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Induction of cytochrome P450 1A1 in MCF-7 human breast cancer cells by 4-chlorobiphenyl (PCB3) and the effects of its hydroxylated metabolites on cellular apoptosis

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Abstract

Several studies suggest an involvement of PCBs in breast cancer formation, but the results are ambiguous and the mechanisms not clear. We propose that local activation of cytochrome P450 enzymes, CYP1A1 and CYP1B1 by PCB3, may generate active metabolites which affect apoptosis and thereby promote mammary carcinogenesis. To test this hypothesis MCF-7 human breast cancer cells were exposed to 300 nM PCB3 and its hydroxylated metabolites, 4OH-PCB and 3,4diOH-PCB3. The enzyme activity for CYP1A1 was assayed using the EROD assay, and CYP1A1 and CYP1B1 protein expression by western blotting. PCB3 increased CYP1A1 activity (~1.5fold) and protein levels within 6 hrs after exposure. No effect on CYP1B1 protein expression was observed. The effects of PCB3 and both its metabolites on staurosporine-induced apoptosis were determined by measuring DNA fragmentation using ELISA and TUNEL assays, and by measuring caspase-8 and caspase-9 activity. We found that PCB3 and both of its hydroxylated metabolites had no effect on caspase-8 and caspase-9 activity when cells were grown in medium deprived of estrogen, but reduced caspase-9 activity when cells were grown in medium supplemented with serum containing estradiol. Interestingly, a decrease of DNA fragmentation was observed upon treatment with 3,4diOH-PCB3 in both culture conditions, suggesting that 3,4diOH-PCB3 affects a caspase-independent pathway of cell death. In summary, interactions of PCB3 and its metabolites with estradiol by yet unknown mechanisms inhibit caspase 9-related apoptosis and additional, other death pathways are affected by the catechol metabolite 3,4diOH-PCB3. These anti-apoptotic effects and the change in metabolic activity may contribute to the carcinogenic effect of PCBs.

Keywords

PCB3; 4OH-PCB3; 3,4diOH-PCB3; MCF-7 cell line; CYPs activity; apoptosis

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1. Introduction

The relationship between exposure to polychlorinated biphenyls (PCBs) and breast cancer has been addressed in numerous epidemiological studies since the early 1990s (Guttes et al., 1998; Dorgan et al. 1998; Zheng et al., 2000; Wolff et al., 2000; Lucena et al., 2001). Several mechanisms may be involved in the modulation of breast cancer risk by specific PCB congeners, but the exact mechanisms are still unclear.

A number of studies have found a correlation between high PCB levels and breast cancer in connection with specific CYP isoforms (Moysich et al., 1999; Laden et al., 2002; Zhang et al., 2004; Li et al., 2005). This indicates that the metabolism of either an exogenous compound like PCBs, or of endogenous compounds like estradiol, may play a role. The metabolism of estradiol and PCBs includes oxidation (mainly hydroxylation) mediated by cytochrome P450s (CYPs) (Safe, 1989; Martucci and Fishman, 1993). CYP1A1, which catalyzes the 2-hydroxylation of estradiol, is induced by PCBs (Bandiera et al., 1982). Human CYP1B1, which catalyzes the 4-hydroxylation of estradiol, also activates numerous procarcinogen and promutagens (Shimada et al., 1996). The potential roles of CYP1A1 and CYP1B1 in carcinogenesis and tumor progression have led to the development of specific inhibitors of CYP1A1 and CYP1B1 as potential anti-mutagenic agents (Chun et al., 2001; Ciolino et al., 2002). The metabolic activation of PCBs by CYPs leads to the formation of arene oxide intermediates and reactive quinones which can bind covalently to macromolecules like DNA, RNA and proteins and induce cell death (Amaro et al., 1996; Oakley et al., 1996; Srinivasan et al., 2001; Zhao et al., 2004).

