene (S) PCR were used. The T/S ratio was calculated for each corresponding well pair. The relative change in telomere length due to exposure was calculated using the T/S ratio of the PCB153 sample and the T/S ratio of the corresponding control. Each sample was analyzed in duplicate.

Measurement of intracellular superoxide levels by Dihydroethidium (DHE) oxidation

Steady state levels intracellular levels of superoxide were determined by measuring to the DHE oxidation in cells. Briefly, cells obtained during subculturing were washed once with PBS containing 5 mM pyruvate and then incubated with DHE (10 μ M, in PBS with 0.1% DMSO) for 40 min at 37°C. After dye loading the cells were kept on ice and analyzed using

a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA). The mean fluorescence intensity (MFI) of 15,000 cells was analyzed in each sample and corrected for auto fluorescence from unlabeled cells. The MFI data were normalized to control levels (Slane et al., 2006).

Determination of cell growth and cell viability

Trypsinized cells were counted in a Neubauer hemocytometer and the total number of cells per dish in the PCB-treated groups was plotted against the control group to compare the cell growth in these groups.

Remaining cells were washed with PBS, incubated with propidium iodide (PI) and PI-fluorescence of cells was measured using a Becton Dickinson FACSCalibur flow cytometer. Data from 10,000 cells per sample were used to determine the percentages of PI-positive (non-viable) and PI-negative (viable) cells using the WinMDI software.

Measurement of cell cycle distribution

Approximately 1×10^6 of trypsinized cells were fixed in 70% ethanol overnight. Then cells were treated with RNase (100 µg/ml) for thirty minutes and PI (50 µg/ml) for one hour. Cell cycle analysis was performed using a Becton Dickinson FACSCalibur flow cytometer. Data were collected from 10,000 cells in list mode and the percentage of cells in each phase of the cell cycle was analyzed using MODFIT software. In these studies control cultures treated with colcemide (0.2 µg/ml) for 7 hrs before harvesting were used as positive control.

Statistical Analysis

Statistical analysis was performed using student's t-test. All experiments were performed twice in duplicates and results are presented as mean $\pm$ standard deviation of both duplicates. The data analysis was performed in SAS software, Version 9.2 of the SAS System for Windows. Treatment groups were considered statistically different at $p < 0.001$, 0.01, or 0.05.

Results

PCB153 produces mild cytotoxicity at high concentrations

To identify a non-toxic concentration of PCB153 in HaCaT and NFK exponentially growing asynchronous cells were exposed to 2–20 µM PCB for six days with one medium-plus-PCB/solvent change on day three after which the number of viable cells in treated and control cultures were compared. No reduction in living cells compared to controls was observed with 5 and 2 µM PCB153 in HaCaT and NFK, respectively (Supplement Figure 1). Higher concentrations caused a very small reduction of living cells up to the highest concentration tested. To avoid non-specific effects due to toxicity all following experiments were therefore performed at a PCB153 concentration of 5 µM with HaCaT cells and 2 µM with NFK cells.

Telomerase activity is reduced by PCB153 in HaCaT cells, constitutively very low in NFK

To explore whether PCB153 has an effect on telomerase activity, it was determined in HaCaT on days 6, 18, 30, 42 and 48 of exposure to. A significantly reduced telomerase activity in PCB153-exposed cells compared to controls was observed at each of these days (Figure 1). An around 20% reduced telomerase activity was observed on day 6 and 18, and this reduction increased from day 30 to day 48 to 30%. No effect was seen with DMSO solvent alone. Thus, a pronounced decrease in telomerase activity was observed in PCB153 treated HaCaT cells.

Untreated, solvent and PCB153-treated NFK cells were assayed on day 6, 18, and 24 of exposure. Compared to HaCaT the basal level of telomerase activity was extremely low in untreated control NFK cells. By increasing the cell number 10-times a little telomerase activity could be measured in both control and PCB153-treated NFK cells, however, it was too low to discern any significant difference between the two groups (data not shown). Therefore no further attempts were made to measure telomerase activity in these primary keratinocytes.

PCB153 exposure shortens telomere length in HaCaT, but not NFK cells

To analyze whether telomere length was modified by PCB153, mean telomere length was measured in both cell types at several time points during exposure. In HaCaT cells no significant change in telomere length was observed on day 6 of PCB153 exposure (Figure 2, top). On day 18 a 20% reduction in mean telomere length was measured which increased to 40% reduction on day 30 and 42 with a slight decrease to about 32% reduction on day 48 of PCB153-treatment. No effect was seen with DMSO solvent alone compared to no treatment. Thus, PCB153 caused a significant shortening in telomere length following a decrease in telomerase activity. No shortening of telomeres was observed in untreated control cells over the 48 days of the experiment.

In NFK cells no significant difference in telomere length was observed between treatment and control groups on any of the days analyzed (Figure 2, bottom). In these cells telomere length shortened in all groups from days 6 to 24 independent of treatment indicating that the cells constantly lost telomeric DNA (data not shown).

PCB153 increased DHE oxidation in HaCaT and NFK cells

Since oxidative stress is suggested to influence telomerase activity and telomere length we determined the intracellular superoxide level in HaCaT and NFK during the exposure period using the DHE oxidation assay. A significant increase (~25%) in superoxide level was observed from day 6 to day 48 in PCB153-treated HaCaT cells compared to untreated controls (Figure 3, top). In NFK cells PCB153 produced a similar significant (~20%) increase in intracellular superoxide level compared to the corresponding untreated control from day 6 to day 24 (Figure 3, bottom).

