Effect of Dietary Selenium on the Promotion of Hepatocarcinogenesis by 3,3′,4,4′-Tetrachlorobiphenyl and 2,2′,4,4′,5,5′-Hexachlorobiphenyl

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Polychlorinated biphenyls (PCBs) are persistent organic pollutants that have promoting activity in the liver. PCBs induce oxidative stress, which may influence carcinogenesis. Epidemiological studies strongly suggest an inverse relationship between dietary selenium (Se) and cancer. Despite evidence linking Se deficiency to hepatocellular carcinoma and liver necrosis, the underlying mechanisms for Se cancer protection in the liver remain to be determined. We examined the effect of dietary Se on the tumor promoting activities of two PCBs congeners, 3,3′,4,4′-tetrachlorobiphenyl (PCB-77) and 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB-153) using a 2-stage carcinogenesis model. An AIN-93 torula yeast-based purified diet containing 0.02 (deficient), 0.2 (adequate), or 2.0 mg (supplemental) selenium/kg diet was fed to Sprague-Dawley female rats starting ten days after administering a single dose of diethylnitrosamine (150 mg/kg). After being fed the selenium diets for 3 weeks, rats received four i.p. injections of either PCB-77 or PCB-153 (150 μmol/kg) administered every 14 days. The number of placental glutathione S-transferase (PGST)-positive foci per cm^3 and per liver among the PCB-77–treated rats was increased as the Se dietary level increased. Unlike PCB-77, rats receiving PCB-153 did not show the same Se dose-response effect; nevertheless, Se supplementation did not confer protection against foci development. However, the 2.0 ppm Se diet reduced the mean focal volume, indicating a possible protective effect by inhibiting progression of preneoplastic lesions into larger foci. Cell proliferation was not inhibited by Se in the liver of the PCB-treated groups. Se did not prevent the PCB-77–induced decrease of hepatic Se and associated reduction in glutathione peroxidase (GPx) activity. In contrast, thioredoxin reductase (TrxR) activity was not affected by the PCBs treatment or by Se supplementation. These findings indicate that Se does not inhibit the number of PGST-positive foci induced during promotion by PCBs, but that the size of the lesions may be inhibited. The effects of Se on altered hepatic foci do not correlate with its effects on GPx and TrxR. Exp Biol Med 233:366–376, 2008

Key words: selenium; polychlorinated biphenyls (PCBs); glutathione peroxidase; thioredoxin reductase; tumor promotion; cell proliferation; altered hepatic foci

Introduction

The beneficial effect of Se in cancer chemoprevention has been recognized for nearly nine decades (1). The Se supplementation trial by Clark et al. (2), primarily designed to prevent skin cancer recurrence, demonstrated that treatment with Se decreased the risk of cancer of the prostate, lung, and colon and rectum, although no significant inhibitions were observed for several other types of cancer, including skin cancer, breast cancer, and liver cancer.

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Although this study and similar clinical trials as well as epidemiologic studies and animal studies pointed to the potential use of Se for cancer prevention and therapy, the mechanisms by which Se could protect from cancer have not been well defined. Proposed mechanisms that may explain the anti-cancer effect of Se involve the antioxidant effect of selenoproteins and source of Se metabolites that affect carcinogenesis (3). These mechanisms include, but are not limited to, antioxidant protection from glutathione peroxidases (GPx) and thioredoxin reductase (TrxR), cell proliferation inhibition, increased apoptosis, effects on the cell cycle, transcription factor activation, increased expression of the tumor suppressor gene p53, impaired glutathione (GSH) metabolism, and formation of Se metabolites that are anti-tumorigenic (4–6).

PCBs are persistent organic pollutants that have remained widely distributed because of their environmental mobility and their ability to biomagnify in the food chain (7, 8). PCBs were produced and commercially used as mixtures of congeners; there are 209 PCB congeners that have varying toxicities based on the number and position of chlorine molecules around the biphenyl ring. PCBs that have no chlorine substitutions at the ortho position can assume a coplanar configuration, and due to their strong affinity to the aryl hydrocarbon receptor (like 2,3,7,8-tetrachlorodibenzo-p-dioxin [TCDD]) are referred to as dioxin-like or coplanar PCBs (9, 10). However, PCB congeners that are ortho-substituted do not bind to the Ah receptor and have increased affinity for the constitutive androstane receptor (CAR) (11–13). Studies have shown that the toxic effects, carcinogenicity, and biochemical mechanism of these two groups differ (14). Two PCB congeners, one representing each group, were selected for this study: 3,3′,4,4′-tetrachlorobiphenyl (PCB-77), a coplanar PCB and Ah receptor agonist; and 2,2′,4,4′,5,5′-hexachlorobipenyl (PCB-153), a di-ortho substituted PCB and CAR agonist.

Epidemiologic studies have associated PCBs with cancer risk (15–18), albeit inconclusively. However, animal studies strongly suggest that PCBs are carcinogenic (19, 20). PCB compounds and individual congeners have been found to have promoting activity in animal studies (21); recently it has been shown that some PCB congeners and metabolites are possibly cancer initiators (22).

Several multistage-carcinogenicity studies have focused on the prevention of chemical induced hepatocarcinogenesis by dietary selenium; however, none has addressed the potential of Se for inhibiting the promotion of carcinogenesis by PCBs. With the growing popularity of Se supplementation, it is necessary to understand how Se interacts with persistent environmental pollutants such as PCBs. The aim of this study was to determine the chemopreventive effect of dietary Se on the hepatic tumor promoting activities of two PCBs congeners: a coplanar PCB, PCB-77; and a non-coplanar PCB, PCB-153.