One mechanism of cell death is by apoptosis, also called programmed cell death. Resistance to apoptosis is closely linked to tumorigenesis, as it enables malignant cell populations to expand even in a stressful environment. Apoptosis has therefore taken a central position in cancer research. Apoptosis is a tightly regulated form of cell death, which can be initiated by two different types of signals: intracellular stress signals (intrinsic pathway) and extracellular ligands (extrinsic pathway) (de Bruin and Medema, 2008). In both cases various caspases are involved. Interestingly, it has been demonstrated that PCBs can both activate and inhibit the caspase pathway of apoptosis (Whysner and Wang, 2001; Tharappel et al., 2002; Gregoraszcuk et al., 2003; Gregoraszcuk et al., 2005; Pocar et al., 2005; Ptak et al., 2006). However, cells can also die by non-apoptotic mechanisms, such as autophagy, mitotic catastrophe and necrosis.

Recently, high levels of lower chlorinated biphenyls were measured in indoor and outdoor air and a surprisingly high percentage of PCB exposure through inhalation was calculated (Harra et al., 2006; Ishikawa et al., 2007). One of these lower chlorinated biphenyls is 4-chlorobiphenyl (PCB3). PCB3 is a substrate for hepatic CYPs and is metabolized to mono- and dihydroxylated species; the latter once can be activated by peroxidases and other mechanisms to electrophilic quinones (McLean et al., 1996). Recently PCB3 and two of its metabolites were shown to induce pre-neoplastic foci in the liver of exposed rats, but the mechanisms is unknown (Espandiari et al., 2004). To analyze the potential role of PCB3 and its metabolites in mammary carcinogenesis we measured the ability of the PCB 3 to activate CYP1A1 and CYP1B1 in the MCF-7 cells line, and the effect of PCB3 and two of its hydroxylated metabolites on cell apoptosis determined by measuring the activities of caspase-8 and caspase-9, as well as by TUNEL and DNA fragmentation assays.

2. Materials and Methods

2.1. Chemicals

PCB3 and its hydroxylated metabolites, 4OH-PCB3 (4'-hydroxy-4-chlorobiphenyl) and 3,4diOH-PCB3 (3',4'-dihydroxy-4-chlorobiphenyl) were synthesized using the Suzuki coupling reaction as described by Bauer et al. (1995) and Lehmler and Robertson (2001). Details about their characterization and purity have been described previously (Ptak et al., 2005). This PCB congener and these two metabolites were chosen as test compounds, since PCB3, 4OH-PCB3 and the oxidized form of 3,4-diOH-PCB3 had been positive in the rat liver initiation assay (Espandiani et al., 2004). Stock solutions of these test compounds in DMSO were prepared and added to the culture medium immediately before use as described below. The final concentration of DMSO in the medium was 0.1% in each case. DMSO at this concentration has no effect on cell viability (data not shown).

DMEM Medium without phenol red, Fetal Bovine Serum (FBS, heat inactivated), Penicilinum, Streptomycinum, Trypan blue, and Charcol-dextran, were obtained from Sigma Chemical Co., MO, USA.

2.2. Cell culture

MCF-7 human breast cancer cells (ATCC) were routinely cultured in DMEM supplemented with 10% heat-inactivated FBS (Sigma), 100 U/ml penicillin and 100 µg/ml streptomycin. Cells were grown in 75 cm² tissue culture dishes (Nunc) in a 37°C incubator with a humidified mixture of 5% CO₂ and 95% air. Forty-eight hours before experiments, the medium was removed and replaced by DMEM without phenol red supplemented with: 1) 5% charcoal/dextran-treated FBS (5% FBS CD; steroids removed) or 2) 5% FBS. The following day cells were re-plated in the same medium and 24 h later treated with 300 nM PCB3 and its metabolites (4OH-PCB3 and 3,4diOH-PCB3) for different periods of time, depending on the experiment. This concentration of PCB3 and its metabolites was chosen based on our previous findings (Gregoraszczyk et al., 2008).

