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The Effect of Dietary Glycine on the Hepatic Tumor Promoting Activity of Polychlorinated Biphenyls (PCBs) in Rats

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Abstract

Polychlorinated biphenyls (PCBs) are ubiquitous lipophilic environmental pollutants. Some of the PCB congeners and mixtures of congeners have tumor promoting activity in rat liver. The mechanism of their activity is not fully understood and is likely to be multifactorial. The aim of this study was to investigate if the resident liver macrophages, Kupffer cells, are important in the promoting activity of PCBs. The hypothesis of this study was that the inhibition of Kupffer cell activity would inhibit hepatic tumor promotion by PCBs in rats. To test our hypothesis, we studied the effects of Kupffer cell inhibition by dietary glycine (an inhibitor of Kupffer cell secretory activity) in a rat two-stage hepatocarcinogenesis model using 2,2',4,4',5,5'-hexachlorobiphenyl (PCB-153, a non-dioxin-like PCB) or 3,3',4,4'-tetrachlorobiphenyl (PCB-77, a dioxin-like PCB) as promoters.

Diethylnitrosamine (DEN, 150 mg/kg) was administered to female Sprague Dawley rats, which were then placed on an unrefined diet containing 5% glycine (or casein as nitrogen control) starting two weeks after DEN administration. On the third day after starting the diets, rats received PCB-77 (300 µmol/kg), PCB-153 (300 µmol/kg), or corn oil by i.p. injection. The rats received a total of 4 PCB injections, administered every 14 days. The rats were euthanized on the 10th day after the last PCB injection, and the formation of altered hepatic foci expressing placental glutathione S-transferase (PGST) and the rate of DNA synthesis in these foci and in the normal liver tissue were determined. Glycine did not significantly affect foci number or volume. PCB-153 did not significantly increase the focal volume, but increased the number of foci per liver, but only in the rats not fed glycine; PCB-77 increased both the foci number and their volume in both glycine-fed and control rats. Glycine did not alter the PCB content of the liver, but did increase the activity of 7-benzoyloxyresorufin O-dealkylase (BROD) in liver microsomes from PCB-153 treated rats. However, glycine did not affect the induction of ethoxyresorufin O-dealkylase activity by PCB-77 in liver microsomes. Glycine diminished hepatocyte proliferation in PGST-positive foci, but not in normal tissue. Overall these results do not support the hypothesis that dietary glycine inhibits the promoting activities of PCBs. The observations that PCB-153 increased the number of foci per liver in control rats but not glycine-fed rats and that dietary glycine reduced cell proliferation in PGST-positive foci, however, do not

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allow us to completely rule out a role for dietary glycine. But the data overall indicate that Kupffer cells likely do not contribute to the tumor promoting activities of PCB-77 and PCB-153.

Keywords

Kupffer cells; glycine; 2,2',4,4',5,5'-hexachlorobiphenyl; 3,3',4,4'-tetrachlorobiphenyl; tumor promotion; hepatocyte proliferation

INTRODUCTION

Polychlorinated biphenyls (PCBs) are ubiquitous lipophilic environmental pollutants which tend to accumulate into the food chain and to concentrate toward the top of this chain, i.e. into humans. At least some of the PCB congeners and mixtures are hepatic tumor promoters in rats. As reviewed by Glauert *et al.* (2001), strong evidence shows that PCB mixtures and medium- and highly-chlorinated individual congeners are hepatic tumor promoters in rats. The rodent model is considered a very good indicator of potential hepatic damage in humans (Hayes *et al.* 1982). Seven studies in rats revealed that PCBs promote gross liver tumors and 42 studies demonstrated that PCBs promote altered hepatic foci in rats (Dean *et al.* 2002; Glauert *et al.* 2005; Glauert *et al.* 2001; Tharappel *et al.* 2002; Whysner and Wang 2001).