Cell growth was altered in PCB-exposed HaCaT and NFK cells, but no cytotoxicity was observed

To examine whether PCB153 had an effect on cell growth, the number of cells per dish was counted at each passaging. With HaCaT, cell numbers per plate were decreased in PCB-treatment groups at all time points (Figure 4, top, bars). The total number of cells was about 10.2×10^6 per dish in the untreated and solvent treated controls throughout the experiment and 8.4×10^6 per dish in PCB153-treated cultures on day 6, going down to about 8.0×10^6 per dish on day 48. No significant increase in the percent of dead cells, determined by PI-staining, was observed in PCB153 exposed groups (Figure 4, top, symbols). The morphology of cells was continuously monitored and no changes were observed throughout the course of the experiment in any of the treatment groups.

With NFK cells the cell numbers were decreased in all PCB-treatment groups from day 6 until day 24 (Figure 4, bottom, bars). The total number of cells was about 2.0×10^6 per dish in the control groups and now significant change in cells per plate was observed during the 3.5 weeks of the experiment. In the PCB153-treated group the cell number per plate decreased with length of exposure, from 1.8×10^6 on day 6, to 1.5×10^6 on day 18 and 1.4×10^6 on day 24. No significant increase in the percent of dead cells, determined by PI-staining, was observed in PCB153 cultures (Figure 4, bottom, symbols). No treatment-

related changes in the morphology of cells were seen throughout the course of the experiment, but on day 24 some cells in each of the treatment groups seemed larger than usual.

PCB153 increase cells in S and G2-M phase in HaCaT cells and G2-M phase in NFK

To analyze whether the reduction in cell number was due to cell cycle inhibition the distribution of cells in the different cell cycle phases was determined by flow cytometry on selected days during the experiment. In HaCaT cells the normal cell cycle distribution is about 60% - 32% - 8% in G0/1 - S - G2/M, respectively (Table 1, Supplemental Figure 2). DMSO alone did not cause any changes in the cell cycle profile compared to the no-treatment group. In the PCB153 group the percentage of G₁ cells was decreased to approximately 90% coinciding with a significant increase (approximately 10%) in the percentage of S, and G₂/M cells on the 6th day of exposure, indicating that PCB153 perturbed progression through the cell cycle. This change in distribution was similar on day 30 and day 48. The spindle poison Colcemide, our positive control, produced an increase in G2/M.

NFK cells have a normal cell cycle distribution of about 75% - 17.5% - 7.5% in G0/1 - S - G2/M, respectively (Table 2, Supplemental Figure 2). In untreated controls a small, non-significant increase in G0/1 at the expense of S-phase cells was seen on day 24. The cell cycle distribution in DMSO-treated cultures was similar to the untreated controls. In PCB153-exposed cells a significant decrease in G0/1 by ~8% and S-phase cells by ~15% along with a significant increase (~doubling) of cells in G2/M phase was observed from day 6 to the end of the experiment on day 24 (Table 2).

Discussion

PCBs are ubiquitous environmental pollutants known to cause a multitude of biological effects in animals and humans (ATSDR, 2000). Although the production of PCBs was discontinued in the USA in the late 1970s, humans continue to be exposed to these contaminants through diet, air, water and soil. Of all 209 possible individual PCB congeners, PCB153 is found at the highest molar concentration in human blood, tissues and breast milk (ATSDR, 2000). A large 2-year exposure study with female SD rats found effects on thyroid and thyroid hormone levels, liver hypertrophy, fatty changes, cholangiomas, and other changes, as well as inflammation of uterus and ovary (NTP, 2006). Occupational exposure to PCB 153, for example among farmers and industrial workers, has also been associated with increased incidence of immunotoxicity (Ritchie *et al.*, 2003; Duffy-Whritenour *et al.*, 2010). Molecular mechanisms involved in these adverse biological effects are not completely understood. PCB153 is a CAR agonist, inducing CYP2B expression, and was reported to increase intracellular oxidative stress and DNA damage mediated by increased intracellular steady-state levels of O₂^{•-} and H₂O₂ (Venkatesha *et al.*, 2008; Zhu *et al.*, 2009). Telomeres and telomerase are strongly involved in cell proliferation and maintenance and dysfunctional telomere complexes or telomerase activity are responsible for diseases ranging from premature aging to cancer (Blasco *et al.*, 1997; Buckingham and Klingelutz, 2011). Oxidative stress is one of the mechanisms reported to modulate telomere length and telomerase activity (von Zglinicki, 2002).

The present study investigates the hypothesis that PCB153 exposure affects telomerase activity and telomere length through ROS formation resulting in the deregulation of cell growth. Immortal human keratinocytes (HaCaT), which express considerable telomerase activity, were exposed continuously for 48 days to a relatively high (5 µM) but non-toxic concentration of PCB153. From the first time point on, after 6 days of exposure, a significant ~20% reduction of telomerase activity was observed which increased with

exposure time to about 40%. This time course is different from the effects reported previously after exposure of HaCaT cell to 5 μ M concentrations of the lower chlorinated biphenyl congeners PCB28 (2,4,4'-trichloro-) and PCB52 (2,2',5,5'-tetrachlorobiphenyl). Here a 40% reduction of telomerase activity was observed during the complete exposure period, but the magnitude of the effect was very similar (Senthilkumar *et al.*, 2011). This seems to indicate that the level of chlorination of the biphenyl molecule may not be a determining factor for this activity. Interestingly a PCB mixture resembling Chicago air (CAM) was slightly less effective.