2.3. EROD activity

To determine CYP1A1 activity, cells were grown in 96-well plates and exposed to the test compounds for 6, 24 and 48 hours. Media were then removed and the cells were washed with cold phosphate buffered saline (PBS) and stored at -70°C for later determination of CYP1A1 activity. Cells were lysed by removing them from the freezer and allowing them to thaw for 10 min. To determine ethoxyresorufin-O-deethylase (EROD) activity, 50 µl BSA solution (1.33 mg/ml in 50 mM Tris, pH 7.2; final reaction concentration) and 100µl ethoxyresorufin solution (10 µM in 50 mM Tris, pH 7.2; final reaction concentration) were placed into each well. Each blank well received in addition 50 µl Tris buffer (50 mM, pH 7.2). Plates were incubated for 15 min at 37°C with gentle shaking. To start the reaction, 50 µl NADPH solution (final reaction concentration of 1.67 mM in 50 mM Tris, pH 7.2) was added to each reaction well but not to the blank wells. The samples were incubated at room temperature without shaking and the increase in fluorescence due to CYP1A-mediated oxidation of non-fluorescent ethoxyresorufin to fluorescent resorufin was measured at 15 min intervals for up to 2 hr with a fluorescence plate reader (FLx 800, Bio-Tek, USA) at 530 nM excitation and 590 nm emission wavelengths. Results were calibrated against a resorufin standard curve (0-100 nM) and BSA standard curve (0-1000 µg).

2.4. CYP1A1 and CYP1B1 protein level

For western blot analysis, cells were transferred into ice-cold lysis buffer after 3, 6, 24 and 48 hours of exposure to test compounds and stored at -20°C. The protein concentrations in the

lysates were determined by the Bradford assay (Bio-Rad Protein). Equal amounts of protein (20 µg) from each treatment group were separated by 10% SDS-PAGE and transferred to PVDF membranes using a Bio-Rad Mini-Protein 3 apparatus (Bio-Rad Laboratories, Inc., USA). The blots were blocked overnight with 5% dry milk and 0.1% Tween 20 in 0.02 M TBS buffer. Blots were incubated overnight at 4°C with antibodies specific for CYP1A1 (sc-9828) and CYP1B1 (sc-31667) (from Santa Cruz Biotechnology Inc, CA) and β-actin (A5316) (Sigma Chemical Co., MO, USA). After incubation with the primary antibody, the membranes were washed three times and incubated for 1 hour with a horseradish peroxidase-conjugated secondary antibody: P0447 (DakoCytomation, Denmark) for β-actin; sc-2020 (Santa Cruz Biotechnology Inc., CA, USA) for CYP1A1 and CYP1B1. Immunopositive bands were visualized using the Amplified Opti-4CN Kit (Bio-Rad Laboratories, Inc., USA) and were quantified using a densitometer (EasyDens, Cortex Nowa, Poland).

2.5. Caspase activity assays

Caspase assays were performed using colorimetric assay kits as per the manufacturer's instructions. MCF-7 cells were exposed to the solvent alone and test compounds for 24h and an additional 3h in the presence of Staurosporine (St). Media were then removed and the cells were lysed with the kit buffer. The protein concentrations in the lysates were determined by the Bradford method (Bio-Rad Protein). Equal amounts of cytosolic extract (50 µg protein) from each sample were analyzed. The Caspase-8/FOLICE Colorimetric Protease Assay Kit (BioSource International, Inc. USA) is based on the hydrolysis of the peptide substrate Ile-Glu-Thr-Asp-p-nitroanilide (IETD-pNA) by Caspase-8 which results in the release of a p-Nitroaniline (p-NA) moiety. The Caspase-9/FOLICE Colorimetric Protease Assay Kit (BioSource International, Inc. USA) is based on hydrolysis of Leu-Glu-His-Asp-p-nitroanilide (LEHD-pNA) by Caspase-9 which results in the release of a p-Nitroaniline (p-NA) moiety. p-NA was measured colorimetrically at 405nm for both Caspase assays. Appropriate controls were included as described in the manufacturer's instructions.

