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Effects of selected polychlorinated biphenyl (PCB) congeners on hepatic glutathione, glutathione-related enzymes, and selenium status: implications for oxidative stress☆

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Abstract

Polychlorinated biphenyls (PCBs) induce drug metabolism that may lead to the bioactivation of PCBs themselves or alternatively may lead to oxidative events within the cell. The goal of the present study was to determine the influence of congeneric PCBs, selected as substrates for or inducers of drug metabolism, upon hepatic glutathione, glutathione-related enzymes, and selenium status. Male and female Sprague–Dawley rats received two i.p. injections per week of PCB 3 (4-chlorobiphenyl), PCB 28 (2,4,4′-trichlorobiphenyl), PCB 38 (3,4,5-trichlorobiphenyl), PCB 77 (3,3′,4,4′-tetrachlorobiphenyl), PCB 153 (2,2′,4,4′,5,5′-hexachlorobiphenyl), or both PCBs 77 and 153 (100 μmol/kg/injection) and were killed at the end of 1, 2, or 3 weeks. Whole liver homogenates, hepatic cytosol, and microsomes were prepared. Both glutathione reductase and glutathione transferase activities were increased significantly in both male and female rats receiving PCB 77, an aryl hydrocarbon receptor agonist, as well as in those receiving both PCBs 77 and 153. No significant trend was observed in the levels of hepatic total glutathione. PCB 77 treatment decreased hepatic selenium-dependent glutathione peroxidase (SeGPX) activity in both male and female rats significantly. This decrease in activity following PCB 77 treatment was accompanied by a decrease in the cytosolic selenium-dependent glutathione peroxidase gene (GSPx1) transcript, as well as a decrease in hepatic total selenium levels. These data support the concept that exposure to the coplanar PCB 77 suppresses, via gene regulatory mechanisms, the cellular antioxidant enzyme SeGPX and that this decrease involves selenium. Lower halogenated PCBs that may be bioactivated to reactive oxygen species (ROS)-producing metabolites, and higher halogenated PCBs that are not Ah receptor agonists, were inactive.

Keywords: Polychlorinated biphenyls (PCBs); Glutathione; Glutathione peroxidase; Glutathione reductase; Glutathione transferase; Selenium

1. Introduction

PCBs are industrial chemicals, manufactured as mixtures that have been commercially available since 1929. As a group, PCBs are relatively stable compounds under a broad range of chemical, thermal, and electrical conditions (properties that made them useful in many industrial settings). Uses included: in transformers and capacitors as dielectrics, in hydraulic systems as cooling fluids, in the formulation of lubricating and cutting oils, in pesticides and flame retardants, and as plasticizers in paints, copying paper, adhesives, sealants, and plastics. Although the production of PCBs has been banned or limited in many countries since the 1970s, they still persist in our environment. Due to their lipophilic nature and their stability, PCBs bioconcentrate and bioaccumulate and are therefore routinely detected in fatty tissues [1,2].

PCBs, especially higher chlorinated PCBs, may selectively induce cytochrome P450s [3]. Induced cytochrome P450 isozymes may be the source of ROS [4,5] or, alternatively, may catalyze the oxidation of a broad range of

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Abbreviations: PCBs, polychlorinated biphenyls; GST, glutathione transferase; GPX, glutathione peroxidase; SeGPX, selenium-dependent glutathione peroxidase; GR, glutathione reductase; MOPS, 3-{*N*-morpholino}propane sulfonic acid buffer; CDNB, 1-chloro-2,4-dinitrobenzene; PB, phenobarbital; 3-MC, 3-methylcholanthrene; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; AhR, aryl hydrocarbon receptor; XRE, xenobiotic response element; ROS, reactive oxygen species; and GSPx1, cytosolic selenium-dependent glutathione peroxidase gene.

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endogenous and exogenous substances, including PCBs themselves. The cytochrome P450-catalyzed oxidation of lower chlorinated biphenyls gives rise to mono- and dihydroxy metabolites. The latter can autoxidize or can be enzymatically oxidized to semiquinones and/or quinones [6]. Some PCB-quinones can undergo redox cycling, with the formation of ROS, thus becoming another source of oxidative stress [7]. PCB metabolites may react with *N*- and *S*-nucleophiles, including amino acids, proteins, and peptides, like GSH [8].

GSH (gamma-l-glutamyl-l-cysteinylglycine), present in the liver in millimolar concentrations, functions as an electrophile, radical scavenger, and a redox partner [9]. GSH may also serve as a cofactor for (a) several drug-metabolizing enzymes (i.e. GSTs) where it is consumed, or (b) for antioxidant enzymes (i.e. GPX) where it functions as a redox partner [10]. During oxidation, GSH forms a dimer, glutathione disulfide (GSSG), which, in turn, can be reduced by the enzyme GR at the expense of NADPH [11].