Epidemiological studies, however, reached variable conclusions. Some studies showed that overall mortality rates are not higher in PCB exposed groups than in non-exposed groups (Bosetti *et al.* 2003; Kuratsune *et al.* 1987; Sinks *et al.* 1992). Some studies find no association between cancer mortality and PCBs (Kimbrough *et al.* 2003; Rusiecki *et al.* 2004). But other data suggest a correlation between PCB exposure and carcinogenesis. A study performed in Poland found that adipose tissue samples of patients who have died of liver cancer contained 4.7 µg/g of PCBs (23% of which was 2,2',4,4',5,5'-hexachlorobiphenyl [PCB-153]), while in samples from cancer-free subjects, the level was less than 1.9 µg/g of PCBs (Falandysz *et al.* 1994). Twelve deaths among 2061 victims of the Yucheng 1979, Taiwan rice-oil poisoning, 3.5 years after the accident were due to hepatoma, liver cirrhosis, or liver diseases with hepatomegaly (Hsu *et al.* 1985). Autopsies of 12 Yusho patients revealed five cases of carcinomas: two of the liver, two of the lung, and one of the esophagus (Kikuchi 1984). Another study on Yusho patients reported that in males the incidence of liver cancer was significantly higher in those patients than in unexposed males (Kuratsune *et al.* 1987). Synergistic factors or different congener composition may account for the different conclusions of the conflicting studies.

The mechanisms underlying the correlation between PCBs and cancer are not fully understood and might be multifactorial. PCB-153 is one of the most abundant congeners in the environment, and in both animals and humans (Chu *et al.* 2003; Duarte-Davidson *et al.* 1994; Falandysz *et al.* 1994; Gill *et al.* 2004; Lanting *et al.* 1998; Mariottini *et al.* 2000). Besides being one of the most abundant congeners in the environment, PCB-153 has been previously shown to increase hepatocyte proliferation in rats (Lu *et al.* 2003). PCB-77 is present in some dietary supplements for human consumption, such as cod liver oil (Storelli *et al.* 2004). In addition, PCB-77 was present in much higher concentration than PCB-126 and PCB-169 in human adipose tissue samples from Concepción, Chile (Mariottini *et al.* 2000). Both PCB-77 and PCB-153 have tumor promoting activity (Glauert *et al.* 2001). The mechanisms of their promoting activities are still unclear, though one possibility is an imbalance between increased cell replication and suppressed apoptosis in the hepatocyte population. Most of the PCB congeners do not directly interact with DNA, but they may promote or amplify in the hepatocyte the consequences of mutagenic events that would otherwise be repaired. The increased cell proliferation and decreased apoptosis increase the probability that a spontaneous mutation

could be propagated and that successive mutations accumulate into the genotype of some cells with a net result of promotion and progression of a tumor.

We have hypothesized that the activation of Kupffer cells by PCB-153 and PCB-77 is necessary for the formation of hepatic preneoplastic foci. Our working model is that PCBs activate Kupffer cells. It is known that Kupffer cells, when activated, release factors (such as TNF- α , HGF, IL-1 β , and PGE₂), which can cause tissue injury and altered gene expression in hepatocytes, leading to an increased mitogenic rate (Cimuro et al. 1997; Edwards et al. 1993). Exacerbated Kupffer cell activation might have multiple causes, one being the presence of xenobiotic toxic compounds, as in the case of hepatotoxicity induced by peroxisome proliferators. Peroxisome proliferators are a class of nongenotoxic carcinogens. Wy-14,643, a member of this class, activates NF- κ B in the Kupffer cells (Rose et al. 2000), and stimulates the Kupffer cells to release mitogenic cytokines such as TNF- α (Rose et al. 1997b) and reactive species such as superoxide (Rose et al. 1999b; Rusyn et al. 2000).