Telomerase came to the attention of scientists because of the correlation between telomerase activation and cellular immortalization and the high telomerase activity in many cancers, suggesting that telomerase is required for tumor growth (Bednarek *et al.*, 1995; Broccoli *et al.*, 1996). However, the lack of telomerase activity was correlated with tumor formation and cancer susceptibility (Blasco *et al.*, 1997; Perona *et al.*, 2009). In addition, lack of telomerase activity leads to several other diseases like the premature aging disorder dyskeratosis congenita (Westin *et al.*, 2007), aplastic anemia (Vulliamy *et al.*), and impaired glucose metabolism and insulin secretion due to deficient pancreatic beta cell regeneration, leading to diabetes mellitus (Kuhlow D, 2010). Thus a reduction or loss of telomerase activity can have severe negative health effects.

Consistent with the hypothesis that lack of telomerase activity is needed to maintain the telomeres of proliferating cells, a shortening of telomere length was observed in HaCaT cells from day 18 of PCB153 exposure. PCB153 produced a more than 40% reduction in telomere length at the 30 and 42-day exposure time points. This effect was by far stronger than the telomere reduction seen with CAM (~5%) or PCB28 (~10%), and seen earlier than with PCB52 (~40% at day 42) (Senthilkumar *et al.*, 2011). In this case higher chlorination correlated with shorter telomeres if these 3 pure congeners are compared; CAM, with an average chlorination of .., was only slightly active, indicating that chlorination level alone is not determining the effect on telomeres. In addition, our previous studies with HaCaT cells showed for the first time that a PCB metabolite, 4-monochloro-2',5'-quinone (PCB-3-Q) shortened telomeres (Jacobus *et al.*, 2008b) and shortened telomere length. It has been reported that cokeoven workers with high exposure to polycyclic aromatic hydrocarbons (PAHs), which are known lung carcinogens, shorten telomere length (Pavanello *et al.*, 2010). Ultraviolet radiation, a skin carcinogen, also caused telomere shortening (Oikawa *et al.*, 2001). Critically shortened telomeres produce a very high likelihood of ploidy changes, which may be associated with the development of cancer (Artandi, 2002; Lange *et al.*, 2010). Telomere shortening also increases the metastatic potential along with genome instability in lung cancer (Perera *et al.*, 2008) and Bisoffi and coworkers proposed that altered telomere length i.e. either shortening or lengthening of telomeres, can be used as a prognostic marker for solid tumors (Bisoffi *et al.*, 2006). Telomere shortening also lead to coronary heart disease (Wang *et al.*, 2010), ageing (Saretzki and Von Zglinicki, 2002), chronic liver disease (Kitada *et al.*, 1995), and migraine (Ren *et al.*, 2010). Thus, independent of the mechanism, telomere shortening can be considered an indicator of various diseases.

One factor that was shown to influence telomerase activity and telomere length are reactive oxygen species (ROS) (von Zglinicki, 2002; Indran *et al.*, 2010). PCB mixtures and individual congeners have been shown repeatedly to increase ROS *in vivo* (Banudevi *et al.*, 2006; Schlezinger *et al.*, 2006; Brown *et al.*, 2007) and in cells in culture (Oakley *et al.*, 1996; Srinivasan *et al.*, 2001; Lee and Opanashuk, 2004; Zhu *et al.*, 2009). To examine whether PCB153 produced ROS we measured intracellular superoxide level at various time points of exposure. In HaCaT cells, PCB153 produced a significant increase in intracellular superoxide level at all time points. This increase in superoxide is consistent with previous

results obtained in human prostate RWPE-1 cells (Zhu *et al.*, 2009) and human mammary MCF10A cells (Chaudhuri *et al.*, 2010). This result suggests that increased ROS could be one of the causes for the reduction in telomerase activity and shortening of telomere length.

Telomerase activity and telomere length are factors that may influence cell growth. In our study, a persistent ~20% reduction in cell growth was seen in HaCaT from day 6 to day 48 in PCB153 exposed cells. Since no increase in cell death was visible, this pointed towards an increase in population doubling time due to a slower progression and/or accumulation of cells in one or more cell cycle phases. Cell cycle analysis at three different time points (days 6, 30, 48) showed a significant increase in S and G2/M phase with concomitant decrease in the G0/G1 cell population compared to untreated control. In quiescent human mammary MCF10A cells and prostate RWPE-1 cells, two immortal but non-tumorigenic cell lines, exposure to PCB153 reduced clonogenicity and cells re-entering into the S-phase after subculturing at low cell density (Zhu *et al.*, 2009; Chaudhuri *et al.*, 2010). The authors suggested increased intracellular steady-state levels of ROS such as $O_2^{\cdot-}$ and H_2O_2 , derived at least in part from a down-regulation of anti-oxidant enzymes, of as cause for the alterations in cell growth.