Previously, we observed that the PCB mixture, Aroclor 1254, and the polybrominated biphenyl mixture, fireMaster BP-6, cause a reduction in the activity of SeGPX in the rat liver at time points greater than 1 week [12]. The specific aims of the present study were to examine the effects of congeneric PCBs on glutathione and glutathione-related enzymes and to investigate the PCB-mediated decreases in SeGPX activity. Furthermore, this study was designed to elucidate which PCBs present in these mixtures are responsible for the observed decreases in SeGPx activity.

2. Materials and methods

2.1. Chemicals

All chemicals were obtained from the Sigma-Aldrich Chemical Co. unless stated otherwise.

2.2. Syntheses of PCBs

PCB 3 (4-chlorobiphenyl) [13], PCB 28 (2,4,4′-trichlorobiphenyl) [14], PCB 38 (3,4,5-trichlorobiphenyl) [15], PCB 77 (3,3′,4,4′-tetrachlorobiphenyl), and PCB 153 (2,2′,4,4′,5,5′-hexachlorobiphenyl) [12] were synthesized and characterized as described. **Caution: PCBs and their metabolites should be handled as hazardous compounds in accordance with NIH guidelines (1981).**

2.3. Animals

All animal experiments were conducted with approval from the University of Kentucky Institutional Animal Care and Use Committee. Male and female Sprague–Dawley rats (150–174 g) (Harlan Sprague–Dawley) were housed in cages in a controlled environment maintained at 22° with a 12-hr light–dark cycle. Rats received an unrefined rat chow (Purina rodent laboratory chow, Purina Mills) and water *ad lib.* Rats received one i.p. injection of vehicle (stripped corn oil; Acros Chemical Co.) or PCBs 3, 28, 38, 77, 153, or both 77 and 153 (100 μmol/kg/injection) on Monday and one on Thursday of each week and were killed at the end of 1, 2, or 3 weeks (on Mondays). Rats were always injected in the order listed and were euthanized also in the same order to avoid diurnal fluctuations. Livers were removed surgically, snap-frozen in liquid nitrogen, and stored at −80°. Whole liver homogenates, cytosol, and microsomes were prepared as previously described [16]. Protein concentrations were determined by the method of Lowry *et al.* [17].

2.4. Experimental procedures

Total glutathione was determined using the 96-well plate method of Baker *et al.* [18], while GPX activity was determined using methods described by Paglia and Valentine [19], as modified by St. Clair and Chow [20]. Accordingly, SeGPX activity was determined using H_2O_2 as substrate, and total GPX activity was determined using cumene hydroperoxide. GR was determined using methods described by Paglia and Valentine [19]. GST was determined by the procedure outlined by Habig and Jakoby [21] using CDNB as substrate. These enzyme assays were performed on a Shimadzu MPS-2000 spectrophotometer.

For northern analysis [22], total RNA was isolated from frozen liver using TRI REAGENT and BCP (Molecular Research Center, Inc.) essentially as described in the manufacturer's protocol. Following isolation and quantitation using OD_{260}, an aliquot of 20 μg RNA was denatured and subjected to electrophoresis through a 1% agarose, 1X MOPS, 2.2 M formaldehyde gel using 1X MOPS as the running buffer [23]. Subsequent to electrophoresis, RNA was transferred using 3 M NaCl, 0.3 M sodium citrate, pH 7.2 (20X SSC, Gibco BRL), followed by overnight hybridization to 10^6 cpm/mL of a ^{32}P-labeled GSPxl probe using 50% formamide, 6X SSC, 5X Denhardt's solution (USB), 0.5% SDS, and 0.1 mg/mL of denatured salmon sperm DNA (ssDNA, Gibco BRL) at 42° overnight. This GSPx1 probe was generated from random priming a 717-bp template consisting of two of the three exons of the mouse GSPx1 gene [24]. Following hybridization, blots were washed to a stringency of 0.1% SDS, 1X SSC for 15 min at 42°, exposed to a phosphoimaging screen, and analyzed on a STORM PhosphoImager using ImageQuant software (Molecular Dynamics). Then the membranes were stripped and rehybridized to a ^{32}P-end-labeled 28S rRNA oligo using 6X SSC, 5X Denhardt's solution, and 0.1 mg/mL of ssDNA at 42° overnight. After hybridization, membranes were washed using 6X SSC and 0.1% SDS for 15 min at 42° followed by 0.1X SSC and 0.1% SDS for 15 min at room temperature, exposed to a phosphoimaging screen, and analyzed as described for GSPx1.