2.6. DNA fragmentation (ELISA)

DNA fragmentation was determined using the 'Cellular DNA Fragmentation ELISA' kit (Roche Molecular Biochemicals). This assay is based on the quantitative detection of bromodeoxyuridine(BrdU)-labeled DNA fragments. Briefly, after exposure to BrdU for 18 hr, cells were reseeded into a 96-well microplate (10⁵ cells/well) and treated for 24 hr with the test compounds and an additional 3 hr with Staurosporine. The supernatant was removed and the cells were lysed with the kit buffer. The cytoplasmic fraction was transferred separately into an anti-DNA precoated microtiter plate and analyzed using the ELISA procedure as recommended by the manufacturer. DNA fragmentation was measured spectrophotometrically at 450 nm. The results presented are the average value of three independent experiments.

2.7. TUNEL assay

To monitor DNA fragmentation via the Terminal dUTP nick-end labeling (TUNEL) assay, we used the APO-BrdU TUNEL assay Kit (Calbiochem, CBA040), and the procedure was performed in accordance with the manufacturer's instructions. MCF-7 cells (1×10⁶ cells/ml) were exposed to the test compounds for 24 hr and an additional 3 hr with Staurosporine. After cell fixation with 1% formaldehyde in PBS (pH 7.4) for 60 min, and centrifugation at 300×g for 5 min, the samples were washed with PBS and stored in 70% ethanol (1 ml) at -20° C until the TUNEL assay was performed. For the TUNEL assays, cells were pelleted by centrifugation at 300×g for 5 min and then washed twice with the provided wash buffer. The cells were resuspended in 50µl of DNA-labelling solution containing terminal deoxynucleotide transferase (TdT) reaction buffer, TdT enzyme, and bromodeoxyuridine triphosphate (BrdUTP), and incubated in a 37° C water bath for 60 min with shaking every 15 min. Cells were

washed twice with the provided rinse buffer and the cell pellets resuspended in 100 μ l antibody solution containing fluorescein-labelled anti-BrDU antibody and incubated at room temperature for 30 min in the dark. Propidium iodide/RNase A solution (500 μ l) was added, the cells were incubated at room temperature for 30 min in the dark and then analyzed by flow cytometry (DAKO Galaxy, USA) using the parameters outlined in the APO-BrDU protocol as per the manufacturer's instructions. Four independent experiments were carried out.

2.8 Statistical analysis

Each experiment was repeated three times (n=3) in quadruplicates. The average of the quadruplet values was used for statistical calculations. Statistical analysis was performed using Statistica 6.0. Data were analyzed by 1-way analysis of variance (ANOVA) followed by the Tukey honestly significant difference (HSD) multiple range test. Groups that are significantly different from each other are indicated in the figures with asterisk. (*p<0.05), (**p<0.01), (***)p<0.001).

3. Results

3.1. Effect of PCB3 on CYP1A1 activity

CYP1A1 enzymatic activity was determined using the EROD assay. The basal CYP1A1 activity in the controls decreased from 65.9 ± 5.4 pmol/100 μ g protein/min at the 6 hr time point to 9.74 ± 1.3 pmol/100 μ g protein/min after 48 hr of culture. PCB3 increased the CYP1A1 activity at all time points compared to the controls, i.e. after 6 hr (p<0.001), 24 hr (p<0.05) and 48 hr (p<0.01) of exposure (Fig.1).

3.2. Effects of PCB3 on CYP1A1 and CYP1B1 protein levels

CYP1A1 and CYP1B1 protein levels were measured by immunoblot analysis in control cells and cells exposed to 300 nM of PCB3 for 3, 6, 24 and 48 hours. β -actin was used as control for equal loading of the lanes. The endogenous level of CYP1A1 in untreated cells was low, but increased after six hours of exposure to PCB3 (Fig. 2). PCB3 treatment had no effect on CYP1B1 expression at all time points tested (Fig. 3).

3.4. Effects of PCB3 on MCF-7 cell apoptosis

Apoptosis was induced by 3 hr exposure to staurosporine at the end of a 27 hr exposure to the test compounds. PCB3 and 4OH-PCB at a concentration of 300 nM, statistically significant decreased DNA fragmentation measured by ELISA (78% and 77% of control values, respectively; p<0.05) (Fig. 4a). The strongest inhibition of DNA fragmentation measured by ELISA (62% of the control) and TUNEL assay (59% of the control) was observed under the influence of 3,4diOH-PCB3 (p<0.001; Fig. 4a, b). None of the compounds tested changed caspase-8 activity compared to the control (Fig. 4c). A statistically significant decrease in caspase-9 activity to 80%, 80%, and 77% was observed in the presence of PCB3, 4OH-PCB3, and 3,4diOH-PCB3, respectively (p<0.01; Fig. 4d).