To test our hypothesis of Kupffer cell involvement in tumor promotion, we studied the effects of Kupffer cell inhibition on two-stage rat hepatocarcinogenesis using PCB-153 (a non-coplanar PCB) or PCB-77 (a coplanar PCB) as promoters. Diethylnitrosamine (DEN, 150 mg/kg) was used as the initiator. There are several ways to inhibit Kupffer cell secretory activity, including dietary administration of the amino acid glycine (Ikejima et al. 1996; Rose et al. 1997a), and we chose this model. Glycine or casein (as nitrogen control) was fed in an unrefined diet at 5% starting two weeks after DEN administration. The rats received four biweekly i.p. injections of vehicle (corn oil) or of PCB-77 or PCB-153. We quantified the formation of preneoplastic focal lesions expressing placental glutathione S-transferase (PGST) and the rate of DNA synthesis in these foci and in the normal tissue.

MATERIALS AND METHODS

Chemicals

PCB-153 (2,2',4,4',5,5'-hexachlorobiphenyl) and PCB-77 (3,3',4,4'-tetrachlorobiphenyl) were synthesized and characterized as described previously (Lehmler and Robertson 2001; Schramm et al. 1985). The purity of each PCB congener was greater than 99%, as assayed by gas-chromatography. Vitamin E-stripped corn oil was obtained from Acros Organics (Morris Plains, NJ). The diet for the entire study was Harlan Teklad, Global 18% protein Rodent Diet 2918 (Madison, WI). Glycine was from Sigma Chemical Company (St. Louis, MO). Casein was from Teklad (Madison, WI). Alzet osmotic pumps (model 2ML1) were obtained from Alza Scientific Products (Palo Alto, CA). The anti-5-bromo-2'-deoxyuridine (BrdU) antibody was purchased from Becton-Dickinson (San Jose, CA). Anti-placental glutathione S-transferase (PGST) antibody was from Novocastra (New Castle upon Tyne, England). Vectastain ABC Kit was from Vector Laboratories, (Burlingame, CA). The Antigen Retrieval Citra solution was purchased from BioGenex (San Ramon, CA). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

Experimental Design

Fifty female Sprague-Dawley rats weighing 100-125g at arrival (Harlan Sprague Dawley, Indianapolis, IN), were housed three per hanging-wire, stainless-steel cage in a temperature- and light-controlled room and were fed a powdered irradiated unrefined rodent diet (Harlan Teklad, Global 18% protein Rodent Diet 2918) and water ad libitum. The experimental protocols and procedures that involved rats were approved by the Institutional Animal Care and Use Committee of the University of Kentucky and were in accordance to all policies for the use and care of laboratory research animals as stipulated by the NIH. Upon arrival, the animals were allowed to adjust for 1 week before starting the experiment. DEN was dissolved

in saline and administrated by gavage, 150 mg/kg body weight. This dose was chosen based on previous studies (Berberian et al. 1995; Glauert et al. 2005; Stemm et al. 2005; Tharappel et al. 2002). Glycine or casein (casein contains 1.8% glycine) was fed in the unrefined diet at 5% starting on the 14th day after DEN initiation until the end of the experiment. Glycine was fed at the 5% level because of previous studies showing that this level inhibited the induction of cell proliferation and hepatic tumors by peroxisome proliferators (Rose et al. 1999a; Rose et al. 1997a). The diet was administered ad libitum in glass feeding cups. On the third day after starting the diets, PCB-77 (300 $\mu\text{mol/kg}$), PCB-153 (300 $\mu\text{mol/kg}$), or corn oil were injected i.p.; the number of rats in each group is shown in Table 1. Both PCB-77 and PCB-153 have been shown to have promoting activity in rat liver using a dose of 300 $\mu\text{mol/kg}$ (Glauert et al. 2001). The rats received a total of 4 PCB injections, administered every 14 days. Euthanasia was by overexposure to carbon dioxide, on the 10th day after the last PCB injection. 72 hours before the euthanasia, the rats were surgically implanted with Alzet osmotic pumps containing BrdU (20mg BrdU/mL PBS, 10 $\mu\text{L/h}$) for the measurement of DNA synthesis. The livers were immediately removed and weighed; a portion of each was frozen in liquid nitrogen and stored at -80°C until used. For each rat, a piece of liver was randomly taken from 4 different lobes and fixed in 10% buffered formalin.

