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ORAL ADMINISTRATION OF PCBs INDUCES PROINFLAMMATORY AND PROMETASTATIC RESPONSES

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Abstract

Exposure to specific congeners of polychlorinated biphenyls (PCBs) can induce proinflammatory alterations, which may contribute to the formation of blood-borne tumor metastasis. The main aim of the present study was to establish an experimental model of PCB exposure in which PCBs are administered by oral gavage, which resembles the human exposure through the food chain. To determine structure-function relationship, we studied induction of inflammatory responses in the livers, lungs and brains of mice treated with PCB77 (a major coplanar PCB), PCB104 (a non-coplanar PCB with multiple *ortho*-chlorine substituents), and PCB153 (a major non-coplanar PCB) after a single gavage dose (150 µmol/kg body weight). The strongest expression of proinflammatory proteins occurred 24 h following the PCB administration independent of the class of PCB congeners. These data indicate that food-chain exposure to PCBs can induce proinflammatory mediators in organs that are potential targets for PCB-induced toxicity.

Keywords

Polychlorinated biphenyls; tumor metastasis; inflammation

1. Introduction

Polychlorinated biphenyls (PCBs) are a class of 209 discrete congeners in which the physical, chemical, and primarily biological properties vary according to the degree and position of chlorine substitution on the biphenyl ring. Two major groups of PCBs can be distinguished, namely dioxin-like and non-dioxin-like PCBs. Dioxin-like congeners do not have chlorine substitution at the *ortho*-positions; therefore, they can adopt a coplanar structure and bind to the aryl hydrocarbon receptor (AhR). Non dioxin-like PCBs have at least two chlorine substitutions at the *ortho*-positions and are non coplanar due to steric hindrance. They exert

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their toxic effects via mechanisms independent on the AhR binding (Kimbrough, 1995; Hansen, 1999; Hennig et al., 2005).

Due to their stability and lipophilic character, PCBs persist in the environment and accumulate in fatty tissues of animals and humans (Kimbrough, 1995; Hansen, 1999). PCBs are well known as potential neurotoxic (Seegal et al., 2005), teratogenic, and embryotoxic agents, effecting the embryonic development in fish (Arzuaga et al., 2004), birds (Hoffman et al., 1996; Summer et al., 1996), rodents (Goodwill et al., 2007; Kimura-Kuroda et al., 2007) and humans (Pocar et al., 2006; Roegge and Schantz 2006). PCBs can also contribute to the development of cancer in experimental animals (Carpenter, 2006; Knerr and Schrenk, 2006; Lehmann et al., 2007) and are classified as the probable carcinogens in humans (class 2A carcinogens according to the IARC classification) (Shields, 2006).

Recently, several vascular effects of PCBs have been described (Hennig et al., 2002; Hennig et al., 2007). Our group reported that selected PCBs can activate endothelial cells, disrupt endothelial monolayers, and stimulate both extravasation and transmigration of the cancer cells (Toborek et al., 1995; Choi et al., 2003; Eum et al., 2004; Eum et al., 2006a; Eum et al., 2006b). Several signal transduction pathways, such as the PI3K, epidermal growth factor receptor (EGFR) and Janus kinase 3 (JAK3), and c-Src kinase signaling pathways appear to be involved at different stages of these effects (Eum et al., 2006a; Eum et al., 2006b). Based on these observations, we hypothesize that PCBs can not only induce carcinogenic effects, but also contribute to the development of cancer metastases.

Metastasis is a complex process by which tumor cells spread out from the primary tumor. The direct adhesive interaction between tumor cells and the vascular endothelium is the critical step in the formation of blood-borne metastasis. It requires binding of tumor cells to specific adhesion molecules on the surface of endothelial cells. Chemokines, such as monocyte chemoattractant protein-1 (MCP-1) (Lebrecht et al., 2004; Kuroda et al., 2005) and several adhesion molecules, including vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) (Silva et al., 2006; Kobayashi et al., 2007), are involved in this process. It is striking to notice that MCP-1, VCAM-1 and ICAM-1 are classical inflammatory mediators which mediate transendothelial migration of leukocytes (Desideri and Ferri, 2005). This fact illustrates a close relationship between cancer and inflammatory processes (Balkwill, 2004; Aggarwal et al., 2006).