Some differences in apoptotic endpoints were observed when cells were cultured in medium supplemented with charcoal-dextran-treated (hormone-depleted) FBS. PCB3 and 4OH-PCB3 at a concentration of 300 nM had no effect on DNA fragmentation measured by ELISA and TUNEL assay. However, a statistically significant decrease in DNA fragmentation measured by ELISA was noted in cells exposed to 3,4diOH-PCB3 (P<0.01; Fig. 5a,b). None of the compounds tested had an effect on caspase-8 (Fig. 5c) and caspase-9 activity in the hormone-depleted medium (Fig. 5d).

4. Discussion

Estrogens and PCBs are both believed to be involved in breast cancer development possibly by several different mechanisms and reactive metabolites. To gain further insight into the role of a lower chlorinated biphenyl (PCB3) in this process, we analyzed cytochrome P450 induction by PCB3 and inhibition of apoptosis by PCB3 and two of its hydroxylated metabolites in MCF-7 cells, which express the arylhydrocarbon receptor (AhR) and estrogen receptor alpha (ER α).

CYPs activation

The results presented here show that PCB3 increases CYP1A1 activity from 6 until 48 hr of exposure with the stimulatory action being highest after 6 hr of exposure. This increase in activity was accompanied by an increase in CYP1A1 protein after 6 hr of exposure to PCB3. No effect on CYP1B1 protein expression was observed. The highest CYP1A1 activity observed after 6 hr of exposure was comparably small, only about 2.5fold of control, but this effect was seen with only 300 nM of PCB3. For comparison, 1 nM TCDD increased CYP1A1 and 1B1 mRNA in MCF-7 about 60fold (Hayes et al 1996). The 40fold increase in mRNA of these CYPs by indolo[3,2-b]carbazole resulted in about equal increase (20fold) in 2- and 4-hydroxylation of E2 (Hayes 1996). Thus it cannot be said how great the influence of the PCB3-induced increase in CYP1A1 activity on the metabolism of exogenous and endogenous compounds like PCBs and estradiol, respectively, will be. Using rat liver microsomes from animals that had been treated with different CYP inducers McLean *et al.* (1996) observed that CYP1A was very efficient in PCB3 metabolism and produced predominantly two catechols (2',3'- and 3',4'-) and to a lesser extent the hydroquinone (2',5'-) in the non-chlorinated ring. The resulting change in metabolite profile may be important for carcinogenesis, since in a rat liver cancer initiation study the 3,4-quinone of PCB3 was found to be active, but not the 2,5-quinone (Espandiar et al., 2004).

It must also be pointed out that the change in the expression level of CYP1A1 not only alters the metabolism of PCB3 and other exogenous compounds but also affect the estrogen in breast tissues and cells. It is well known that endogenous estrogens can be hydroxylated at multiple positions by cytochrome P450 enzymes. A major metabolite of estradiol, 2-hydroxyestradiol, is mainly produced by CYP1A1 in extrahepatic tissues (Aoyama et al., 1990). Steroid hormones are metabolized by many of the same cytochrome P450 enzymes that metabolize xenobiotics, thus changes in xenobiotic metabolism should also be reflected by an altered metabolism of steroid hormones. Pang et al. (1999) reported that exposure of MCF-7 cells to PCBs 81, 126 and 39 caused highly elevated CYP1A1 and CYP1B1 mRNA levels and caused marked stimulation of E2 metabolism in this cell line. It is believed that 4-hydroxylation of estradiol is the more dangerous metabolic pathway than 2-hydroxylation. Since PCB3 induced CYP1A (2-hydroxylation) but not CYP1B (4-hydroxylation), this change in metabolic capacity may actually be protective and lower the risk of breast cancer. However, estrogen is believed to be carcinogenic by at least 3 different mechanisms, genotoxic effects by its 3,4-hydroxylated metabolite, stimulation of cell proliferation through the estrogen receptor, and induction of aneuploidy (Russo and Russo 2006). Moreover, PCB77, a typical AhR agonist and CYP1A inducer, was found to also increase ER α mRNA and a complicated cross-talk between estrogen- and Ah-receptor signaling pathways has recently been described (Mortensen and Arukwe, 2007). Thus more aspects of the complex interactions of PCBs with breast tissue cells need to be considered.