PCB Analysis

PCBs 77 and 153 were extracted from liver samples using an Accelerated Solvent Extractor ASE 200 (Dionex Corporation, Sunnyvale, CA) as described previously (Kania-Korwel et al. 2007). A recovery standard solution consisting of PCBs 14, 65, and 166 (50 μL ; 4 mg PCB/L in hexane) was added to the extraction cells prior to extraction using hexane:acetone. The PCB extract was concentrated to 1 mL and an internal standard solution consisting of PCBs 30 and 204 (300 μL ; 100.8 μg PCB/L in hexane) was added. Sulfur impurities were removed using standard EPA procedures prior to PCB analysis (Kania-Korwel et al. 2005; Kania-Korwel et al. 2007; Lehmler et al. 2003).

The PCB analysis was performed using a HP 6890 gas chromatograph with 60-m DB-5 capillary column and a ^{63}Ni $\mu\text{-ECD}$ detector (Kania-Korwel et al. 2005). The detector temperature was 300°C . The oven temperature program was as follows: 100°C held for 1 min, then increased by $1^{\circ}/\text{min}$ from 100 to 240°C , $10^{\circ}/\text{min}$ from 240 to $280^{\circ}/\text{min}$, hold for 20 min. An aliquot of the PCB extract (4 μL) was injected and the concentration of PCB 77 and 153 in each sample was determined using the internal standard method.

Method blanks (samples containing only Celite and Florisil) and tissue blanks (liver tissue samples from control animals) were analyzed with each set of samples. The PCB 77 and PCB 153 levels in the method blanks were 8 ± 5 and 4 ± 2 ng, respectively. The recoveries rates of the surrogate standards were $98\pm 9.0\%$, $94\pm 11\%$, and $90\pm 11\%$ for PCBs 14, 65 and 165, respectively. Corrections of total PCB concentration were made for recoveries $< 100\%$ using the recovery rates of PCB 65. The method detection limit for PCB 77 was 16 ng, and for PCB 153 6 ng. The minimum limit of detection was for PCB 77 52 ng, and for PCB 153 20 ng.

Lipid Content Determination

Lipids were extracted from tissue samples with a chloroform:methanol mixture (2:1 v/v) using an Accelerated Solvent Extractor ASE 200 (Dionex Corporation, Sunnyvale, CA) as described previously (Kania-Korwel et al. 2007). The lipid content was determined gravimetrically after evaporation of the solvents and used to calculate lipid adjusted PCB levels in the liver.

Alkoxyresorufin O-dealkylation Assay

The microsomal fraction was isolated from the whole-liver homogenate using the method described by Schramm et al. (1985). Microsomal 7-benzoyloxyresorufin and ethoxyresorufin

were used as specific substrates for the CYP2B1/2 and CYP1A1 isozymes, respectively, in the alkoxyresorufin O-dealkylase method (Burke and Mayer 1974). The absorbance of resorufin was detected with a fluorescence spectrophotometer at an excitation wavelength of 556 nm and an emission wavelength of 589 nm.

Immunohistochemical Staining

Tissues were double-stained for PGST and for BrdU. The formalin-fixed liver tissues were paraffin-embedded, sectioned, laid on glass slides, stained with an anti-PGST antibody to identify altered hepatic foci (using Vector[®] Red as the staining agent), stained with an anti-BrdU antibody to identify nuclei that had incorporated BrdU (using diaminobenzidine (DAB) as the staining agent), and counter-stained with hematoxylin (Wynford-Thomas and Williams 1986). The Vectastain ABC Kit (Vector Laboratories, Burlingame, CA) was used, according to the protocol provided by the manufacturer. Three lobes per each slide were counted randomly, using at least 1000 hepatocellular nuclei per lobe and not less than 3500 total per slide, with an average of 400 nuclei being from PGST-positive foci. The labeling index was expressed as the percentage of number of labeled hepatocyte nuclei out of total number of hepatocyte nuclei and was calculated separately for focal and non-focal hepatocytes.