Materials and Methods

Chemicals. PCB-77 and PCB-153 were synthesized and characterized as described previously (23). All dry constituents of the AIN-93 purified diet were from Harlan Teklad Test Diets (Madison, WI). The anti-placental glutathione S-transferase (PGST) antibody was purchased from Novocastra Laboratories Ltd. (Newcastle, England). The Vectastain staining kit was from Vector Laboratories (Burlingame, CA). The sodium selenite (Na_2SeO_3), thioredoxin (Trx), and all other chemicals were from Sigma-Aldrich Chemical Co. (St. Louis, MO).

Experimental Design and Animal Treatment. This study was approved by the University of Kentucky Institutional Animal Care and Use Committee. Female Sprague-Dawley rats, weighing 100–125 grams, were obtained from Harlan Sprague Dawley (Indianapolis, IN) and housed three rats per cage in a temperature- and light-controlled room.

The experimental protocol is shown in Figure 1. After one week of acclimatization, all rats were injected p.o. with diethylnitrosamine (DEN) dissolved in saline (150 mg DEN/kg). After a 10-day recovery period, rats were randomly divided into three diet groups (27–28 per group) and fed a purified diet (Table 1) based on the AIN-93 diet formulation (24) ad libitum until the rats were euthanized. Se (as Na_2SeO_3) was mixed with the diet at a dose of 0.02, 0.2, and 2.0 mg selenium/kg diet corresponding to low, adequate, and high Se diet, respectively.

After three weeks, randomly grouped rats (9–10 rats/treatment/diet) were injected i.p. with vehicle (corn oil), PCB-77, or PCB-153 (300 μmol/kg). The rats received a total of 4 i.p. injections administered every 14 days, and were euthanized 10 days after the last injection. Three days prior to euthanasia, Alzet osmotic pumps containing bromodeoxyuridine (BrdU) (20 mg/ml, 10 μL/hr) were implanted s.c. as described (25). Rats were sacrificed using carbon dioxide asphyxiation followed by cervical dislocation. Liver pieces were removed and fixed in 10% buffered formalin. The remaining liver was frozen in liquid nitrogen and stored at −80°C.

Tissue Processing and BrdU-Placental Glutathione S-Transferase (PGST) Immunostaining. At the time of necropsy, liver slices from all lobes were cut and fixed in formalin followed by paraffin embedding. The sections (6 μm) were stained using a BrdU/PGST double immunohistochemical staining method (26) with modification using a Vector Laboratories protocol.

Quantitation of PGST-Positive Altered Hepatic Foci. The number and volume of PGST-positive altered hepatic foci were measured using a quantitative stereology computer program, STEREO, as described previously (25, 27–29). The STEREO program was a generous gift from Drs. Yihua Xu and Henry Pitot, University of Wisconsin. Briefly, utilizing a microscope (Nikon Eclipse E800), images of the stained liver section were taken and processed

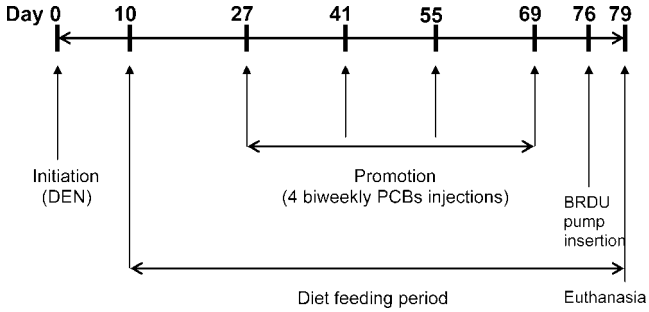

Figure 1. Experimental protocol. Female Sprague-Dawley rats were initiated with DEN (150 mg/kg body weight p.o.) before feeding with AIN 93-based purified diet containing varying levels of selenium (0.02, 0.2, 2.0 mg Se (as Na_2SeO_3)/kg). The promotion period consisted of four biweekly i.p. injections of corn oil, PCB-77 or PCB-153.

with a Scion Image software, Microsoft Photoshop, and a background correction program to generate data on tissue outline, X-Y coordinates, focal transection diameter, and location. The data were exported and organized using the STEREO program, and the resulting files were used to calculate volume % of foci in liver (Delesse), number of foci/cm^3 (Saltykov), number of foci/liver (Saltykov), and mean volume of foci (Saltykov) for each rat and group.

Counting of BrdU-Stained Nuclei. Representative images of all liver sections were taken and processed with the Scion image program and Photoshop. The NLIA program, a component of the STEREO program (29, 30), was employed to automatically count the BrdU labeled nuclei in each image. A total of approximately 4,000–6,000 nuclei were counted per slide. Cells that had brown nuclei were identified as BrdU labeled. All labeled and total hepatocytes in the non-focal area were counted. The labeling index was the percentage of number of labeled nuclei per total nuclei counted.

Protein Assay. The protein concentration of supernatants, dialysates, and cytosolic fractions was determined using the BCA method (Pierce Chemical Company).

Homogenate and Dialysate Preparation. Frozen liver tissues (0.45–5.0 g) were homogenized in phosphate-buffered saline (PBS) pH 7.4 with 1 mM EDTA solution for 30 seconds. The homogenate was centrifuged at 1300 *g* for 30 minutes and the supernatant collected for dialysis. The supernatant was dialyzed to remove endogenous GSH in PBS. Supernatant was pipetted into prepared dialysis tubing and placed into a beaker containing PBS pH 7.4 solution (100 ml PBS/1 ml supernatant) for 16 hours at 4°C. The dialysate was collected and aliquoted. The protein concentration of the dialysate was adjusted to 1 mg protein/ml (31, 32).