Extravasation of tumor cells also requires activation of matrix metalloproteinases (MMPs). MMPs are a family of zinc enzymes responsible for degradation of extracellular matrix components including basement membrane collagens, interstitial collagen, fibronectin, and various proteoglycans during tissue remodeling and repair processes. Thus, MMPs are involved in angiogenesis, cell migration, proliferation, and apoptosis (Folgueras et al., 2004). MMPs also contribute to the development of a number of pathological conditions where accelerated turnover of extracellular matrix plays an important role, such as rheumatoid arthritis, osteoarthritis, atherosclerotic plaque rupture, aortic aneurysms, periodontitis, autoimmune skin disorders, and chronic ulcerations. Finally, MMPs play a critical role in the growth, invasion, and metastasis of malignant tumors (Deryugina and Quigley, 2006; Jodele et al., 2006). We have shown that specific MMP-3 is involved in PCB-induced migration of tumor cells across the vascular endothelium (Eum et al., 2006b).

The vast majority of studies on PCB toxicity are based on *in vitro* models or animal experiments in which mice or rats are intraperitoneally injected with PCBs. However, the most common route of PCB exposure is through the food chain. Therefore, the aim of the present study was to establish an *in vivo* mouse model in which PCBs are administered orally and enter the body

through the gastro-intestinal tract. Due to their role in cancer metastases, the present study focused on PCB-mediated induction of inflammatory mediators and MMPs.

2. Materials and Methods

2.1 Reagents and experimental animals

PCB77 (3,3',4,4',-tetrachlorobiphenyl), PCB104 (2,2',4,6,6'-pentachlorobiphenyl), and PCB153 (2,2',4,4',5,5'-hexachlorobiphenyl) were purchased from AccuStandard (New Haven, CT) and were > 99% pure. Individual PCBs were dissolved in vitamin stripped corn oil. The PCB solutions were administered by oral gavage with a 18 G gavage needle, 3" long, curved, and with 2¼ mm ball diameter (Popper and Sons, New Hyde Park, NY).

Male C57Bl/6 mice (7–8 weeks old; weight 22–25 g) were obtained from the Charles River Laboratories (Wilmington, MA) and kept in a temperature- and humidity-controlled environment. Food and water were provided ad libitum, and the animals were subjected to a 12 h light-dark cycle. The animals were acclimated to the facility for 7 days before starting the experiments. All procedures and handling techniques were approved by the University of Kentucky Institutional Animal Care and Use Committee. Before tissue harvest, mice were anesthetized with pentobarbital and perfused with cold PBS. Liver, lung and brain samples were collected for RNA and protein expression. The samples for RNA were kept in RNeasy lysis buffer (Qiagen, Valencia, CA). Tissues were frozen immediately in liquid nitrogen and stored at –80° C until analysis.

2.2 Experimental groups

To study time-dependent effects of specific PCB congeners, mice received PCB77, PCB104 or PCB153 at the dose of 150 µmol/kg body weight by oral gavage. Literature data (Twaroski et al., 2001; Lue et al., 2003 and 2004) and our own published results (Hennig et al., 2002) indicated that similar doses of PCB when administered intraperitoneally to mice or rats can induce inflammatory responses. The control group of mice was administered with vehicle. Mice were sacrificed and samples were collected 12, 24, and 48 h after PCB administration. Due to its potent biological activity, PCB77 was selected for the dose-dependent experiments. Mice received this PCB congener at the doses of 150, 200 or 300 µmol/kg body weight. Samples were collected 24 h following PCB administration.

2.3 Real-time RT-PCR

Quantitative real-time PCR was performed using the ABI Prism 7000 Sequence Detection system (Applied Biosystems, Foster City, CA) as described earlier (Eum et al., 2006a; Eum et al., 2006b). Briefly, total RNA was extracted from the tissues using the RNeasy kit (Qiagen, Valencia, CA) and 1 or 2 µg RNA were reverse transcribed using Reverse Transcription system (Promega, Madison, WI, USA) in a total volume of 20 µL. All analyses were performed in duplicate. Specific primers and FAM/TAMRA-labeled probes were purchased from Applied Biosystems. β-actin mRNA was determined as an internal reference. Expression of target mRNA was calculated based on the standard curves generated by serial dilutions of the standard cDNA and normalized according to β-actin expression. Relative gene expression in tissues of PCB treated mice compared with control was calculated by dividing the normalized expression in PCB treated samples by the expression in control tissue (target gene/β-actin in PCB-treated mice)/(target gene/β-actin in control).