2.5. Selenium determination

The determination of selenium concentrations in liver tissue was carried out at the Research Reactor Center at the University of Missouri-Columbia.

2.6. Preparation of rat liver samples for analysis

Each sample was divided into equal parts to prepare a duplicate sample. Three ashless filter paper disks were placed at the bottom of a pre-cleaned 0.2 mL high-density polyethylene (HDPE) vial. Replicate samples were placed into the numbered HDPE vial, ranging in mass from approximately 28–33 mg, and weighed (wet weight) using a Mettler AT261 balance. The samples were thoroughly frozen (overnight), then freeze-dried, and the dry weights determined. The vials were closed with a friction-fit polyethylene cap.

2.7. Neutron activation analysis (NAA)

The content of selenium in the liver samples was determined via NAA. For the analysis of selenium, the neutron-capture reaction is: $_{34}Se^{76} + {}_{o}n^{1} \Rightarrow {}_{34}Se^{77m}$. This is the activation step. Selenium-76 is one of the stable isotopes of selenium (abundance = 9.0%); it captures a neutron to produce selenium-77m, which is radioactive. Selenium-77m decays by isomeric transition emitting a gamma-ray: $_{34}Se^{77m} \Rightarrow {}_{34}Se^{77} + \gamma$-ray.

The samples were loaded into an HDPE shuttle rabbit and closed with a screw cap. Each loaded rabbit was irradiated for 7 sec via the MURR pneumatic tube system. The thermal neutron flux in this irradiation position is approximately 8×10^{13} n · cm^{-2} · sec^{-1}. After irradiation, the samples decayed for 15 sec, and then were real-time counted for 25 sec using a 30% high-purity germanium (HPGe) detector. The gamma-ray ($E\chi$ = 161.9 KeV) from the decay of Se-77m ($T^{1}/_{2}$ = 17.45 sec) was measured quantitatively, and concentrations were determined by standard comparison.

The induced activity was measured using the MURR gamma-ray spectroscopy network, which is based on the Canberra Nuclear VMS system. This system consists of an ORTEC 30% HPGe detector and high voltage power supply, Canberra digital signal processing and loss-free counting modules connected to a Compaq Alpha computer network through a Canberra acquisition interface module.

2.8. Standard reference materials

NIST SRM-1577 bovine liver was used as a quality control. Selenium was quantified in the same way as described for the samples. The mean ± SD measured in six samples was 1.103 ± 0.028. The certified value is 1.1 ± 0.1 ppm.

2.9. Statistics

Statistical analysis was performed using ANOVA followed by Bonferroni post hoc to test for differences in treatment means. Treatment groups were considered statistically different at $P < 0.05$. Statistics were performed using Toxstat, V. 3.2, developed by David D. Gulley at the University of Wyoming.

3. Results

We have demonstrated previously that a PCB mixture can diminish liver SeGPX activity in the rat [12]. To elucidate which PCB congeners comprising this mixture may be mediating this effect on SeGPX, as well as to determine if GSH and other GSH-related enzymes are affected by these PCBs, we analyzed rats treated with individual PCB congeners for 1, 2, or 3 weeks for GSH and GSH-dependent enzyme activity. Male and female Sprague–Dawley rats treated with several structurally distinct PCB congeners showed only sporadic significant changes in total hepatic glutathione levels. There was also no obvious trend that was either gender- or time-dependent (Table 1).

In contrast to the results seen with GSH, the activities of GST in rats treated with PCB 77 or PCB 153 were increased significantly when compared with controls, as analyzed using CDNB as the substrate (Table 2). In addition, the increase in GST activity with PCB 77 occurred in both male and female rats and at all time points examined. PCB 153 increased GST activity in males at weeks 2 and 3, but only at week 3 in females. Interestingly, PCB 153 was less efficacious at increasing GST activity in female rats than in male rats. In addition, male and female rats treated with the combination of PCBs 77 and 153 showed significant increases in GST activity at all time points examined, but these increases suggest little if any additivity as compared with individual treatments of PCB 77 and PCB 153.

The effects of PCBs on the activity levels of GR were similar to the results seen with GST activity; GR activity levels were increased significantly in both male and female rats receiving PCB 77, but the activity in the males reached a plateau by week 2 and was in decline by week 3, whereas the activity in the females was still increasing at the week-3 time point (Table 2). Interestingly, there was no effect seen in the female rats receiving the PCB 153 treatment. Male rats receiving both PCBs 77 and 153 had their largest activity increases by week 2 and then activity declined in the week-3 samples. In female rats receiving both PCBs, activity continued to increase through week 3.