Analysis of PGST-Positive Foci

The number and volume of PGST-positive foci were measured using a computer digitizing system developed at University of Wisconsin (Campbell et al. 1982; Campbell et al. 1986; Xu et al. 1998). The images were captured using a Nikon Eclipse E800 microscope equipped with MACRO 0.5x and 1.0x lenses. The number of foci/cm³ (Saltykov method), foci/liver (Saltykov method), the mean focal volume (Saltykov method), and the volume fraction (Delesse method) were quantified.

Statistical Analysis

Most of the data, including those for the body and liver weights, labeling index, mean focal volume, and volume fraction, were analyzed by 2 × 3 analysis of variance (ANOVA). However, data for PCB levels were analyzed by 2 × 2 ANOVA because PCB-77 was not quantified in PCB-153-treated rats and PCB-153 was not quantified in PCB-77-treated rats. Individual differences between means were determined using the Bonferroni post hoc test. The number of foci per liver and number of foci per cubic centimeter data were analyzed by negative binomial regression model with logarithm as the link function. The statistical comparison was made between the PCB treatment groups with the respective corn oil groups (control groups), separately for casein and glycine diets. The goodness of fit of the model was assessed by Pearson X² value adjusted for overdispersion and the parameters of the model were estimated by the method of maximum likelihood. The Wald's asymptotic procedure was used to determine the p values for significance of the differences between the PCB treatment groups and the control groups. The statistical software PROC GENMOD of SAS, version 8, was used to carry out the mentioned statistical analyses. We used the negative binomial regression model for the analysis of data obtained for the number of foci per liver and number of foci per cubic centimeter because of the discrete nature of these measurements (Espandari et al. 2003). SAS version 8 (SAS Institute, Cary, NC) was used for the above analysis. The results were expressed as means ± standard deviations (SD). The results were considered significant at p < 0.05.

RESULTS

The effects of dietary glycine on two-stage hepatocarcinogenesis promoted by PCB-153 and PCB-77 were assessed in female Sprague-Dawley rats initiated with DEN. The body weights were not significantly modified by any of the treatments (Table 1). The absolute and relative

liver weights were significantly increased in the rats treated with PCB-77 ($p < 0.05$) but not with PCB-153, and the glycine treatment had no effect (Table 1).

The livers of control animals (which did not receive PCBs) contained detectable background exposure of about 50 ng/g wet weight of PCB-77 and PCB-153. These values were significantly lower than those of the PCB treated groups (Table 2). With the same dosage, the PCB-153 treated animals have about 2.5 times higher respective PCB levels than animals treated with PCB-77. However, dietary glycine did not significantly affect hepatic PCB levels.

The activities of the cytochrome P-450 1A and 2B families were examined by quantifying EROD and BROD activities, respectively. BROD and EROD activities were induced by PCB-153 and PCB-77, respectively (Figure 1). Dietary glycine significantly augmented the PCB-153-induced increase in BROD activity, but had no significant effect on PCB-77-induced EROD activity.

PCB-77 significantly increased the number of PGST-positive foci per liver and per cubic centimeter as well as the mean focal volume and the total focal volume as a percentage of liver volume, both in the casein and in the glycine groups (Table 3.). PCB-153 significantly increased the number of foci per liver compared to the corn oil group for the casein diet only. The number of foci per cubic centimeter was slightly increased for the casein diet by PCB-153 ($p = 0.08$) but not for the glycine diet. PCB-153 did not significantly increase the mean focal volume or the percentage of liver occupied by foci. There were no significant differences in focal number or volume between the casein and glycine diets in rats administered vehicle, PCB-77, or PCB-153.