HaCaT is a spontaneously immortalized human keratinocyte cell line (Boukamp *et al.*, 1988). In addition to this cell line we studied the effect of PCB153 in Normal Human Foreskin Keratinocytes (NFK). These normal primary cells reach senescence very fast so that the PCB exposure study could only be conducted for 24 days at which time the NFK cells started to go into senescence. NFK were slightly more sensitive to PCB153 so that the exposure concentration had to be reduced to 2 μ M of PCB153 instead of 5 μ M with HaCaT cells in order to avoid confounding effects due to toxicity. Human keratinocytes of the epidermis basal layer have been shown to be positive for telomerase activity (Harle-Bachor and Boukamp, 1996a). Our NFK cells also expressed telomerase activity but only a very low level, so low, in fact, that we were not able to identify significant differences between treated and untreated groups. Furthermore, telomere length shortened during the time of the experiment, indicating that telomerase activity was too low to maintain telomere length. No additional shortening was observed in PCB153 exposed cells until day 24, when the experiment had to be terminated due to senescence. This could indicate that the shortening of telomeres is not due to a direct action of PCB153 or a downstream intermediate like ROS on telomeres, but an effect on telomerase which then results in loss of telomere length. Thus only cells that express telomerase at a sufficient level to maintain their proliferative capacity, like cancer cells or stem cells, would be at risk of this type of damage by PCBs.

We also measured intracellular superoxide level in NFK cells. Interestingly, a significant increase in intracellular superoxide level was found on all exposure days and unlike the persistent level of superoxide in HaCaT, the ROS level in NFK increased with exposure time. The increase in ROS may be related to progress of these cells towards senescence, which may further reduce the de-novo syntheses of proteins, including anti-oxidant proteins, which were shown to be down-regulated by PCB153 (Zhu *et al.*, 2009; Chaudhuri *et al.*, 2010). More importantly, this shows that increased ROS doesn't necessarily need to a decrease in telomere length and further supporting our hypothesis that the telomerase activity is the major target of PCB153. It is noteworthy that the differences in telomere damage pathways and susceptibility between normal and malignant cells was recently proposed as novel target for chemotherapy (Lansdorp, 2005). In addition to increasing the superoxide level in NFK cells, PCB153 also reduced cell proliferation (~20%), with a small decrease in G0-G1 and S phase cells along with a roughly doubling of cells in G2-M phase. Since no effect on telomere length was seen and a role of telomerase activity is unlikely, although it cannot be excluded, we assume that the increase in intracellular ROS produced this effect on cell growth, as has previously been shown with PCB153-treated MCF10A and

RWPE-1 cells (Zhu *et al.*, 2009; Chaudhuri *et al.*, 2010). We therefore also assume that the reduction in cell growth of HaCaT cells is most likely not a consequence of telomere shortening and reduced telomerase activity.

These results show for the first time that PCB153, the most prevalent PCB congener in human bodies, affects telomerase activity and telomere length. It should be noted that the concentration used in these experiments was relatively high (5 μ M). A study with arsenic, another environmental pollutant, reported a ~50% decrease in telomerase activity in HaCaT cells after exposure to 10 μ M arsenic for 3 days, but an increase in telomerase activity after exposure to 0.5 μ M arsenic or less (Zhang *et al.*, 2003a). Also, the mechanism of PCB153's effect on telomeres and telomerase may or may not be related to ROS. Our study cannot give a final answer to this question. Finally, we cannot know whether other cell types that have telomerase activity, like hematopoietic stem cells or germ cells, have a similar susceptibility to telomerase and telomere damage by PCB153. Careful dose-response studies with different cell types and various radical scavengers and enzyme modulation with siRNA or other techniques is needed before a statement about the risk to human health through this new pathway can be estimated.

Highlights

- > Human immortal (HaCaT) and primary (NFK) keratinocytes were exposed to PCB153.
- > PCB153 significantly reduced telomerase activity and telomere length in HaCaT.
- > No effect on telomere length and telomerase activity was found in NFK.
- > Increased intracellular superoxide levels and reduced cell growth was seen in both.
- > PCB153 may damage telomerase expressing cells like stem cells

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

PCB153	2,2',4,4',5,5' hexachlorobiphenyl
HaCaT	Immortalized human skin keratinocytes
NFK	normal human foreskin keratinocytes