As shown in Table 3, activity levels of both total GPX and SeGPX were decreased significantly as compared with controls in both male and female rats treated with PCB 77 at all time points examined. The reduction in activity levels was more dramatic in female rats, where activity decreased to approximately 50% of control levels. Conversely, there

Table 1
Levels of total hepatic glutathione

		Total glutathione (mM/g protein)	
		Male	Female
Vehicle	1 week	29.1 ± 6.5	23.4 ± 2.3
	2 weeks	27.2 ± 4.2	23.8 ± 2.7
	3 weeks	25.1 ± 2.1	16.0 ± 4.0
PCB 3	1 week	21.2 ± 1.0	25.2 ± 3.3
	2 weeks	24.6 ± 1.2	17.5 ± 0.7
	3 weeks	25.5 ± 1.3	19.1 ± 1.1
PCB 28	1 week	22.3 ± 1.9	26.9 ± 3.4
	2 weeks	19.4 ± 1.5	23.3 ± 1.6
	3 weeks	22.4 ± 3.2	18.5 ± 1.1
PCB 38	1 week	21.0 ± 1.4	19.4 ± 2.8
	2 weeks	22.2 ± 2.2	17.9 ± 1.8
	3 weeks	26.3 ± 2.7	18.6 ± 0.9
PCB 77	1 week	25.4 ± 4.6	22.9 ± 1.7
	2 weeks	25.9 ± 4.5	27.0 ± 2.6
	3 weeks	27.1 ± 5.9	27.2 ± 2.4*
PCB 153	1 week	18.6 ± 1.6	20.1 ± 1.7
	2 weeks	20.5 ± 1.5	16.9 ± 1.5**
	3 weeks	23.5 ± 1.3	19.4 ± 0.7
PCBs 77 and 153	1 week	12.2 ± 1.0**	25.8 ± 2.9
	2 weeks	21.7 ± 2.2	17.5 ± 1.0
	3 weeks	20.0 ± 1.3	23.6 ± 1.0*

Results are expressed as means ± SEM with N = 4–6 rats receiving 100 μmol/kg/injection.

* Signifies a statistically significant ($P < 0.05$) increase as compared with equivalent gender and time-point vehicle control.

** Signifies a statistically significant ($P < 0.05$) decrease as compared with equivalent gender and time-point vehicle control.

was no observed effect on GPX activity in the presence of PCB 153 in either sex. As with GR and GST, the same trends as those seen with PCB 77 alone were seen in male and female rats receiving both PCBs 77 and 153.

To elucidate possible mechanisms for the decreases observed in SeGPX activity in both female and male rats receiving PCB 77, northern analysis was conducted. Using a mouse GSPx1 probe, a single, strongly hybridizing band was observed in both male and female rats. This band was decreased in intensity in both male and female rats treated with PCB 77 as compared with the controls at all time points (Fig. 1, top). Equal loading and RNA integrity was confirmed as determined by the equal signal intensity observed following hybridization to a ^{32}P-labeled 28S rRNA probe. As shown in the bottom panel of Fig. 1, after normalizing the abundance of GSPx1 mRNA to 28S rRNA using densitometry, significant decreases in mRNA were observed in females treated with PCB 77 at 1 and 3 weeks, although non-significant decreases were also observed at 2 weeks. In addition, male rats treated with PCB 77 also demonstrated decreased GSPx1, although this decrease was only significant following 3 weeks of treatment.

We were quite interested in determining if the observed decreases in SeGPX activity and GSPx1 transcript levels following PCB 77 treatment were mirrored by total hepatic selenium levels. Liver samples from rats treated with PCB 77 or vehicle alone were analyzed by neutron activation methods. Selenium levels in PCB 77-treated female rats were decreased to almost half of those of control rats by week 3. Male rats treated with PCB 77 showed a significant decrease, but decreases were not as dramatic as in the female rats (Fig. 2).

4. Discussion

PCB congeners were chosen for this study according to the following criteria: PCB 3 is a prototype of lower halogenated biphenyls and is rapidly metabolized [6]. PCB 38 was included because its metabolite is a potent inducer of oxidative DNA damage (as measured by 8-oxo-deoxyguanosine levels) [25]. PCB 28 was included because it is a minor component of Aroclor 1260 (0.03%) but is present in human breast milk at a much higher concentration (3.6% of total PCBs) [26]. PCB 77 is an AhR agonist (a so-called co-planar PCB) [3], and PCB 153 is a higher chlorinated biphenyl that induces cytochrome P450 like PB, resists metabolism, and is a major component of environmental PCBs [26,27].

These PCB congeners demonstrated no significant effects on hepatic total glutathione concentrations. Our results extend those of previous studies in which changes in hepatic GSH levels following Aroclor 1254 were not observed [28]. Although the total concentration of GSH appears not to be affected by PCBs, Dogra *et al.* [29], as well as our own preliminary data (data not shown), suggest that the oxidative stress effects may be more readily seen in the ratio of GSH to GSSG than in total glutathione values. These effects are currently under investigation in our laboratory.