Cell apoptosis

Apoptosis is recognized as a major barrier which must be circumvented by tumor cells to allow them to survive and proliferate under stressful conditions (Hanahan and Weinberg, 2000).

Moreover, resistance to apoptosis plays an important role in tumorigenesis (Johnstone et al., 2002, de Bruin and Medema, 2008). The two most common mechanisms of apoptosis in a cell are through either an extrinsic pathway which involves the activation of plasma membrane death receptors (Fas receptor/caspase-8 pathway) or an intrinsic pathway which depends upon mitochondrial release of cytochrome c (cytochrome c/caspase-9 pathway).

Exposing MCF-7 cells to the apoptosis inducer staurosporine, we found that PCB3 and its hydroxylated metabolites had no effect on caspase-8 and caspase-9 activity when cells were grown in medium deprived of estrogen, however, all three test compounds, decreased caspase-9 activity when cells were grown in medium supplemented with 5% normal FBS which contains a small (~0.76 pg/ml) but sufficient amount of estradiol to support cell proliferation of these cells. These results indicate that neither of the PCBs induced apoptosis through the plasma membrane death receptor or the mitochondrial pathway, but all three compounds reduced the apoptotic effect of staurosporine, which induces apoptosis primarily through the mitochondrial cytochrome c/caspase-9 pathway, in the presence of estradiol. Currently only limited information is available about the apoptotic effects of PCBs. Several studies have shown that some PCB mixtures and individual congeners can increase cell proliferation and inhibit apoptosis in rodent liver *in vivo* and hepatocytes *in vitro* (Bohnenberger et al., 2001; Haag-Gronlund et al., 2000; Kolaja et al., 2000; Tharappel et al., 2002; Whysner and Wang, 2001). Another study indicated that exposure to PCB126 and PCB153 decreased the incidence of apoptotic bodies and caspase-3 activity in cells from large porcine follicles (Gregoraszczyk et al., 2003). To our knowledge the observation that the anti-apoptotic effect of a PCB congener or metabolite is hormone dependent is novel. Estradiol has been reported to prevent apoptosis in breast cancer cells (Perillo et al., 2000). More specifically, Seeger and coworkers (2006) showed no effect of E2 on concentration of FasL (caspase-8 pathway), but a decrease of the concentration of cytochrome c in MCF-7 cells. PCB3 and both hydroxylated metabolites were shown to bind to and activate the estrogen receptor (ER α) in transfected yeast, T47D, and MCF-7 cells (Layton et al., 2002; Machala et al., 2004; Wu et al., 2008). We suggest that the observed decrease of caspase-9 activation by PCBs in the presence of E2 could be due to changes caused by E2 in the cells providing an additive or synergistic effect with the PCBs. E2 and PCBs could act either through the same mechanism, for example ER α activation, or through different mechanisms, for example if PCB effects depend on the maintenance of sufficient cell proliferation by E2. The elucidation of the exact mechanism(s) needs further studies.