2.4 PCB analysis in tissue samples

Before analysis, the samples were thawed, and weighed. An amount of 0.2–0.5 g of the sample was spiked with surrogate standards (PCB65 and PCB166) and mixed with 1.0 g of

diatomaceous earth. The mixture was transferred to a glass mortar, grained into fine powder using a glass pestle, and transferred into the extraction cell. Extraction was performed using an ASE 200 accelerated solvent extractor (Dionex Corporation, Sunnyvale, CA). After extraction with hexane, the extracts were concentrated using Rotavapor from 22–23 ml to approximately 4 ml. Lipids were removed by addition of 2 ml of concentrated sulfuric acid. The samples were vortexed thoroughly and left overnight to achieve phase separation. Afterwards, the hexane layer containing PCBs was collected. In addition, the aqueous layer was extracted three times with 2 ml of hexane each time. The extracted aliquots were combined and the samples were concentrated by Rotavapor to approximately 2 ml. The samples were further concentrated down to 100 μ l using a gentle stream of nitrogen. Then 10 μ l of 1.0 ng/ μ l of internal standard PCB209 was added to each sample. Next, the samples were transferred to a glass microvial (with a 150 μ l insert) for analysis. Gas chromatographic analysis was performed with a GC- μ ECD system (Agilent GC 6890N, autosampler G2913A, μ ECD detector) (Agilent Technologies, Santa Clara, CA) using a HP-5MS 5% Phenyl Methyl Siloxane (30 m length, 0.25 mm internal diameter, 0.25 μ m film thickness) column. The column temperature was held at 60 $^{\circ}$ C for 1 min, then increased rapidly to 200 $^{\circ}$ C at 40 $^{\circ}$ C/min rate, and then increased slowly at a rate of 4 $^{\circ}$ C/min to 280 $^{\circ}$ C where it was held for 5.5 min. The injector temperature was set at 250 $^{\circ}$ C and the detector temperature was set at 280 $^{\circ}$ C. The carrier gas was ultra pure helium, and the makeup gas was nitrogen. PCBs were identified on the basis of their retention times relative to standards. Quantification was achieved based on calibration curves obtained using PCB standards, the recovery efficiency calculated from the surrogates, and the sample weight.

2.5 Statistical Analysis

The data were analyzed by one-way or two-way ANOVA using SigmaStat 2.03 software (SPSS, Chicago, IL, USA). Statistical probability of $p < 0.05$ was considered significant.

3. Results

3.1 Alterations of ICAM-1 expression as the result of PCB treatment

The effects of time exposure on changes in ICAM-1 mRNA levels in different organs of PCB-injected animals are reflected in Figures 1A–1C. In the livers, ICAM-1 mRNA levels significantly increased 12 and 24 h after administration of PCB77 and PCB104 (Figure 1A). In the lungs, a significant increase in ICAM-1 mRNA levels was detected in mice after administration of all studied PCBs at the 24 h time point (Figure 1B). In addition, ICAM-1 mRNA levels significantly increased in the brains 48 h after exposure to PCB104 and PCB153 (Figure 1C).

3.2 Alterations of VCAM-1 expression as the result of PCB treatment

The effects of PCB treatment on VCAM-1 mRNA expression are illustrated in Figures 2A–2C. In the livers, VCAM-1 mRNA levels were markedly elevated in the PCB77 and PCB104 groups 24 h post PCB administration. In addition, VCAM-1 mRNA levels were increased in the livers of mice 48 h after exposure to PCB104 (Figure 2A). In the lungs, exposure to all studied PCBs resulted in elevated VCAM-1 mRNA levels 24 h post administration (Figure 2B). However, treatment with PCBs did not affect VCAM-1 mRNA levels in the brains (Figure 2C).

3.3 Dose-dependent effects of PCBs on ICAM and VCAM mRNA expression

The dose-response effects of PCB77 on ICAM-1 mRNA expression are shown in Figure 3A. In these experiments, analyses were performed 24 h after exposure to PCB77. Consistent with the time-dependent experiments, treatment with PCB77 increased ICAM-1 mRNA levels in

the livers and the lungs. The induced effects were similar for all three doses of PCB77 used in the present study. Similarly, all doses of PCB77 markedly increased VCAM-1 mRNA expression in the livers and lungs 24 h after administration (Figure 3B).