Numerous xenobiotics increase the activity of GSTs, increases which may be detected by several substrates that are somewhat selective for individual GST isozymes. Inducers of GST include classical inducers of cytochrome P450, like PB and 3-MC, as well as congeneric PCBs [12]. PCB induction of GSTs has been investigated within the individual cell types of the liver and found to be present in both parenchymal and non-parenchymal cells [30,31]. In the present study, treatment with PCB 77, a dioxin-like compound that is a constituent of Aroclor 1254 [2], increased GST activity in both male and female rats beginning at 1 week, and continued to increase it throughout the duration of this study. The observed increases in GST activity with PCB 77 agree with the effects on GST activity observed with the AhR agonist, TCDD, which also increases GST activity [32]. Treatment with PCB 153, a PB-like inducer of cytochrome P450, also causes a significant increase in GST activity levels in both male and female rats [33]. Interestingly, activity levels in male rats were increased to a much

Table 2
Activities of glutathione *S*-transferase and glutathione reductase

		Glutathione *S*-transferase (nmol/μg protein/min)		Glutathione reductase (nmol/mg protein/min)	
		Male	Female	Male	Female
Vehicle	1 week	62.3 ± 4.2	45.2 ± 2.2	5.40 ± 0.3	3.59 ± 0.2
	2 weeks	49.4 ± 4.0	48.0 ± 2.2	4.43 ± 0.6	3.23 ± 0.2
	3 weeks	64.4 ± 1.5	43.9 ± 2.8	7.39 ± 0.4	3.38 ± 0.1
PCB 3	1 week	66.7 ± 4.8	49.1 ± 2.9	6.45 ± 0.6	3.89 ± 0.2
	2 weeks	58.3 ± 1.7	41.8 ± 3.6	5.92 ± 0.3	2.85 ± 0.1
	3 weeks	66.0 ± 3.8	43.7 ± 1.4	6.84 ± 0.4	2.77 ± 0.2
PCB 28	1 week	55.1 ± 1.7	47.5 ± 3.5	5.49 ± 0.2	3.66 ± 0.3
	2 weeks	57.5 ± 2.9	43.9 ± 1.3	5.86 ± 0.3	3.26 ± 0.1
	3 weeks	67.6 ± 3.1	45.1 ± 1.4	7.56 ± 0.7	3.34 ± 0.3
PCB 38	1 week	47.9 ± 4.2*	43.8 ± 2.4	4.26 ± 0.2	2.73 ± 0.1
	2 weeks	55.5 ± 2.4	48.0 ± 1.7	5.59 ± 0.3	3.18 ± 0.1
	3 weeks	66.5 ± 1.9	45.7 ± 2.3	5.79 ± 0.2	3.15 ± 0.1
PCB 77	1 week	86.4 ± 4.8**	80.6 ± 1.1**	7.59 ± 0.3**	3.59 ± 0.3
	2 weeks	95.8 ± 5.9**	91.7 ± 4.9**	8.24 ± 1.0**	4.23 ± 0.1**
	3 weeks	98.0 ± 5.2**	95.0 ± 2.6**	7.10 ± 0.8	5.04 ± 0.2**
PCB 153	1 week	70.0 ± 0.8	52.1 ± 1.7	7.70 ± 0.1**	3.85 ± 0.2
	2 weeks	101 ± 4.9**	53.5 ± 5.4	11.1 ± 0.9**	3.29 ± 0.2
	3 weeks	91.4 ± 8.7**	54.4 ± 2.8**	8.97 ± 1.2	3.64 ± 0.1
PCBs 77 and 153	1 week	89.2 ± 3.4**	88.6 ± 2.6**	8.04 ± 0.6**	4.61 ± 0.3**
	2 weeks	109 ± 7.6**	96.2 ± 2.4**	11.0 ± 1.4**	4.87 ± 0.1**
	3 weeks	115 ± 6.0**	115 ± 6.0**	9.35 ± 0.8	5.59 ± 0.3**

Results are expressed as means ± SEM with N = 4–6 rats receiving 100 μmol/kg/injection.
* Signifies a statistically significant ($P < 0.05$) decrease as compared with equivalent gender and time-point vehicle control.
** Signifies a statistically significant ($P < 0.05$) increase as compared with equivalent gender and time-point vehicle control.

more substantial level than in female rats treated with PCB 153. These results are in agreement with those of Lamartiniere *et al.* [33], who observed increased GST activity in male and female Sprague–Dawley rats exposed to PCB 153 in utero.