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References

- Artandi S. Complex roles for telomeres and telomerase in breast carcinogenesis. *Breast Cancer Res.* 2002; 5:37–41. [PubMed: 12559044]
- ATSDR. Toxicological profile for polychlorinated biphenyls (PCBs):. U.S. Dept. Health Services, Public Health Service; 2000.
- Bandiera S, Safe S, Okey AB. Binding of polychlorinated biphenyls classified as either phenobarbitone-, 3-methylcholanthrene- or mixed-type inducers to cytosolic Ah receptor. *Chemico-Biological Interactions.* 1982; 39:259–277. [PubMed: 6804100]
- Banudevi S, Krishnamoorthy G, Venkataraman P, Vignesh C, Aruldas MM, Arunakaran J. Role of alpha-tocopherol on antioxidant status in liver, lung and kidney of PCB exposed male albino rats. *Food Chem Toxicol.* 2006; 44:2040–2046. [PubMed: 16979807]
- Bates MN, Hannah DJ, Simon JB, Taucher JA, Maanen Tv. Chlorinated Organic Contaminants in Breast Milk of New Zealand Women. *Environmental Health Perspectives.* 1994; 102:211–217. [PubMed: 8187711]
- Bednarek A, Budunova I, Slaga TJ, Aldaz CM. Increased Telomerase Activity in Mouse Skin Premalignant Progression. *Cancer Research.* 1995; 55:4566–4569. [PubMed: 7553630]
- Bisoffi M, Heaphy CM, Griffith JK. Telomeres: Prognostic markers for solid tumors. *International Journal of Cancer.* 2006; 119:2255–2260.
- Blasco MA, Lee H-W, Hande MP, Samper E, Lansdorp PM, DePinho RA, Greider CW. Telomere Shortening and Tumor Formation by Mouse Cells Lacking Telomerase RNA. *Cell.* 1997; 91:25–34. [PubMed: 9335332]
- Boukamp P, Petrussevska RT, Breitkreutz D, Hornung J, Markham A, Fusenig NE. Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. *J Cell Biol.* 1988; 106:761–771. [PubMed: 2450098]
- Broccoli D, Godley L, Donehower L, Varmus H, de Lange T. Telomerase activation in mouse mammary tumors: lack of detectable telomere shortening and evidence for regulation of telomerase RNA with cell proliferation. *Mol. Cell. Biol.* 1996; 16:3765–3772. [PubMed: 8668193]
- Brown JF Jr, Mayes BA, Silkworth JB, Hamilton SB. Polychlorinated biphenyls modulated tumorigenesis in Sprague Dawley rats: correlation with mixed function oxidase activities and superoxide (O₂^{*}) formation potentials and implied mode of action. *Toxicol Sci.* 2007; 98:375–394. [PubMed: 17510085]
- Buckingham EM, Klingelutz AJ. The role of telomeres in the ageing of human skin. *Experimental Dermatology.* 2011; 20:297–302. [PubMed: 21371125]
- Cawthon RM. Telomere measurement by quantitative PCR. *Nucleic Acids Research.* 2002; 30:e47. [PubMed: 12000852]
- Chao HR, Wang SL, Lee CC, Yu HY, Lu YK, Pöpke O. Level of polychlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls (PCDD/Fs, PCBs) in human milk and the input to infant body burden. *Food and Chemical Toxicology.* 2004; 42:1299–1308. [PubMed: 15207381]
- Chaudhuri L, Sarsour EH, Kalen AL, Aykin-Burns N, Spitz DR, Goswami PC. Polychlorinated biphenyl induced ROS signaling delays the entry of quiescent human breast epithelial cells into the proliferative cycle. *Free Radical Biology and Medicine.* 2010; 49:40–49. [PubMed: 20307652]
- Cogliano VJ. Assessing the cancer risk from environmental PCBs. *Environ Health Perspect.* 1998; 106
- Danis B, Cattini C, Teyssié JL, Villeneuve JP, Fowler SW, Warnau M. Coplanar and non-coplanar congener-specificity of PCB bioaccumulation and immunotoxicity in sea stars. *Aquatic Toxicology.* 2006; 79:105–113. [PubMed: 16837075]
- Duffy-Whitnour JE, Kurtzman RZ, Kennedy S, Zelikoff JT. Non-coplanar polychlorinated biphenyl (PCB)-induced immunotoxicity is coincident with alterations in the serotonergic system. *Journal of Immunotoxicology.* 2010; 7:318–326. [PubMed: 20843273]
- Fürst P, Fürst C, Wilmers K. Human milk as a bioindicator for body burden of PCDDs, PCDFs, organochlorine pesticides, and PCBs. *Environ Health Perspect.* 1994; 102:187–193.
- Greider CW, Blackburn EH. The telomere terminal transferase of tetrahymena is a ribonucleoprotein enzyme with two kinds of primer specificity. *Cell.* 1987; 51:887–898. [PubMed: 3319189]