Thioredoxin Reductase (Trxr) Activity Assay. The TrxR activity in dialysates was determined using a method of Holmgren and Bjornsted (33) as modified by Hill et al. (32). Briefly, a reaction mixture was prepared containing 1 ml of 10 mg/ml insulin, 400 µl of 1M HEPES buffer (pH 7.6), 80 µl of 0.2M EDTA, and 80 µl of 40 mg/ml freshly prepared NADPH. 60 µl of the reaction mixture was added into tubes. Two tubes were allocated to each dialysate sample. To one tube, 15 µl of 60 µM *Escherichia coli* thioredoxin (test) was added, while 15 µl of distilled water was added to the other tube (control) to represent the non–TrxR-thioredoxin system-dependent reaction. After adding 105 µl ($\approx$105 µg protein) of dialysates into the test and control tubes, the reaction was incubated at 37°C for 15 minutes. The reaction was stopped by adding 750 µl of 0.4 mg/ml DTNB (5,5′-dithiobis(2-nitrobenzoic acid) in 6 M guanidinium hydrochloride, and the absorbance of both test and control mixtures was measured at 412 nm. The TrxR-thioredoxin system-dependent NADPH reduction of insulin was determined by subtracting the absorbance of the control from that of the test reaction mixture. TrxR activity was expressed as A_{412} units $\times$ 1000/(min $\times$ mg protein).

Table 1. Composition of Purified Diet

Constituent	Percent of diet
Torula yeast	30.0
Corn starch	36.0
Dextrose monohydrate	19.95
Cellulose fiber	5.0
AIN-93M mineral mix, without selenium	3.5
AIN-93M vitamin mix	1.0
Choline bitartrate	0.25
DL-methionine	0.3
Soybean oil	4.0

Cytosolic Fraction Preparation. Approximately 0.5 gram liver was used in the preparation of liver homogenate. Briefly, the liver was homogenized in 0.25 M sucrose/0.1 mM EDTA, pH 7.4, then centrifuged at 10,000 $\times$ *g* for 20 minutes. The supernatant was collected and then centrifuged at 100,000 $\times$ *g* for one hour. After separating the cytosolic fraction (supernatant) from the microsomal pellet, the cytosolic fraction was aliquoted for protein determination and enzyme assay.

Glutathione Peroxidase Activity Assay. The GPx activity of the cytosolic fraction was determined using the method of Paglia and Valentine (34) as modified by St. Clair and Chow (35). The enzyme activity was expressed as nmoles NADPH/min/mg protein.

Selenium Determination. Liver samples were analyzed using the neutron activation analysis (NAA). Briefly, the samples were weighed and freeze-dried. An aliquot of the freeze-dried sample was irradiated for 7 seconds at a flux of approximately 5×10^{13} $ncm^{-2}sec^{-1}$, decayed for 15 seconds, and counted for 25 seconds. The samples were analyzed using the gamma-ray (Energy = 161.9 Kev) from the decay of Se-77m (half-life = 17.45 sec and 52.4% abundant). The standard comparator method was used to obtain the absolute selenium concentration. In addition to the HPGe detector, the spectrometer system included a Tennelec 244 coupled to a Canberra 599 loss-free counting

Table 2. Effect of Selenium and PCBs on Body and Liver Weights[a]

Selenium	Treatment	Liver weight	Body weight	LW/BW ratio
Low	Corn oil	9.74 ± 0.70	258.11 ± 9.75	3.75 ± 0.16
	PCB-77	14.36 ± 0.50[b]	246.67 ± 4.56	5.82 ± 0.17[b]
	PCB-153	11.22 ± 0.18[b]	262.80 ± 4.46	4.40 ± 0.11[b]
Adequate	Corn oil	9.30 ± 0.19	254.78 ± 3.88	3.64 ± 0.05
	PCB-77	13.19 ± 0.38[b]	240.44 ± 5.43	5.50 ± 0.15[b]
	PCB-153	11.58 ± 0.57[b]	252.90 ± 5.16	4.41 ± 0.15[b]
High	Corn oil	9.86 ± 0.27	251.00 ± 3.82	3.93 ± 0.08
	PCB-77	13.70 ± 0.46[b]	246.22 ± 2.97	5.56 ± 0.14[b]
	PCB-153	12.29 ± 0.40[b]	258.80 ± 4.23	4.74 ± 0.11[b]

[a] Results are expressed as mean ± SEM. Each group contained 9–10 animals.
[b] Values are significantly different from their respective controls treated with corn oil ($P < 0.05$).

module and a Canberra 9660 DSP. Data acquisition and peak extraction were done using VAX Station 3100 model 38 Canberra ND application software. Se was expressed as mg selenium/kg wet tissue.

Statistical Analysis. For the analysis of the number of foci/cm^3 and number of foci/liver, a negative binomial regression model with logarithm as the link function was used, as previously described (22). All other results were analyzed by two-way analysis of variance followed by Tukey's post-hoc test for comparison of group means.

Results

Effect on Body and Liver Weights. The liver weights and the relative liver weights (as percentage of body weight) were significantly increased in rats treated with PCB-77 or PCB-153 at all levels of selenium, with the highest increase seen in the PCB-77 groups (Table 2). In contrast, PCBs had no effect on final body weight (Table 2) or weight gain (not shown). The dietary Se level did not affect the body weight or the gross or relative liver weights.