To measure hepatocyte proliferation in normal and PGST-positive hepatocytes, we quantified the labeling indexes after 3 days of continuous BrdU infusion using Alzet pumps. In normal hepatocytes, the BrdU labeling index was similar among all groups (Figure 2). In PGST-positive hepatocytes, rats fed glycine had a lower labeling index, but neither PCB affected the labeling index. The labeling index was not different between different lobes of the same animal, regardless of the treatment. As expected, cell proliferation was higher in the PGST-positive foci than in the normal tissue.

DISCUSSION

The importance of this study resides in the fact that human exposure to PCBs (especially PCB-153) is ubiquitous and once the PCBs are in the body, important quantities of the parent congeners or their metabolites are retained for long periods of time (Chu et al. 2003; Guo et al. 1997). Therefore, the real human health concern is the long term effects of PCBs in the body, such as cancer.

In this study, we tested the hypothesis that the secretory activity of Kupffer cell is required for the promoting activity of polychlorinated biphenyls. For this, we examined the ability of dietary glycine to inhibit PCB-induced tumor promotion, since several studies have shown that glycine decreases the production of superoxide and TNF- α by Kupffer cells (Ikejima et al. 1996; Rose et al. 1999a; Rose et al. 1997a; Rusyn et al. 1999). The inhibition by glycine of TNF- α production by Kupffer cells is not as strong as using agents such as gadolinium chloride or methyl palmitate, but that may be an advantage for a therapeutic agent (Rentsch et al. 2005). Kupffer cell inhibition by glycine has also been shown to affect lipid metabolism in the liver (Neyrinck 2004). There were no adverse effects of glycine in this study, based on changes in body weight. However, since glycine receptors are present in other tissues, for example the central nervous system (Cascio 2004; Rajendra et al. 1997), it is likely that the administration of high levels of glycine exerted other biochemical effects. More recently it has been shown that glycine might affect tumor promotion by inhibiting angiogenesis (Yamashina et al.

2005). However, the ability of glycine to inhibit the production of cytokines such as TNF- α was not measured in this study. Therefore, any changes in glycine-fed rats cannot be unequivocally linked to lower levels of TNF- α or other cytokines, and the lack of an effect of glycine on altered hepatic foci production could be due to a lack of an effect on the levels of TNF- α or other cytokines.

Our present data show that the PCB treatment leads to increased PCB congener levels in the liver and increased cytochrome P-450 related enzyme activities, showing that the administration of the PCBs brought about the expected biochemical changes in the liver. With the same dosage, the PCB-153 treated animals have about 2.5 times higher respective PCB levels than animals treated with PCB-77. This difference is to be expected since PCB-77 is metabolized faster than PCB-153 (Robertson and Hansen 2001). Glycine increased cytochrome P-450 activity only for PCB-153. It was reported that, if Kupffer cells are co-cultured with hepatocytes and treated with LPS (a well known Kupffer cell activator) and simultaneously treated with phenobarbital, the induction by phenobarbital of cytochrome P-450 2B1 mRNA and the activity of PROD are significantly reduced (85%) (Milosevic et al. 1999). In our experiment, the activity of cytochrome P-450 2B1/2 was increased by PCB-153 + glycine by about 50% compared to PCB-153 + casein. Cytokines, released by macrophages, are possibly responsible for the down-regulation of cytochrome P-450 2B1/2B2 activities (Milosevic et al. 1999). Reports show that TNF- α , IL-1 β , IL-2 are able to reduce cytochrome P-450 2B1 and 1A1 levels (Cantoni et al. 1995; Ferrari et al. 1992; Milosevic et al. 1999; Pous et al. 1990).