GR activity levels were increased significantly in both male and female rats receiving PCB 77, but the activity in the males peaked by the 2-week time point and was in decline by week 3, whereas the activity in the females was still increasing at the 3-week time point. Interestingly, GR activity was not affected by PCB 153 treatment in female rats. Increased GR activity following treatment with PCB mixtures has been reported for male Sprague–Dawley rats receiving Phenoclor DP6 [34], and for male Wistar rats receiving Kanechlor 500 [35]. Increased GR activity has also been observed in female CD-1 mice treated with Aroclor 1254 [36] and multiple strains of male mice also exposed to Aroclor 1254 [28].

Although an increase in both GST and GR was observed following PCB 77 treatment, an opposing effect was observed in GPX activity. GPX activity exhibited a decrease that was sustained with increasing reduction of activity over time in rats treated with PCB 77. Furthermore, the observed decrease in total GPX activity was attributable, in part, to decreases in SeGPX activity. In addition, our results from the northern blots confirmed that the decreased SeGPX activity correlated with decreased GSPx1 mRNA in rats treated with PCB 77. The observed decreases in SeGPX activity and mRNA levels extend previous rodent studies of GPX activity. Accordingly, SeGPX activity decreased with time following Aroclor 1254 treatment in male Sprague–Dawley rats [12]. A decrease in SeGPX activity was also seen in male Sprague–Dawley rats treated with a mixture of tetrachlorobiphenyls [37]. Finally, this decrease in SeGPX was seen in male mice treated with two co-planar PCBs, PCBs 77 and 126 [38].

The AhR is activated in the presence of agonists, TCDD or PCB 77, and binds to XRE in target genes, therefore modulating their transcription [39]. Although to date no functional XREs have been identified in the 5′-upstream region of the rat GSPx1 gene [40], Hori *et al.* [38] treated AhR active (C57BL) and AhR-less active (DBA) mice with two dioxin-like PCBs, PCBs 77 and 126, and found that the observed decrease in SeGPX activity was only apparent in the C57BL mice. These results indicate that the AhR may mediate these PCB effects on GPX. The observed effects on GSPx1 transcript levels may not be due to changes in the transcription rate of GSPx1 and may not directly involve

Table 3
Selenium-dependent and total glutathione peroxidase activities

		Selenium-dependent Glutathione peroxidase (nmol/mg protein/min)		Total Glutathione peroxidase (nmol/mg protein/min)	
		Male	Female	Male	Female
Vehicle	1 week	587 ± 21.3	1230 ± 20.0	725 ± 31.6	1360 ± 19.9
	2 weeks	518 ± 41.8	1120 ± 75.2	627 ± 55.5	1260 ± 68.2
	3 weeks	666 ± 20.5	1080 ± 72.1	787 ± 5.72	1230 ± 60.5
PCB 3	1 week	665 ± 25.5	1500 ± 56.1*	829 ± 40.7	1630 ± 47.1*
	2 weeks	641 ± 17.5*	1200 ± 37.3	734 ± 26.6	1350 ± 46.1
	3 weeks	723 ± 52.5	1140 ± 43.7	841 ± 63.2	1260 ± 47.3
PCB 28	1 week	564 ± 12.8	1060 ± 58.5	706 ± 23.7	1210 ± 65.6
	2 weeks	560 ± 31.6	927 ± 31.5**	677 ± 23.9	1080 ± 39.7**
	3 weeks	518 ± 20.9**	1120 ± 63.4	645 ± 28.6	1260 ± 61.6
PCB 38	1 week	577 ± 51.0	1130 ± 26.5	686 ± 49.9	1300 ± 33.9
	2 weeks	533 ± 27.0	1040 ± 30.0	606 ± 33.4	1210 ± 38.8
	3 weeks	631 ± 46.7	1230 ± 65.9	767 ± 47.5	1340 ± 50.7
PCB 77	1 week	458 ± 24.1**	621 ± 23.9**	555 ± 23.8**	779 ± 19.1**
	2 weeks	410 ± 23.1**	528 ± 18.7**	556 ± 35.0**	708 ± 35.6**
	3 weeks	430 ± 23.8**	551 ± 20.5**	545 ± 32.4**	727 ± 23.0**
PCB 153	1 week	579 ± 23.4	1180 ± 37.3	681 ± 26.4	1310 ± 44.0
	2 weeks	628 ± 21.4*	1090 ± 35.4	766 ± 20.3**	1200 ± 42.3
	3 weeks	503 ± 31.3**	1160 ± 20.5	635 ± 41.5	1270 ± 26.5
PCBs 77 and 153	1 week	430 ± 13.3**	649 ± 20.4**	537 ± 16.9**	816 ± 18.0**
	2 weeks	368 ± 20.2**	570 ± 10.8**	537 ± 24.1	706 ± 20.0**
	3 weeks	339 ± 27.3**	536 ± 22.0**	483 ± 24.7**	737 ± 29.0**

Results are expressed as means ± SEM with N = 4–6 rats receiving 100 μmol/kg/injection.
* Signifies a statistically significant ($P < 0.05$) increase as compared with equivalent gender and time-point vehicle control.
** Signifies a statistically significant ($P < 0.05$) decrease as compared with equivalent gender and time-point vehicle control.