- Haendeler J, Hoffmann J, Brandes RP, Zeiher AM, Dimmeler S. Hydrogen Peroxide Triggers Nuclear Export of Telomerase Reverse Transcriptase via Src Kinase Family-Dependent Phosphorylation of Tyrosine 707. *Mol. Cell. Biol.* 2003; 23:4598–4610. [PubMed: 12808100]
- Hansen LG, DeCaprio AP, Nisbet ICT. PCB congener comparisons reveal exposure histories for residents of Anniston, Alabama, USA. *Fresenius' Environ Bull.* 2003; 12:181–190.
- Harle-Bachor C, Boukamp P. Telomerase Activity in the Regenerative Basal Layer of the Epidermis in Human Skin and in Immortal and Carcinoma-Derived Skin Keratinocytes. *Proceedings of the National Academy of Sciences of the United States of America.* 1996a; 93:6476–6481. [PubMed: 8692840]
- Harle-Bachor C, Boukamp P. Telomerase activity in the regenerative basal layer of the epidermis in human skin and in immortal and carcinoma-derived skin keratinocytes. *Proc Natl Acad Sci U S A.* 1996b; 93:6476–6481. [PubMed: 8692840]
- Hayward D, Wong J, Krynetsky AJ. Polybrominated diphenyl ethers and polychlorinated biphenyls in commercially wild caught and farm-raised fish fillets in the United States. *Environmental Research.* 2007; 103:46–54. [PubMed: 16769049]
- Henle ES, Han Z, Tang N, Rai P, Luo Y, Linn S. Sequence-specific DNA Cleavage by Fe²⁺-mediated Fenton Reactions Has Possible Biological Implications. *Journal of Biological Chemistry.* 1999; 274:962–971. [PubMed: 9873038]
- Indran IR, Hande MP, Pervaiz S. Tumor cell redox state and mitochondria at the center of the non-canonical activity of telomerase reverse transcriptase. *Molecular Aspects of Medicine.* 2010; 31:21–28. [PubMed: 19995569]
- Jacobus JA, Flor S, Klingelhutz A, Robertson LW, Ludewig G. 2-(4'-Chlorophenyl)-1,4-benzoquinone Increases the Frequency of Micronuclei and Shortens Telomeres. *Environ Toxicol Pharmacol.* 2008a; 25:267–272. [PubMed: 18438462]
- Jacobus JA, Flor S, Klingelhutz AJ, Robertson LW, Ludewig G. 2-(4'-chlorophenyl)-1,4-benzoquinone increases the frequency of micronuclei and shortens telomeres. *Environmental Toxicology and Pharmacology.* 2008b; 25:267–272. [PubMed: 18438462]
- Jönsson A, Gustafsson Ö, Axelman J, Sundberg H. Global Accounting of PCBs in the Continental Shelf Sediments. *Environmental Science & Technology.* 2002; 37:245–255.
- Kikuchi M. Autopsy of patients with yusho. *Am J Ind Med.* 1984; 5:19–30. [PubMed: 6422747]
- Kim NW, Wu F. Advances in quantification and characterization of telomerase activity by the telomeric repeat amplification protocol (TRAP). *Nucleic Acids Res.* 1997; 25:2595–2597. [PubMed: 9185569]
- Kitada T, Seki S, Kawakita N, Kuroki T, Monna T. Telomere Shortening in Chronic Liver Diseases. *Biochemical and Biophysical Research Communications.* 1995; 211:33–39. [PubMed: 7779103]
- Kogevinas M, Becher H, Benn T, Bertazzi PA, Boffetta P, Bueno-de-Mesquita HB, Coggon D, Colin D, Flesch-Janys D, Fingerhut M, Green L, Kauppinen T, Littorin M, Lyne E, Mathews JD, Neuberger M, Pearce N, Saracci R. Cancer Mortality in Workers Exposed to Phenoxy Herbicides, Chlorophenols, and Dioxins An Expanded and Updated International Cohort Study. *American Journal of Epidemiology.* 1997; 145:1061–1075. [PubMed: 9199536]
- Kuhlow D, F S, von Figura G, Weimer S, Schulz N, Petzke KJ, Zarse K, Pfeiffer AF, Rudolph KL, Ristow M. Telomerase deficiency impairs glucose metabolism and insulin secretion. *Aging (Albany NY).* 2010; 2:650–658. [PubMed: 20876939]
- Lange K, Holm L, Vang Nielsen K, Hahn A, Hofmann W, Kreipe H, Schlegelberger B, Ouml, hring G. Telomere shortening and chromosomal instability in myelodysplastic syndromes. 2010
- Lansdorp PM. Major cutbacks at chromosome ends. *Trends in Biochemical Sciences.* 2005; 30:388–395. [PubMed: 15936947]
- Lee DW, Opanashuk LA. Polychlorinated biphenyl mixture aroclor 1254-induced oxidative stress plays a role in dopaminergic cell injury. *Neurotoxicology.* 2004; 25:925–939. [PubMed: 15474611]
- Ludewig G, Lehmann L, Esch H, Robertson LW. Metabolic activation of PCBs to carcinogens in vivo--A review. *Environmental Toxicology and Pharmacology.* 2008; 25:241–246. [PubMed: 18452002]