3.4 Alterations of MCP-1 expression as the result of PCB treatment

The effects of PCBs on MCP-1 mRNA induction are reflected in Figures 4A – 4C. In the livers, PCB77 and PCB104 increased MCP-1 mRNA levels 48 h after exposure (Figure 4A). In lung tissue, administration of PCB77 elevated MCP-1 mRNA expression 24 and 48 h post exposure. In addition, PCB104 increased MCP-1 mRNA levels in the lungs 12 and 24 h following administration (Figure 4B). Induction of MCP-1 mRNA was also observed in the brains of animals 24 and 48 h after PCB77 and PCB104 exposure (Figure 4C).

3.5 PCB-mediated induction of MMP mRNA levels

Due to their role in tumor metastasis formation, the present study also focused on the effects of PCBs on the induction of MMP-3 and MMP-7. Hepatic MMP-3 mRNA expression was not affected by PCB treatment (Figure 5A). However, PCB104 and 153 increased MMP-3 mRNA levels in the lungs 24 h after exposure. MMP-3 mRNA levels also increased in the lungs 12 h post PCB104 administration (Figure 5B). In the brains, MMP-3 mRNA expression was increased 12 h after exposure to PCB77 and PCB104 as well as 24 h after exposure to all PCBs evaluated in the present study (Figure 5C).

As illustrated in Figure 6A, all studied PCBs increased MMP-7 mRNA levels in the livers 24 h post exposure. PCB77 and PCB104 also increased MMP-7 mRNA levels in the brains 24 and 48 h post administration (Figure 6C). In contrast, PCBs did not affect MMP-7 mRNA expression in the lungs (Figure 6B).

3.6 Tissue levels of PCBs

In addition to the measurements of gene expression, we assessed concentrations of the injected PCBs in the livers, lungs, and the brains of experimental animals (Table 1). PCB77 preferentially accumulated in the liver, where its levels exceeded those in the lungs approximately 16 times. PCB77 concentrations in the brain were very low and in the range of ~0.2 µg/g tissue. PCB104 levels in all studied tissues (liver, lung and brain) were below 0.5 µg/g tissue. PCB153 levels in the livers and the lungs of exposed animals were similar and in the range of ~8.5 µg/g tissue. In addition, PCB153 accumulated in the brains of treated mice, where its levels exceeded 3 µg/g tissue.

4. Discussion

The biological effects of PCBs are determined by their chemical structure and the interactions with specific cellular targets. Therefore, to establish a structure-function relationship of PCB-mediated induction of proinflammatory mediators *in vivo*, PCBs from several groups were used in the present study. Specifically, we used PCB77 as a typical coplanar PCB and an AhR ligand, PCB104 as an example of highly *ortho*-chlorine-substituted PCB congeners, and PCB153 as an example of a major non-coplanar PCB present in the environment and biological samples (Kimbrough, 1995; Hansen, 1999; Hennig et al., 2005). To mimic exposure to PCBs through the food chain, mice were exposed to these PCBs by oral gavage. In fact, the development of a model PCB toxicity induced by an oral administration of these compounds was one of the main goals of the present study.

Evidence indicates that an underlying mechanism of toxicity of PCBs is cellular oxidative stress (reviewed by Hennig et al., 2005 and 2007). Indeed, the indices of enhanced lipid peroxidation have been increased in Yusho victims 35 years after accidental poisoning with

PCBs (Shimizu et al., 2007). It appears that increased cellular oxidation induced by coplanar PCBs is related to the interaction with the AhR and activation of the cytochrome P450 1A (CYP1A) subfamily (Alsharif et al., 1994; Safe and Krishnan, 1995). It was demonstrated that PCB77 can uncouple the catalytic cycle of CYP1A1, allowing heme iron within the active site of this enzyme complex to act as a Fenton catalyst and generating hydroxyl radicals from hydrogen peroxide (Schlezingner et al., 1999; Schlezingner et al., 2006). Other coplanar PCBs, such as PCB126 and PCB169 also markedly increased cellular oxidative stress in vascular endothelial cells, brain, and hepatic tissues (Twaroski et al., 2001; Hassoun et al., 2002; Hennig et al., 2002; Ramadass et al., 2003) with superoxide anions being a major reactive oxygen species. Thus, induction of CYP1A1 or CYP1A2 may lead to generation of excess levels of reactive oxygen species, resulting in cell injury. In addition, induction of prooxidative responses can be involved in genotoxic effects of coplanar PCBs.