Effect on Altered Hepatic Foci Number and Volume. The number and volume of altered hepatic foci (AHF) were quantified using placental glutathione S-transferase (PGST) as an immunohistochemical marker. A representative PGST-positive focus is shown in Figure 2. The numbers of PGST-positive foci were quantified as foci/cm^3 and as foci/liver, which is calculated by multiplying the number of foci/cm^3 by the liver weight. The volume of the PGST-positive foci is expressed as two endpoints: the mean volume of the foci (in mm^3) and the volume fraction (the percentage of the liver that is occupied by foci), which represents the product of focal number and focal volume (25, 27–29). The numbers of PGST-positive foci per liver and per cm^3 were significantly increased in rats treated with PCB-77 compared with the corresponding corn oil control group for each diet. Surprisingly, this PCB-77 effect significantly increased with increasing dietary Se intake, and this was especially notable in the high Se diet groups (Fig. 3A and B). The percent of the liver that was occupied by PGST positive foci (volume fraction) was also significantly increased in the PCB-77–treated rats. The volume fraction in the high and adequate selenium groups treated with PCB-77 was significantly higher than that in the low selenium group treated with PCB-77, but the high selenium diet group was not increased when compared with the adequate selenium group (Fig. 3C).

In PCB-153–treated rats, although the number of PGST-positive foci per cm^3 in the high dietary selenium group was significantly different compared with the adequate and low dietary selenium groups ($P < 0.05$), this effect was not seen against the corresponding control or high Se corn oil group (Fig. 3B). Among the control (corn oil) groups, the number of PGST-positive foci per cm^3 and per liver in the high Se corn oil group was significantly different compared with the adequate and low dietary Se groups (Fig. 3B). Similarly, although the focal volume fraction was increased by PCB-153 treatment, it was significant only for the group fed with adequate selenium but not with low or high selenium (Fig. 3C). Note that the corn oil groups represent the control for the DEN initiation event only, i.e., no PCBs. In the corn oil control group, Se supplementation produced a 3-fold increase in the number of AHF per liver and per cm^3 compared with the low and adequate Se groups (Fig. 3A and B).

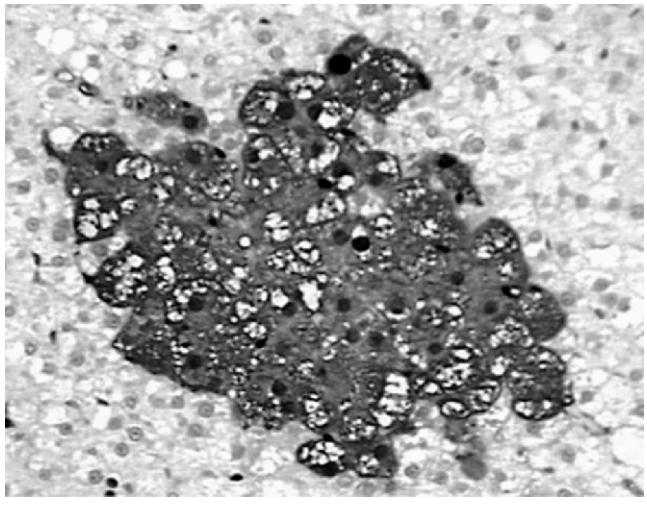

Figure 2. Representative PGST-positive focus.