- McClintock B. The stability of broken ends of chromosomes in *Zea Mays*. *Genetics*. 1941; 26:234–282. [PubMed: 17247004]
- Moyzis RK, Buckingham JM, Cram LS, Dani M, Deaven LL, Jones MD, Meyne J, Ratliff RL, Wu JR. A highly conserved repetitive DNA sequence, (TTAGGG)_n, present at the telomeres of human chromosomes. *Proceedings of the National Academy of Sciences*. 1988; 85:6622–6626.
- NTP. NTP technical report on the toxicology and carcinogenesis studies of 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) (CAS No. 35065-27-1) in female Harlan Sprague-Dawley rats (Gavage studies). *Natl Toxicol Program Tech Rep Ser*. 2006:4–168.
- Oakley GG, Devanaboyina Us, Robertson LW, Gupta RC. Oxidative DNA Damage Induced by Activation of Polychlorinated Biphenyls (PCBs): Implications for PCB-Induced Oxidative Stress in Breast Cancer. *Chemical Research in Toxicology*. 1996; 9:1285–1292. [PubMed: 8951230]
- Oikawa S, Kawanishi S. Site-specific DNA damage at GGG sequence by oxidative stress may accelerate telomere shortening. *FEBS Letters*. 1999; 453:365–368. [PubMed: 10405177]
- Oikawa S, Tada-Oikawa S, Kawanishi S. Site-Specific DNA Damage at the GGG Sequence by UVA Involves Acceleration of Telomere Shortening†. *Biochemistry*. 2001; 40:4763–4768. [PubMed: 11294644]
- Pavanello S, Pesatori AC, Dioni L, Hoxha M, Bollati V, Siwinska E, Mielzyńska D, Bolognesi C, Bertazzi PA, Baccarelli A. Shorter telomere length in peripheral blood lymphocytes of workers exposed to polycyclic aromatic hydrocarbons. *Carcinogenesis*. 2010; 31:216–221. [PubMed: 19892797]
- Perona R, Machado-Pinilla R, Manguan C, Carrillo J. Telomerase deficiency and cancer susceptibility syndromes. *Clinical and Translational Oncology*. 2009; 11:711–714. [PubMed: 19917533]
- Ren H, Collins V, Fernandez F, Quinlan S, Griffiths L, Choo KHA. Shorter Telomere Length in Peripheral Blood Cells Associated With Migraine in Women. *Headache: The Journal of Head and Face Pain*. 2010; 50:965–972.
- Ritchie JM, Vial SL, Fuortes LJ, Guo H, Reedy VE, Smith EM. Organochlorines and risk of prostate cancer. *J Occup Environ Med*. 2003; 45:692–702. [PubMed: 12855910]
- Safe SH. Polychlorinated Biphenyls (PCBs): Environmental Impact, Biochemical and Toxic Responses, and Implications for Risk Assessment. *Critical Reviews in Toxicology*. 1994; 24:87–149. [PubMed: 8037844]
- Sandor Sipka SYE, Kwang Won Son, Shifen Xu, Vasileios G Gavalas, Hennig Bernhard, Toborek Michael. ORAL ADMINISTRATION OF PCBs INDUCES PROINFLAMMATORY AND PROMETASTATIC RESPONSES. *Environmental Toxicology and Pharmacology*. 2008; 25:251–259.
- Saretzki G, Von Zglinicki T. Replicative Aging, Telomeres, and Oxidative Stress. *Annals of the New York Academy of Sciences*. 2002; 959:24–29. [PubMed: 11976182]
- Schleizinger JJ, Struntz WD, Goldstone JV, Stegeman JJ. Uncoupling of cytochrome P450 1A and stimulation of reactive oxygen species production by co-planar polychlorinated biphenyl congeners. *Aquat Toxicol*. 2006; 77:422–432. [PubMed: 16500718]
- Schramm H, Robertson LW, Oesch F. Differential regulation of hepatic glutathione transferase and glutathione peroxidase activities in the rat. *Biochem. Pharmacol*. 1985; 34:3735–3739. [PubMed: 4052112]
- Senthilkumar PK, Klingelutz AJ, Jacobus JA, Lehmler H, Robertson LW, Ludewig G. Airborne polychlorinated biphenyls (PCBs) reduce telomerase activity and shorten telomere length in immortal human skin keratinocytes (HaCat). *Toxicology Letters*. 2011; 204:64–70. [PubMed: 21530622]
- Silberhorn EM, Glauert HP, Robertson LW. Critical Reviews in: Carcinogenicity of Polyhalogenated Biphenyls: PCBs and PBBs. *Critical Reviews in Toxicology*. 1990; 20:440–496. [PubMed: 2165409]
- Slane BG, Aykin-Burns N, Smith BJ, Kalen AL, Goswami PC, Domann FE, Spitz DR. Mutation of Succinate Dehydrogenase Subunit C Results in Increased O₂•–, Oxidative Stress, and Genomic Instability. *Cancer Research*. 2006; 66:7615–7620. [PubMed: 16885361]
- Smith LL, Collier HA, Roberts JM. Telomerase modulates expression of growth-controlling genes and enhances cell proliferation. *Nat Cell Biol*. 2003; 5:474–479. [PubMed: 12717449]