In the present study, oral administration of PCB77 markedly increased expression of pro-inflammatory and pro-metastatic mediators, such as ICAM-1, VCAM-1, MCP-1, MMP3, and MMP7. Oxidative stress-induced transcription factors NF-kappaB (NF- κ B) and activator protein-1 (AP-1) may play critical roles in the induction of these responses. To support this statement, it was demonstrated that the classical AhR agonist TCDD can activate NF- κ B and AP-1. For example, a dose-dependent activation of NF- κ B was observed in fish exposed to coplanar PCBs (Schlezingner et al., 2000). Pre-treating of human leukemic mast cells with the NF- κ B pathway inhibitor pyrrolidine dithiocarbamate suppressed PCB-induced NF- κ B activation and reduced expression of inflammatory mediators, such as cyclooxygenase-2 and interleukin-6 mRNA expression (Kwon et al., 2002). Our published studies based on an AhR-deficient mouse model indicated that a functional AhR is critical for the proinflammatory events mediated by coplanar PCBs in the vascular endothelium (Hennig et al., 2002). Other *in vivo* studies with AhR knockout mice also demonstrated that AhR activation is involved in the inflammatory response induced by the AhR ligands (Thatcher et al., 2007).

As indicated in the present study, PCB77 induced expression of proinflammatory and prometastatic mediators primarily in the liver and lungs of the exposed animals. In contrast, the effects of PCB77 were less pronounced in the brain and were limited to the induction of MCP-1 and MMPs in this tissue (Figure 4C, Figure 5C, and Figure 6C). These findings are in agreement with previous results which indicate that coplanar PCBs do not accumulate in the brain and have limited affinity to this organ (Dewailly et al., 1995; Saghir et al., 2000). In fact, our analysis of PCB tissue levels indicated only minimal presence of PCB77 in the brain (Table 1).

PCB104 appeared to be one of the most biologically active PCBs used in the present study. Treatment with this PCB congener stimulated induction of ICAM-1, VCAM-1, MCP-1 and MMPs. Despite the fact that levels of PCB104 in all organs studied were relatively low (Table 1), PCB104 was active in all examined tissues, i.e., in the livers, lungs, and brains of exposed animals. It appears that a low absorption or high metabolism rate of this PCB may be responsible for its low tissue levels. High biological activity of PCB104 is in the line with our published reports which indicated stimulation of endothelial permeability, tumor cell adhesion to microvascular endothelial cells, and transendothelial migration of tumor cells in response to exposure to this PCB (Eum et al., 2004; Eum et al., 2006a; Eum et al., 2006b).

Our research has focused on the vascular effects of highly *ortho*-chlorine-substituted PCBs and PCB104 is an example of this group of compounds (Choi et al., 2003; Eum et al., 2004; Eum et al., 2006a; Eum et al., 2006b). Exposure of human endothelial cells to PCB104 significantly increases oxidative stress in human vascular endothelial cells and activates redox-responsive transcription factors (Choi et al., 2003; Eum et al., 2004; Eum et al., 2006a; Eum et al., 2006b). We also have evidence that PCB104 can affect inflammatory pathways in

endothelial cells and induce expression of MCP-1, E-selectin and ICAM-1 (Choi et al., 2003). These effects are mediated by redox-regulated signaling, such as activation of AP-1 and NF- κ B as well as stimulation of Src kinase activity, epidermal growth factor receptor, and Janus kinase 3 (Choi et al., 2003; Eum et al., 2004; Eum et al., 2006a; Eum et al., 2006b).

PCB153 is a dominant PCB congener found in wildlife and human tissues and accounts for approximately 25% of all PCBs present in the environment (Derocher et al., 2003; Gladen et al., 2003; Barr et al., 2006). PCB153 is very persistent in the environment and its half-life may exceed 100 years in marine sediments (Jonsson et al., 2003). It is a di-*ortho*-substituted non-coplanar PCB congener which does not bind to the AhR and exhibits no dioxin-like activity. In fact, PCB153 and PCB congeners with similar chemical structure can antagonize the activity of the AhR ligands as demonstrated by measuring of induction of CYP1A1 (Suh et al., 2003). On the other hand, PCB153 is an inducer of cytochrome P450 2B1 and 2B2 (CYP2B1 and CYP2B2) and other phenobarbital-responsive genes (Vezina et al., 2004). In the present study, PCB153 appeared to be relatively less biologically active in the livers as compared to other PCB congeners. PCB153 did not induce expression of hepatic inflammatory mediators and its effects were largely limited to the lungs and brain. As indicated in Table 1, tissue levels of PCB153 reached relatively high concentrations in these organs. Among all PCB congeners evaluated in the study, PCB153 was the only congener which accumulated in the brain. A selective accumulation of *ortho*-substituted PCBs (such as PCB153) in the brain was described in the literature. For example, i.v. injections of rats with the same doses of PCB153 and PCB169 resulted in four to nine time higher levels of PCB153 in the brain as compared to those of PCB169 (Saghir et al., 2000). Similar dominant brain accumulation of *ortho*-substituted PCBs, including PCB153, has been observed in human autopsy samples (Dewailly et al., 1995). In the present study brain levels of PCB153 exceeded those of PCB77 by 17 fold. Thus, it appears that relative high levels of PCB153 in the brain may be responsible for the induction of ICAM-1 (Figure 1C) and MMP-3 (Figure 5) in this tissue. It was also described that treatment with PCB153 can activate NF- κ B (64) and this effect may be responsible for overexpression of inflammatory genes in the lungs and the brains as observed in the present study. In addition, several other toxic effects of PCB153 have been reported, including developmental toxicity (Coccini et al., 2006), estrogenic effects (Jung et al., 2005), alterations of reproductive functions (Oskam et al., 2005), angiogenesis (Tavolari et al., 2006), inhibition of gap junction communication (Machala et al., 2003), and hepatic proliferation (Lu et al., 2004).