In this study, the numbers of PGST-positive foci were significantly increased by both PCBs but were not significantly affected by dietary glycine. PCB-153 increased the number of foci per liver in the casein-fed group but not in the glycine-fed group. The volume of foci was increased by PCB-77 but was not affected by PCB-153 or dietary glycine. Cell proliferation was not increased by PCB treatment in this study either in the normal hepatocytes or in the PGST-positive hepatocytes. As expected, cell proliferation was higher in the PGST-positive foci than in the normal tissue. Previous studies using the same protocol on rats on the same strain, sex and age (Berberian et al. 1995; Glauert et al. 2005; Tharappel et al. 2002) found that cell proliferation in both focal and nonfocal hepatocytes was increased by PCB-77 (unlike our study) but was not affected by PCB-153 (similar result to our study).

In summary, in this study we have examined the hypothesis that Kupffer cell inactivation by dietary glycine would inhibit the promoting activities of PCB congeners. This study is the first to examine if Kupffer cell activation is important in the promoting activity of PCBs. Our findings indicate that dietary glycine likely does not influence the promoting activities of PCB-77 and PCB-153; however, the observations that PCB-153 increased the number of foci per liver in control rats but not glycine-fed rats and the reduction in cell proliferation in PGST-positive foci in glycine-fed rats do not allow us to completely rule out a role for dietary glycine. But the data overall indicate that Kupffer cells likely do not contribute to the tumor promoting activities of PCB-77 and PCB-153. These results clearly differ from those of Rose et al. (1999a), who found that Kupffer cell inactivation by dietary glycine inhibited liver carcinogenesis by the peroxisome proliferator Wy-14,643. The findings of the present study imply that Kupffer cell activation may not be essential for hepatic tumor promotion. In studies published thus far, PCBs (present study) and peroxisome proliferators (Rose et al., 1999a) are the only hepatic tumor promoting agents to be examined in Kupffer cell inactivation models. Only the study of other hepatic tumor promoting agents will provide further evidence as to whether Kupffer cell activation is important for most tumor promoting agents, with PCBs being an exception, or whether it is only important in promotion by peroxisome proliferators.

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List of abbreviations

BrdU

bromodeoxyuridine

BROD	Benzyloxyresorufin O-dealkylase
DEN	diethylnitrosamine
EROD	Ethoxyresorufin O-dealkylase
LPS	lipopolysaccharide
PCBs	polychlorinated biphenyls
PCB-153	2,2',4,4',5,5'-hexachlorobiphenyl
PCB-77	3,3',4,4'-tetrachlorobiphenyl
PGST	placental glutathione S- transferase

Figure 1. Effect of PCBs and glycine on ethoxyresorufin-O-dealkylase (EROD) and 7-benzyloxyresorufin-O-dealkylase (BROD) activities in rat liver microsomes

Values represent means $\pm$ SD. The number of rats per group is the same as in Table 1. Values with different letters are significantly different from one another ($p < 0.05$). **A.** Microsomal ethoxyresorufin was used as specific substrate for the CYP1A1 isoenzyme (Burke and Mayer 1974). PCB-77 induced CYP1A1 with no significant difference between glycine and casein groups. **B.** Microsomal 7-benzyloxyresorufin was used as specific substrate for the CYP2B1/2 isozymes (Burke and Mayer 1974). PCB-153 induced CYP2B1/2 and the effect in the glycine group was significantly greater than in the casein group.

Figure 2. Quantitation of hepatocyte proliferation in normal tissue (A) and in PGST-positive foci (B)

The labeling index was determined by BrdU immunohistochemical staining. Values represent means $\pm$ SD. The number of rats per group is the same as in Table 1. *Rats fed glycine had a lower labeling index in PGST-positive foci ($p < 0.05$ by two-way ANOVA).