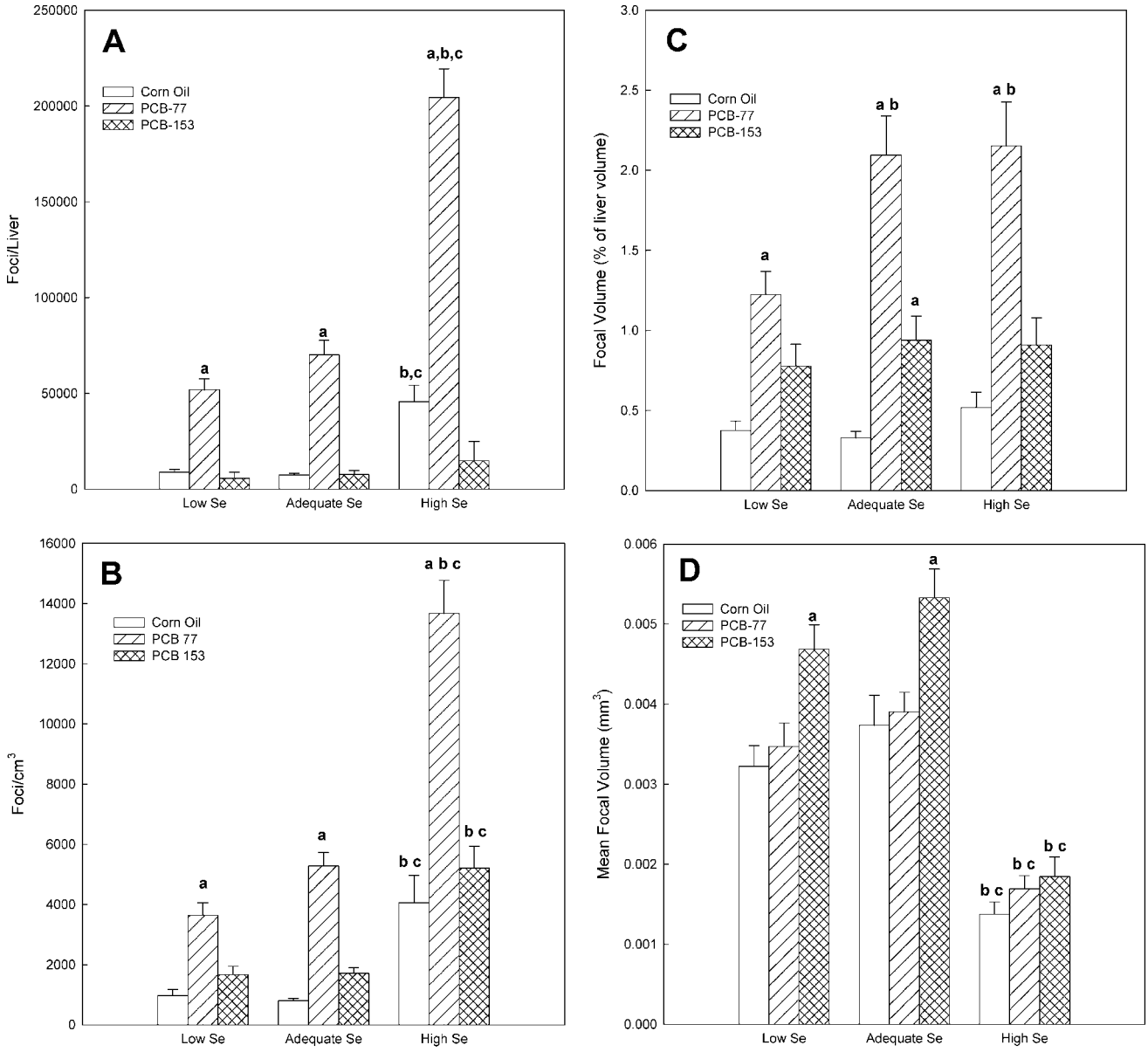

Figure 3. Effect of PCBs and dietary selenium on the induction of PGST-positive foci. A. Foci per liver; B. Foci per cubic centimeter; C. Volume fraction; D. Mean focal volume. Results are expressed as mean ± SEM. [a]Values are significantly different from their respective controls treated with corn oil ($P < 0.05$). [b]Values are significantly different from the low selenium diet group with similar treatment ($P < 0.05$). [c]Values are significantly different from the adequate selenium diet group with similar treatment ($P < 0.05$).

In contrast, the mean focal volume of the PCB-77–treated groups was not increased compared with the corresponding control, whereas PCB-153 treatment produced a significant increase, but only in the low and adequate selenium diet groups (Fig. 3D). The adequate Se diet did not affect the mean focal volume, compared with the Se deficient group. However, in the high Se diet groups, the mean focal volume was drastically reduced by about two-thirds compared to the levels seen in the adequate and low groups. The PCB-treated groups did not differ from the vehicle group in rats fed the high Se diets.

Effect on Cell Proliferation. Cell proliferation in non-focal areas was measured using BrdU labeling. BrdU was incorporated into DNA during DNA synthesis through a 3-day infusion of BrdU using an osmotic pump. The labeled nuclei represent the cells that progressed through the S phase of cell cycle. Both PCB treatments slightly increased the BrdU labeling index of the normal hepatocytes in all Se diet groups; however, this effect was not statistically significant except in the PCB-153–treated group that received adequate Se (Fig. 4).

Effect on GPx Activities. A significant dose-dependent increase in the activity of the cytosolic selenium-dependent GPx was observed in control (corn oil) rats fed the high Se diets (Fig. 5). At a supranutritional dose of 2 ppm selenium, GPx activity continued to increase.

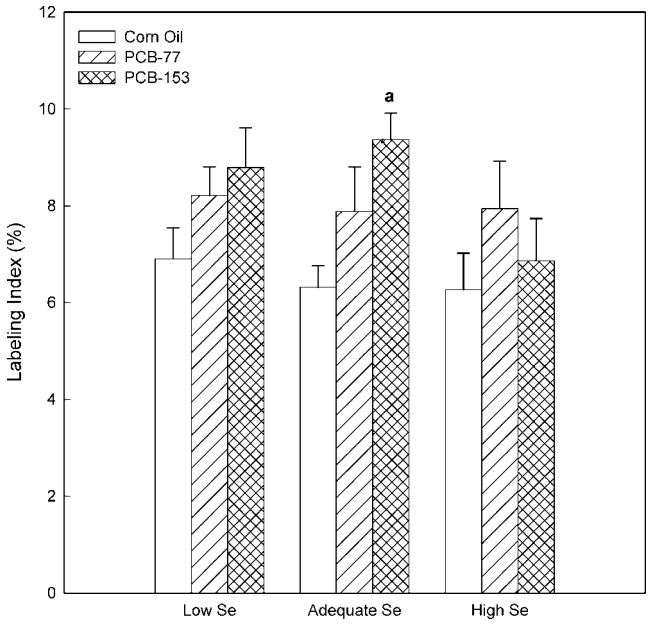

Figure 4. Cell proliferation in rat hepatocytes. Results are expressed as mean ± SEM. [a]Values are significantly different from their respective controls treated with corn oil ($P < 0.05$).

In the PCB-77–treated groups, a 67% decrease in GPx activity was noted in the Se-deficient group. The adequate Se diet produced more than a 2-fold increase in the GPx activity in PCB-77–treated rats compared with the low selenium diet, but GPx activity was still less than in untreated rats fed the adequate selenium diet. Further Se supplementation did not lead to a further increase in the GPx