Besides caspases we also analyzed the effects of PCBs on apoptosis by measuring DNA fragmentation by ELISA and the TUNEL assay. Although all three compounds reduced DNA fragmentation in medium supplemented with 5% normal FCS, confirming the observations with the caspase-9 assay, a decrease of DNA fragmentation was also observed upon treatment with 3,4diOH-PCB3 in hormone-depleted medium suggesting that 3,4diOHPCB3 affects a caspase-independent pathway of cell death. Zhang and coworkers (2003) described such a staurosporine-induced caspase-independent apoptotic pathway that appeared to be involved in late apoptotic events in human melanoma cell lines. Interestingly, different action of 3,4diOH-PCB3 on MCF-7 cell proliferation compared to PCB3 and 4OH-PCB3, i.e. a decrease in proliferation and increase in G2/M and apoptotic cells after very long time exposure, have been observed by us previously (Gregoraszczyk et al., 2008). Also, in ovarian follicular cells, the kinetics of DNA damage induction and removal was different in 3,4diOHPCB3-treated cultures compared to PCB3 and 4OH-PCB3 in that 3,4diOH-PCB3 induced a significant increase in DNA damage, determined by the COMET assay, only at a very late time point, after 72 h of recovery (Ptak et al., 2006). How and/or whether these different effects are mechanistically correlated cannot be elucidated from the current limited information.

Alternative models of programmed cell death have been proposed, including autophagy, paraptosis, and mitotic catastrophe, as well as the descriptive model of apoptosis-like and necrosis-like programmed cell death (Broker et al., 2005). Considering that the oxidized metabolite of 3,4diOH-PCB3, the 3,4-quinone, was described to be the most likely ultimate carcinogenic metabolite of PCB3 (Espandiari et al., 2004) and that breast cancer cells contain peroxidases that can metabolically activate 3,4diOH-PCB3 to this reactive species, a further analysis of this pathway and its mechanisms could help to understand the risks associated with the exposure to such lower halogenated and therefore metabolizable PCB congeners.

In summary, PCB3 modestly increases CYP1A1, but not CYP1B1, protein levels and activity in MCF-7 cells, an alteration that may change metabolic activation pathways of PCB3 itself and of estradiol, and PCB3 and its metabolites interfere in the presence of estradiol with the intrinsic (cytochrome c/caspase-9) apoptosis pathway. Additionally, other non-caspase related death pathways seem to be inhibited by 3,4diOH-PCB3. Further experiments are required to determine the mechanisms of action of PCB3 and particularly 3,4diOH-PCB3 that may be critical for tumor initiation and promotion and their relationship to the inhibition of apoptosis.

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Figure 1. Effect of PCB3 on CYP1A1 activity (EROD assay) after 6, 24 and 48 hours of treatment. Control cells were incubated with 0.1% DMSO alone. Values are mean $\pm$ SEM. An asterisk indicates a value statistically different from the control (* p <0.05); (** p <0.01); (***) p <0.001)

Figure 2.

CYP1A1 protein levels in MCF-7 cells treated for 1 to 48 hours with 300 nM PCB3; a) Western blot, b) densitometry analysis of Western blot. Control cells (C) were treated with 0.1% DMSO only. The amount of protein loaded per lane was 20 μ g. B-actin protein levels were determined to control for loading and the transfer of cell protein.

Figure 3.

CYP1B1 protein levels in MCF-7 cells treated for 1 to 48 hours with 300 nM PCB3; a) Western blot, b) densitometry analysis of Western blot. Control cells (C) were treated with only 0.1% DMSO. The amount of protein loaded per lane was 20 μ g. B-actin protein levels were determined to control for loading and transfer of cell protein

Figure 4.

Effect of PCB3 and its metabolites (300 nM) on: a) DNA fragmentation as determined by ELISA, b) DNA fragmentation as determined by TUNEL assay, c) caspase-8 activity, and d) caspase-9 activity after 27 hr of growth in medium supplemented with 5% FBS. Staurosporine (St; 0.1µM) was added during the last 3 hr to induce apoptosis. An asterisk indicates a value statistically different from the control (*p<0.05); (**p<0.01); (**p<0.001)

Figure 5.

Effect of PCB3 (300 nM) and its metabolites on a) DNA fragmentation as determined by ELISA b) DNA fragmentation as determined by TUNEL assay c) caspase-8 activity and d) caspase-9 activity after 27 hrs of growth in medium deprived of estrogen by treatment with activated charcoal-dextran (5% FBS CD). Staurosporine (St; 0.1µM) was added during the last 3 hr to induce apoptosis. An asterisk indicates a value statistically different from the control (**p<0.01)