- Srinivasan A, Lehmler HJ, Robertson LW, Ludewig G. Production of DNA strand breaks in vitro and reactive oxygen species in vitro and in HL-60 cells by PCB metabolites. *Toxicol Sci.* 2001; 60:92–102. [PubMed: 11222876]
- Tharappel JC, Lee EY, Robertson LW, Spear BT, Glauert HP. Regulation of Cell Proliferation, Apoptosis, and Transcription Factor Activities during the Promotion of Liver Carcinogenesis by Polychlorinated Biphenyls. *Toxicology and Applied Pharmacology.* 2002; 179:172–184. [PubMed: 11906247]
- Tsai PC, Huang W, Lee YC, Chan SH, Guo YL. Genetic polymorphisms in CYP1A1 and GSTM1 predispose humans to PCBs/PCDFs-induced skin lesions. *Chemosphere.* 2006; 63:1410–1418. [PubMed: 16580705]
- Venkatesha VA, Venkataraman S, Sarsour EH, Kalen AL, Buettner GR, Robertson LW, Lehmler HJ, Goswami PC. Catalase ameliorates polychlorinated biphenyl-induced cytotoxicity in nonmalignant human breast epithelial cells. *Free Radical Biology and Medicine.* 2008; 45:1094–1102. [PubMed: 18691649]
- von Zglinicki T. Oxidative stress shortens telomeres. *Trends in Biochemical Sciences.* 2002; 27:339–344. [PubMed: 12114022]
- Vulliamy TJ, Walne A, Baskaradas A, Mason PJ, Marrone A, Dokal I. Mutations in the reverse transcriptase component of telomerase (TERT) in patients with bone marrow failure. *Blood Cells, Molecules, and Diseases.* 34:257–263.
- Wege H, Chui MS, Le HT, Tran JM, Zern MA. SYBR Green real-time telomeric repeat amplification protocol for the rapid quantification of telomerase activity. *Nucleic Acids Research.* 2002; 31:e3. [PubMed: 12527792]
- Wei W, Zhang C, Liu AL, Xie SH, Chen XM, Lu WQ. Effect of PCB153 on BaP-induced genotoxicity in HepG2 cells via modulation of metabolic enzymes. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis.* 2009; 675:71–76.
- Westin ER, Chavez E, Lee KM, Gourronc FA, Riley S, Lansdorp PM, Goldman FD, Klingelhutz AJ. Telomere restoration and extension of proliferative lifespan in dyskeratosis congenita fibroblasts. *Aging Cell.* 2007; 6:383–394. [PubMed: 17381549]
- WHO. Polychlorinated biphenyls and terphenyls. Geneva, Switzerland: World Health Organization; 1993.
- Yoshizawa K, Brix AE, Sells DM, Jokinen MP, Wyde M, Orzech DP, Kissling GE, Walker NJ, Nyska A. Reproductive Lesions in Female Harlan Sprague-Dawley Rats Following Two-Year Oral Treatment with Dioxin and Dioxin-like Compounds. *Toxicologic Pathology.* 2009; 37:921–937. [PubMed: 19843953]
- Zhang TC, Schmitt MT, Mumford JL. Effects of arsenic on telomerase and telomeres in relation to cell proliferation and apoptosis in human keratinocytes and leukemia cells in vitro. *Carcinogenesis.* 2003a; 24:1811–1817. [PubMed: 12919960]
- Zhang TC, Schmitt MT, Mumford JL. Effects of arsenic on telomerase and telomeres in relation to cell proliferation and apoptosis in human keratinocytes and leukemia cells in vitro. *Carcinogenesis.* 2003b; 24:1811–1817. [PubMed: 12919960]
- Zhu Y, Kalen AL, Li L, Lehmler HJ, Robertson LW, Goswami PC, Spitz DR, Aykin-Burns N. Polychlorinated-biphenyl-induced oxidative stress and cytotoxicity can be mitigated by antioxidants after exposure. *Free Radical Biology and Medicine.* 2009; 47:1762–1771. [PubMed: 19796678]

Figure 1.

Telomerase activity in control and PCB153-treated HaCat cells. In HaCaT, PCB153 significantly reduced telomerase activity from day 6 to 48. The solvent alone had no effect. Telomerase activity in NFK cells was too low (data not shown) to be evaluated. *Error bars denote SD, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$*

Figure 2.

Telomere length in control and PCB153-treated HaCat cells. PCB153 produced a significant shortening of telomere length from day 18 to 48. No effect was seen in NFK cells and all solvent-treated cultures. Error bars denote SD, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Figure 3.

Intracellular levels of superoxide, determined by DHE oxidation. PCB153 significantly increase intracellular DHE oxidation levels in HaCaT (top) and NFK (bottom) cells. No effect was seen in solvent-treated cultures. Error bars denote SD, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Figure 4.

Cell growth of control cells and PCB153 cells. PCB153 caused a significant reduction in cell growth in HaCaT (top) and NFK (bottom) cells at all time points tested (bars), but no change in cell viability (symbols and lines) was observed in either of the cell types throughout the experiment. Error bars denote SD, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 1

Effect of PCB153 on cell cycle distribution in HaCat cells

PCB153 produced an increase in S and G2/M -phase with concomitant decrease in G0/G1 phase at all 3 time points. Colcemide (Col) was used as a positive control.

% of cells in	Day 6					Day 30					Day 48				
	Media	DMSO	PCB153	Col		Media	DMSO	PCB153	Col		Media	DMSO	PCB153	Col	
G0/G1	60.5	59.5	53.6***	3.5		60.3	60.1	54.1***	3.2		60.2	60.3	53.2***	3.9	
S	31.3	32.1	35.9***	60.4		31.2	30.9	35.2***	60.5		30.9	30.7	36.5***	60.2	
G2/M	8.2	8.3	10.5**	36.1		8.5	9	10.7**	36.3		8.9	9	10.3**	35.9	

Table 2

Effect of PCB153 on cell cycle distribution in NFK cells

PCB153 produced an increase in G2/M-phase with concomitant decrease in G0/G1 and S phase at all 3 time points.

% of cells in	Day 6			Day 18			Day 24		
	Media	DMSO	PCB153	Media	DMSO	PCB153	Media	DMSO	PCB153
G0/G1	75.09	75.59	71.73***	75.24	75.69	70.53***	77.09	76.79	71.9***
S	17.65	17.18	14.59***	17.49	16.95	14.49***	15.85	15.80	14.92**
G2/M	7.25	7.22	13.68***	7.25	7.35	14.98***	7.05	7.30	13.18***

* $P < 0.05$,

** $P < 0.01$,

*** $P < 0.001$