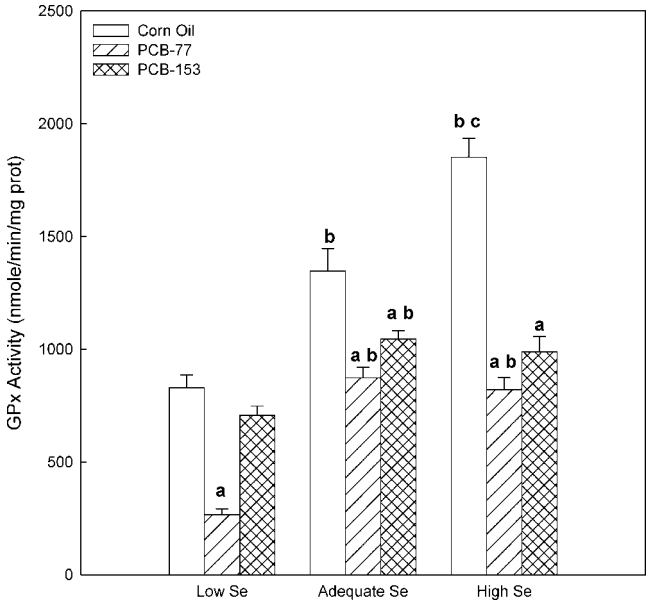

Figure 5. Glutathione peroxidase activity. Results are expressed as mean ± SEM. [a]Values are significantly different from their respective controls treated with corn oil ($P < 0.05$). [b]Values are significantly different from the low selenium diet group with similar treatment ($P < 0.05$). [c]Values are significantly different from the adequate selenium diet group with similar treatment ($P < 0.05$).

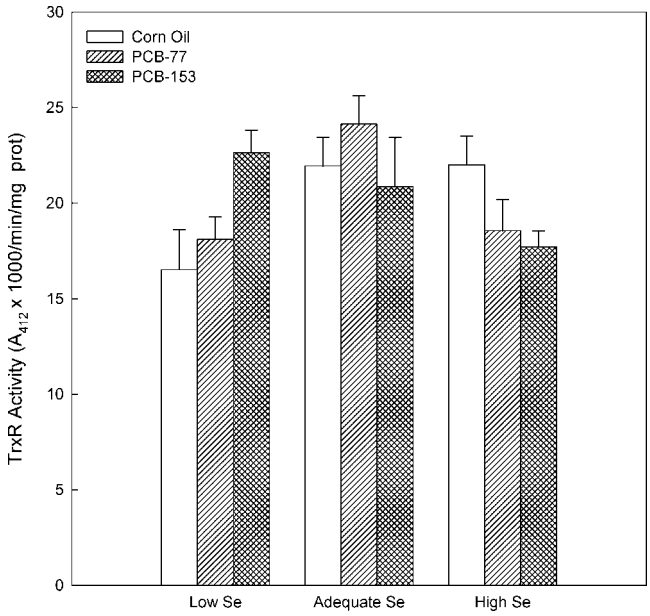

Figure 6. Thioredoxin reductase activity. Results are expressed as mean ± SEM. No significant differences were observed.

activity in PCB-77–treated rats. PCB-153 also significantly reduced the GPx activity in the adequate and high selenium diet groups. For both PCBs, the trends were similar in that adequate Se in the diet contributed to increasing the GPx activity compared with the low Se group. Further Se supplementation did not produce any further increase in activity.

Effect on TrxR Activities. The TrxR activity in the PCB-treated groups varied slightly, but there were no statistically significant effects (Fig. 6). There were no significant differences seen between the Se diet groups.

Effect on Hepatic Selenium. A significant dose-dependent increase in the hepatic Se concentrations can be seen between the low dose Se (0.02 ppm), the adequate dose (0.2 ppm) and the high dose (2 ppm) groups regardless of treatment (Fig. 7). Neither PCB significantly affected hepatic selenium levels in rats fed the 0.02 ppm Se diet, but PCB-77 decreased hepatic selenium at the 0.2 and 2.0 ppm dose levels, whereas PCB-153 only decreased hepatic selenium significantly at the 2.0 ppm dose level.

Discussion

PCBs are known hepatic tumor promoters that have been shown to decrease both Se levels and GPx activity in the liver of experimental animals (23, 36, 37). The ability of PCB-77 and PCB-153 to promote hepatocarcinogenesis in DEN-initiated animals has been demonstrated in several studies (21). The chemopreventive effects of Se against cancer have been observed in both animal and epidemiological studies (5, 38). In the present study, we found that a supranutritional Se diet did not inhibit the promotion of altered hepatic foci by PCB-77 or PCB-153. Instead, the number of PGST-positive foci induced by PCB-77 signifi-

cantly increased with increasing dietary Se, notably in the high Se diet group. Similarly, Se did not influence the number of PGST-positive foci per cm^3 and per liver in rats receiving PCB-153. In contrast, the high Se diet decreased the mean focal volume in rats exposed to either PCB as well as in the control group. Dietary Se had no effect on cell proliferation in liver of both PCBs except in the adequate Se, PCB-153–treated rats. Both GPx activities and Se levels were reduced in the liver by PCB-77 but not PCB-153; however, TrxR activity was not affected by either PCB. The differential effect on TrxR and GPx by PCBs confirmed the contrasting regulation of GPx and TrxR.

PCB-77 only influenced the number of foci induced in this study, whereas PCB-153 only influenced the mean focal volume. Other studies have reported different effects of these PCBs on the focal number and volume. For PCB-77, studies that have quantified mean focal volume as an endpoint have reported different results: Berberian et al. (39) found that PCB-77 increased both focal numbers as well as the mean focal volume; Tharappel et al. (40) found that PCB-77 increased focal number but not mean focal volume; and Kobusch et al. (41) found that PCB-77 increased the size of N-nitrosomorpholine–initiated foci but decreased the number induced. In studies that did not quantify the mean focal volume, Sargent et al. (42) found that PCB-77 did not affect the number of foci or the volume fraction, and several studies (43–45) found that PCB-77 increased the number of foci and/or the volume fraction. In other studies with the non-coplanar PCB-153, Berberian et al. (39) found that it increased the mean focal volume as well as focal number whereas Tharappel et al. (40) found that it did not affect either parameter. Several studies (45–48) found that PCB-153 increased the number of foci and/or the volume fraction but did not quantify mean focal volume as an endpoint.