In summary, the present study demonstrates that oral administration of individual PCB congeners can activate specific inflammatory mediators and MMPs. These effects can be induced by congeners belonging to different PCB classes, such as PCB77, PCB104, or PCB153. Expression of inflammatory mediators and MMPs may contribute to PCB-induced toxicity in the target organs.

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Figure 1. Effects of exposure to different PCB congeners on intercellular adhesion molecule-1 (ICAM-1) mRNA levels

PCB congeners were administered in a single dose of 150 $\mu\text{mol/kg}$ by oral gavage. Mice were sacrificed 12, 24 or 48 h post PCB administration. ICAM-1 levels were determined by real-time RT-PCR in the livers (A), lungs (B), and brains (C) of exposed animals. *Statistically significant as compared to respective controls.

Figure 2. Effects of exposure to different PCB congeners on vascular cell adhesion molecule-1 (VCAM-1) mRNA levels

Experiments were performed as described in the legend to Figure 1. VCAM-1 levels were determined by real-time RT-PCR in the livers (A), lungs (B), and brains (C) of exposed animals.

*Statistically significant as compared to respective controls.

Figure 3. Dose-dependent effects of PCB77 on ICAM-1 and VCAM-1 mRNA levels

PCB77 was administered by oral gavage in a single dose of 150, 200, or 300 µmol/kg. Mice were sacrificed 24 h post PCB77 exposure. ICAM-1 (A) and VCAM (B) mRNA levels were determined by real-time RT-PCR in the livers, lungs and brains of exposed animals.

*Statistically significant as compared to respective controls.

Figure 4. Effects of exposure to different PCB congeners on monocyte chemoattractant protein-1 (MCP-1) mRNA levels

Time-dependent experiments were performed as described in the legend to Figure 1. MCP-1 levels were determined by real-time RT-PCR in the livers (A), lungs (B), and brains (C) of exposed animals. *Statistically significant as compared to respective controls.

Figure 5. Effects of exposure to different PCB congeners on matrix metalloproteinase-3 (MMP-3) mRNA levels

Time-dependent experiments were performed as described in the legend to Figure 1. MMP-3 levels were determined by real-time RT-PCR in the livers (A), lungs (B), and brains (C) of exposed animals. *Statistically significant as compared to respective controls.

Figure 6. Effects of exposure to different PCB congeners on matrix metalloproteinase-7 (MMP-7) mRNA levels

Time-dependent experiments were performed as described in the legend to Figure 1. MMP-7 levels were determined by real-time RT-PCR in the livers (A), lungs (B), and brains (C) of exposed animals. *Statistically significant as compared to respective controls.

Table 1

Tissue levels of PCBs in animals exposed to specific congeners for 36 h. PCBs were administered by oral gavage in a single dose of 150 $\mu\text{mol/kg}$ body weight. Data are mean $\pm$ SEM.

PCB Congeners	Tissue Levels ($\mu\text{g/g}$ tissue)		
	Liver	Lungs	Brain
PCB77	9.35 $\pm$ 0.44	0.58 $\pm$ 0.05	0.19 $\pm$ 0.02
PCB104	0.10 $\pm$ 0.02	0.38 $\pm$ 0.13	0.11 $\pm$ 0.01
PCB153	8.33 $\pm$ 0.55	8.56 $\pm$ 1.39	3.17 $\pm$ 0.75