Table 1
Effect of PCBs and glycine on the body weight, liver weight and relative liver weight

Glycine	PCB Treatment	Number of rats per group	Liver weight (g)	Body weight (g)	Relative liver weight (%)
Absent	Corn oil	9	10.4 ± 0.7	262 ± 12	4.0 ± 0.2
	PCB-77	9	14.0 ± 1.6*	248 ± 15	5.6 ± 0.5*
Present	PCB-153	8	11.7 ± 1.5	264 ± 20	4.4 ± 0.3
	Corn oil	9	10.9 ± 1.6	259 ± 18	4.2 ± 0.4
	PCB-77	9	13.1 ± 1.0*	248 ± 7	5.3 ± 0.5*
	PCB-153	6	11.7 ± 1.5	245 ± 25	4.8 ± 0.3

Results are expressed as mean ± SD. Values with an asterisk (*) indicate a significant PCB effect compared to the respective control treated with corn oil ($p < 0.05$). There were no significant effects due to glycine administration.

Table 2

Effect of PCBs and glycine on hepatic PCB concentrations

	Concentration [µg/g liver]		Concentration [µg/g lipid]		Liver content [µg/liver]	
	casein	glycine	casein	glycine	casein	glycine
Controls, PCB-77	0.04±0.02 (n=4)	0.06±0.06 (n=4)	n.d.	n.d.	0.46±0.10 (n=4)	0.67±0.82 (n=4)
Controls, PCB-153	0.39±0.62 (n=4)	0.03±0.02 (n=4)	n.d.	n.d.	3.8±5.80 (n=4)	0.35±0.22 (n=4)
PCB-77	5.5±3.68* (n=8)	5.5±3.0* (n=9)	59.3±38.70 (n=6)	75.4±49.72 (n=6)	77.1±58.55* (n=8)	73.0±39.00* (n=9)
PCB-153	13.8±3.43* (n=6)	15.1±3.43* (n=6)	220.8±51.2 (n=4)	242.4±64.78 (n=3)	165.4±46.6* (n=6)	175.0±44.09* (n=6)

Results are expressed as means ± SD, n.d. = not determined. The numbers of rats per determination are indicated in parentheses. Values with an asterisk (*) indicate a significant PCB effect compared to the respective control treated with corn oil (p<0.05). There were no significant effects due to glycine administration.

Table 3

Effect of PCBs and glycine on the induction of PGST-positive foci

The formalin-fixed liver tissues slides from the animals receiving corn oil only, PCB-77 or PCB-153 in corn oil were stained with an anti- PGST antibody to identify altered hepatic foci.

Glycine	Treatment (no. of animals)	Foci/cm ³ ($\times 10^3$)	Foci/liver ($\times 10^3$)	Mean focal volume (mm ³ $\times 10^{-3}$)	Focal volume (% of liver volume)
Absent	Corn oil(9)	2.3 $\pm$ 0.9	23.7 $\pm$ 10.2	3.0 $\pm$ 1.2	0.6 $\pm$ 0.3
	PCB-77(9)	7.5 $\pm$ 2.7*	104.7 $\pm$ 39.0*	4.4 $\pm$ 1.5*	3.3 $\pm$ 1.5*
Present	PCB-153(8)	3.0 $\pm$ 0.8	36.1 $\pm$ 15.0*	4.1 $\pm$ 1.1	1.3 $\pm$ 0.6
	Corn oil(9)	2.7 $\pm$ 0.6	29.2 $\pm$ 6.6	3.3 $\pm$ 1.2	0.9 $\pm$ 0.3
	PCB-77(9)	7.0 $\pm$ 1.2*	92.8 $\pm$ 18.9*	4.4 $\pm$ 1.5*	3.1 $\pm$ 0.9*
	PCB-153(6)	3.0 $\pm$ 1.5	35.6 $\pm$ 19.1	3.1 $\pm$ 1.5	1.0 $\pm$ 0.7

Results are expressed as mean $\pm$ SD. Values with an asterisk (*) indicate a significant PCB effect compared to the respective control treated with corn oil ($p < 0.05$). There were no significant effects due to glycine administration.