In the present study, the number of foci induced by PCB-77 was increased by the high selenium diet, but the effect of PCB-153 on the focal volume was abolished in the high selenium group. Several studies have investigated the effect of Se on different phases of hepatocarcinogenesis using varying *in vivo* hepatocarcinogenesis protocols, initiating agents and tumor promoters. Two studies that used DEN as the initiator and phenobarbital (PB) as the promoting agent demonstrated that Se did not affect the induction of hyperplastic hepatic nodules or carcinomas when Se supplementation was given during either the initiation or promotion stages, or throughout the entire study (49, 50). Milks et al. (51) found that feeding higher levels of Se only during aflatoxin B_1 (AFB1) administration inhibited the subsequent development of altered hepatic foci. Conflicting results on focal development were reported by Baldwin and Parker (52): in 2-acetylaminofluorene (AAF) – treated rats supplemented with 6.0 ppm Se, the mean volume and volume fraction of gamma-glutamyl transpeptidase (GGT)-positive foci were decreased, whereas the number of foci/cm^3 of liver was not affected. Similarly, using a Solt-Farber protocol, Bjorkhem-Bergman et al. (53)

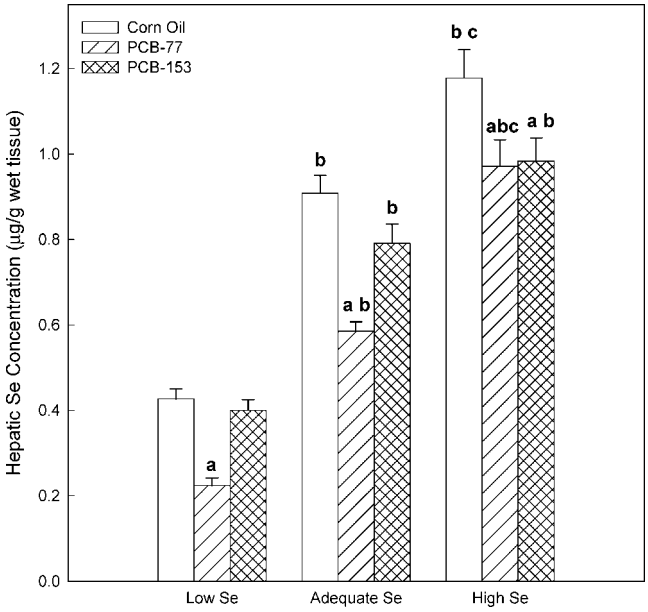

Figure 7. Hepatic selenium concentration. Results are expressed as mean ± SEM. [a]Values are significantly different from their respective controls treated with corn oil at the same dietary selenium level ($P < 0.05$). [b]Values are significantly different from the low selenium diet group with similar treatment ($P < 0.05$). [c]Values are significantly different from the adequate selenium diet group with similar treatment ($P < 0.05$). Selenium levels were measured using the NAA method.

found that 1 and 5 ppm Se administered to rats during initiation had no effect on the number and volume of hepatic nodules, but Se administered during either the selection or 6-month progression stages decreased the volume occupied by the nodules in the liver. LeBeouf et al. (54) also noted that Se (6 ppm) decreased focal growth (mean volume) with no corresponding effect on the number of GGT-positive foci in the liver when fed either after DEN or during AAF administration; when high (6 ppm) Se was fed between DEN initiation and phenobarbital promotion, however, the induction of altered hepatic foci was increased. Other hepatocarcinogenesis studies have shown that Se inhibits focal growth: Lei et al. (55) demonstrated that Se inhibited the induction of altered hepatic foci and tumors by AFB1, and Glauert et al. (56) observed that Se inhibited the incidence of tumors induced by the peroxisome proliferator ciprofibrate as well as the number of altered hepatic foci in rats. The results of the present study agree somewhat with those of Baldwin and Parker (52) and LeBeouf et al. (54) in that the volume of altered hepatic foci was reduced by selenium but not the number of foci.

The nature of the anti-carcinogenic effects of Se remains unclear. Likewise, the mechanism by which Se protects from hepatic cancer is not known. Several mechanisms have been proposed, including inhibition of cell proliferation and induction of apoptosis (57, 58), altered carcinogen metabolism, and antioxidant protection. PCB-77 had been shown to depress hepatic Se and GPx activity (37). Since Se metabolism occurs mainly in the liver, it follows

that decreased available Se will lead to decreased available Se for selenocysteine incorporation or selenoprotein synthesis (59). The synthesis of different selenoproteins follows a certain hierarchy and GPx production has been shown to be less important compared to TrxR or iodothyronine deiodinases (5, 32). The production of reactive oxygen species (ROS) by PCBs and their metabolites, which may lead to oxidative damage, is one mechanism that may contribute to tumor promotion (21). Therefore, the PCB-induced decrease in hepatic Se leading to reduced antioxidant defense may amplify the growth of new preneoplastic lesions resulting from oxidative damage. In addition, another effect of PCBs is glutathione depletion. Selenite reacts with GSH to form selenodiglutathione (GSSeSG), which could produce oxidative stress via redox cycling of the $GSSe^{-}$ anion (60–62). GSSeSG is also the precursor of the primary intermediate, hydrogen selenide, which is then methylated into methylselenol, the key Se metabolite that has been demonstrated to possess anti-cancer properties (4, 38, 60, 63). Studies have argued that selenite causes oxidation and depletion of intracellular GSH, which is more cytotoxic than pro-apoptotic (62, 64, 65). The combined effect on GSH by both PCBs and Se may have contributed to decreased methylselenol production leading to diminution of the focal size.