AhR interaction with the GSPx1 gene but alterations in mRNA stability and translation.

Accordingly, decreases in hepatic SeGPX activity in rats following TCDD treatment have been observed, and these effects on SeGPX appear to diminish upon increased dietary selenium; thus, the authors hypothesize that the effects may be mediated through altered selenium metabolism [41–43]. Other authors have found that Aroclor 1254 treatment of chicks increased the amount of selenium required to protect microsomal fractions from lipid peroxidation. These authors suggest that PCBs potentiate selenium deficiency by interference with the biological utilization of dietary selenium, but also say that there is no biochemical basis for this effect [44]. We observed that there was a significant decrease in the levels of total hepatic selenium following PCB 77 treatment, and hypothesize that it may be part of a redistribution of selenium through selenoprotein utilization. To date, eleven selenoproteins including SeGPX have been identified as proteins that require selenium in their active site for proper function [45]. Interestingly, a 54-kDa selenium binding protein has been identified recently, which is highly inducible by both 3-MC and PCB 126, the most potent PCB AhR agonist, suggesting that this increase may be AhR mediated [46]. This highly inducible selenoprotein may function in a transport role similar to that of another selenoprotein, selenoprotein P [47,48], or may be transported to another organ for utilization itself, thus decreasing the total hepatic selenium pool.

Additional theories regarding selenium and the role it plays in the regulation of SeGPX and other selenoproteins include: first, the fact that selenium helps regulate the translation of new selenoproteins, and second that selenium deficiency decreases stability of selenoprotein transcripts [47]. This may be applicable to our findings; decreased selenium levels may, in fact, be down-regulating the synthesis of SeGPX, and thus subjecting the liver to increased oxidative stress. Another possibility is that SeGPX has a dual function: (a) it is a crucial component of the antioxidant defense system, and (b) it acts as a storage depot for selenium. It could be that during selenium deficiency its role as an antioxidant is superceded by its role as a ready source of selenium for the production of more crucial selenoproteins [45,49]. If this is the case, then maybe SeGPX is being sacrificed for additional production of the uncharacterized selenium-binding protein that is induced by dioxin-like compounds.

Information concerning oxidative stress and PCBs can be categorized as that arising from the metabolism of lower