Cell proliferation in the liver is important in the processes of initiation, promotion, and progression (66). One proposed mechanisms for Se chemoprevention is the inhibition of cell proliferation (54, 67–69). Using the same initiation-promotion protocol as in the present study but without Se intervention, Tharappel et al. (25) observed that PCB-77 increased cell proliferation in both focal and non-focal hepatocytes in female Sprague-Dawley rats. Hence, we hypothesized that Se would decrease the ability of PCBs to increase cell proliferation. In this study, we found that DNA synthesis in the non-focal hepatocytes of PCBs-dosed rats was not significantly affected by adequate or supranutritional Se. However, we found, albeit insignificantly, that the labeling index was consistently higher in the PCB-treated groups compared with their corresponding controls, except for the PCB-153 rats fed with adequate Se. In contrast, Bjorkhem-Bergman et al. (53) found that cell proliferation in the surrounding, non-nodular tissue was significantly increased in the Se supplemented rats, although the opposite was observed in the nodules. The conflicting results support the possibility that inhibition of cell proliferation may not be a common mechanism by which Se protects against carcinogenesis.

Protective mechanisms produced by Se metabolites, namely selenodiglutathione, selenide, and methyl selenol, are proposed to be responsible in part for the anti-cancer effects of Se, but the antioxidant function of selenoproteins may also be important (70–72). Two major selenoenzymes, GPx and TrxR, are essential components of the two major redox systems in the cell, the glutathione and thioredoxin systems. It is known that cytosolic GPx1 has low affinity for Se incorporation when Se is limiting. In addition, GPx1 protein synthesis and expression are drastically decreased in Se deficiency, whereas other selenoproteins are not as affected (73). The finding that GPx1 knockout mice did not develop abnormalities when subjected to hyperoxic condition led to the belief that another mechanism compensates for the loss of GPx1 function (74). The premise that GPx prevents carcinogenesis remains an issue because GPx activity was found to be at its maximum in animals with adequate dietary Se, whereas anti-cancer effects were mostly observed at supranutritional levels (72, 75–78). Also, overexpressing glutathione peroxidase in transgenic mice does not necessarily inhibit carcinogenesis (79). We observed that GPx activity was strongly reduced by PCB-77, especially in rats fed the Se-deficient diet. Although PCB-153 did not display as strong an effect as PCB-77, it also depressed GPx activity in rats fed adequate and supplemented Se diets. Overall, Se supplementation was not able to prevent reduction of the glutathione peroxidase activity by PCBs.

Thioredoxin reductase (TrxR) catalyzes the NADPH-dependent reduction of thioredoxin (Trx). The activated Trx controls cellular redox processes such as transcription, protein-DNA interactions, embryonic development, and DNA synthesis. Some studies demonstrate the protective role of the Trx system in cancer; however, indications that this system may also have pro-tumorigenic effects has been noted (78). Trx has been shown to inhibit apoptosis, which therefore favors tumor growth (78, 80–82). It was observed that rats fed with high Se (1.0 ppm as sodium selenite) diet had a 2-fold increase in hepatic TrxR activity, although there was no accompanying increase in TrxR protein (83). However, after long-term feeding with a high Se diet, hepatic TrxR activity eventually decreased to the level of the control, which was attributed to a decrease in TrxR protein synthesis resulting from a decrease in Se incorporation. This may explain why the high Se diet in this study failed to produce a corresponding increase in the TrxR activity. Furthermore, Gallegos et al. (84) noted that selenite treatment did not affect TrxR1 mRNA stability or protein possibly because an increase in TrxR mRNA level occurred first, followed by an increase in protein levels and then increased activity. Studies have shown that GPx and TrxR are regulated differently (85, 86). Using transforming growth factor-α/*c*-myc mice, a model of accelerated hepatocarcinogenesis, it was shown that GPx expression was decreased in tumors compared with the surrounding normal tissue (85). In contrast, TrxR expression and activity were increased in tumors. The opposing regulation of TrxR and GPx was confirmed in human prostate cell lines from normal and cancer cells, where it was shown that GPx was repressed while TrxR was increased in tumor cells compared with the normal cells. In our study, we did not differentiate the enzyme activity in the foci versus the surrounding normal cells; however, the apparent repression of GPx activity by PCB-77 is strongly associated with the

increased number of foci per liver and increased focal volume ratio. In contrast, TrxR activity was not affected by the PCBs treatment. Moreover, although high dietary Se increased hepatic Se and GPx activity, TrxR activity was not affected. Therefore it does not appear that TrxR plays a role in the effects of selenium on carcinogenesis in this study.

Our findings on hepatic Se concentrations indicate that the effect of PCBs on the Se levels is associated with GPx activity. A dietary selenium-related increase in hepatic Se level was observed for both PCBs and control. Again, PCB-77 has stronger reducing effect on hepatic Se compared with PCB-153. This result confirms a previous finding that the suppression of GPx activity by PCB-77 is associated with its reducing effect on hepatic Se (37). Our group traced the distribution and excretion of Se after a single i.p. dose of PCB-77 and found that PCB-77–induced depletion of hepatic Se may be caused by enhanced Se excretion in urine (87).

In summary, our findings showed that Se supplementation increased the number of PGST-positive foci in PCB-77–treated rats. On the other hand, Se supplementation reduced the mean focal volume of the foci in untreated, PCB-77–treated, and PCB-153–treated rats. The inhibition of cell proliferation does not appear to be one of the mechanisms by which Se confers protection against PCB induced tumor promotion. Se supplementation did not prevent the PCB-77–induced decrease in hepatic Se, which was accompanied by a corresponding reduction in GPx activity. In contrast, TrxR activity was not affected by the PCBs treatment or Se supplementation.

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