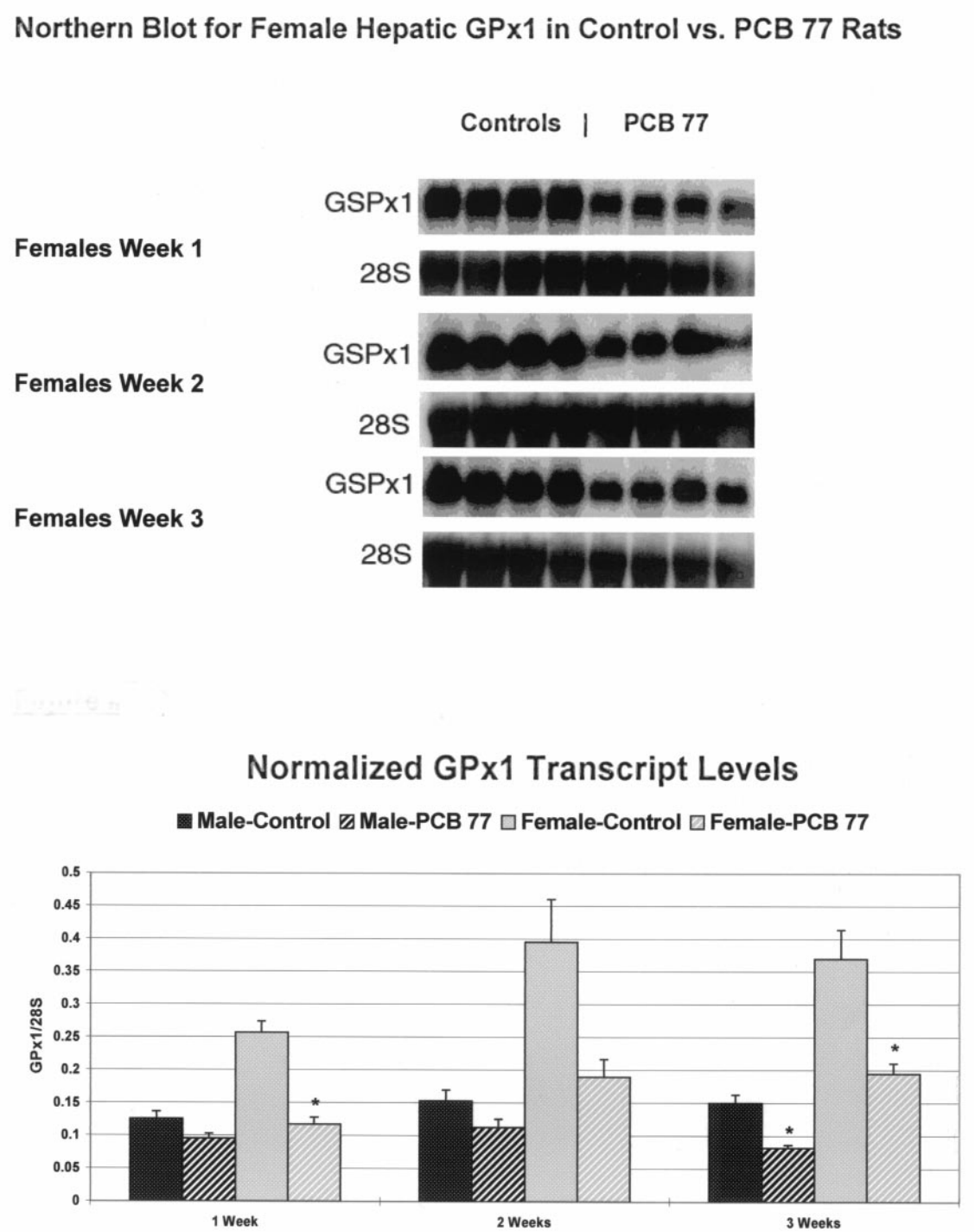

Fig. 1. Analysis of the hepatic mRNA levels for SeGPX. (Top) Northern analysis of the female hepatic GSPx1 signal as well as the 28S signal which was used as a loading indicator. (Bottom) GSPx1 transcript levels normalized for loading inequalities to 28S rRNA. Values are means ± SEM, N = 4. Key: (*) signifies a statistically significant ($P < 0.05$) decrease in GSPx1 transcript levels in PCB 77-treated rats as compared with vehicle controls.

chlorinated biphenyls themselves: redox cycling [7] and increased superoxide production [50], increased 8-oxo-deoxyguanosine content [25], and DNA strand breaks [50]. On the other hand, higher chlorinated biphenyls appear to drive oxidative stress-sensitive alterations in gene regulation and activation. These include increased DNA binding of transcription factors, such as NF-κB [51] and AP-1 [14], induction of oxidative capacity, e.g. cytochrome P450 [4,5,52], and increased lipid peroxidation [34,35,37]. Against this background of PCB-induced oxidative stress, the present study shows that the activity of a critical antioxidant enzyme, SeGPX, is diminished and that the lower activity is associated with decreased mRNA content and decreased hepatic selenium. Future studies should focus on the role of

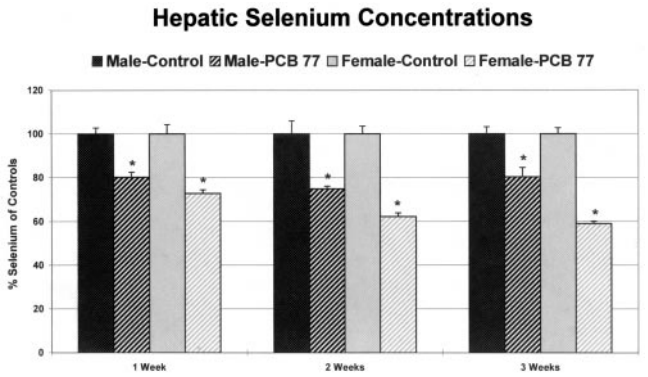

Fig. 2. Hepatic selenium concentrations as determined by neutron activation analysis. Results (means ± SEM) are expressed as a percentage of the respective gender and time point control with N = 4 rats receiving 100 μmol/kg/injection. Actual values for the controls: males 1 week, 0.59 ppm; males 2 weeks, 0.63 ppm; males 3 weeks, 0.59 ppm; females 1 week, 0.78 ppm; females 2 weeks, 0.90 ppm; and females 3 weeks, 1.0 ppm. Key: (*) signifies a statistically significant ($P < 0.05$) decrease as compared with equivalent gender and time-point vehicle controls.

selenium as a possible mediator of one category of PCB-induced oxidative events.

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