**BLARINA BREVICAUDA AS A BIOLOGICAL MONITOR OF POLYCHLORINATED BIPHENYLS: EVALUATION OF HEPATIC CYTOCHROME P450 INDUCTION**

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(Received 30 June 2003; Accepted 19 January 2004)

**Abstract**—We assessed the value of short-tailed shrews (*Blarina brevicauda*) as a possible biomonitor for polychlorinated biphenyl pollution through measurement of the induction of hepatic cytochrome P450 and associated enzyme activities. First, we checked the inducibility of four monooxygenases (benzoxysterorufin-O-dealkylase [BROD], ethoxyysterorufin-O-dealkylase [EROD], methoxyysterorufin-O-dealkylase [MROD], and pentoxyesterorufin-O-dealkylase [PROD]) by measuring the activity of these enzymes in hepatic microsomes prepared from shrews injected with β-naphthoflavone (βNF) or phenobarbital (PB), typical inducers of cytochrome P4501A (CYP1A) and CYP2B enzyme families, respectively. Enzyme activity was induced in shrews that received βNF but not in shrews that received PB; PROD was not induced by either exposure. Later, shrews were exposed to a mixture of polychlorinated biphenyls (PCBs) (Aroclor 1242:1254, in 1:2 ratio) at 0.6, 9.6, and 150 ppm in food, for 31 d. Induction in these shrews was measured by specific enzyme activity (BROD, EROD, and MROD) in hepatic microsomes, by western blotting of solubilized microsomes against antibodies to CYP1A or CYP2B, and by duration of sodium pentobarbital-induced sleep. Three CYP enzymes were induced in shrews by PCBs at similar levels of exposure as in cotton rat (*Sigmodon hispidus*). Neither sleep time nor the amount of CYP2B family protein were affected by PCB exposure. *Blarina brevicauda* can be a useful biomonitor of PCBs that induce CYP1A, especially in habitats where they are the abundant small mammal.

**Keywords**—Short-tailed shrew  Cytochrome P450 induction  Polychlorinated biphenyls

**INTRODUCTION**

Polychlorinated biphenyls (PCBs) are persistent, global contaminants of great ecological concern [1], as they are lipophilic and bioaccumulate. Characteristics of the short-tailed shrew (*Blarina brevicauda*) make this small mammal a good candidate for monitoring terrestrial PCB contamination [2]. *Blarina brevicauda* is abundant and widespread in a variety of habitats in northeastern United States and southern Canada [3]; it is exposed to contaminants primarily through food; and because it is a secondary consumer, *B. brevicauda* (order Insectivora) is likely to be the recipient of biomagnified doses of lipophilic contaminants. Shrews are fossorial, and hence additionally may be exposed to PCBs by ingestion or inhalation of contaminated soil [4,5], although likely at a lesser rate than through food. Shrews have rather small home ranges (<1 ha; [6]), allowing any observed toxicological responses to be related to local exposure.

However, few field studies have measured PCB concentrations in short-tailed shrews. Other lipophilic pollutants such as mirex and DDT, and also heavy metals, are found in greater concentrations in shrews than in other species of small mammals found in the same habitat that feed at lower trophic levels than *B. brevicauda* [7–9]. Indeed, bioaccumulation of PCBs does occur in other shrew species, as high as 1 mg/kg in *Sorex araneus* [10], but no data exist for *B. brevicauda*.

The goal of this study is to evaluate the induction of hepatic microsomal CYPs and associated monooxygenases in *B. brevicauda* as a biomarker of PCB exposure. Induction of hepatic CYPs is an established biochemical marker of PCB exposure in mammals and is a useful tool for monitoring exposure of PCBs in rodents [8,11]. Induction of these enzymes has been measured in white-toothed shrews (*Crocidura russula*) treated with benzo-α-pyrene [12], but no information is available on induction of hepatic monooxygenases in short-tailed shrews. In this study, we evaluated the inducibility of hepatic monooxygenases in short-tailed shrews exposed to prototype inducers of cytochrome P4501A (CYP1A) or CYP2B enzyme families, and the induction of monooxygenases in shrews fed PCBs.

**MATERIALS AND METHODS**

Test animals and experimental design

Short-tailed shrews were trapped at the U.S. Fish and Wildlife Services (U.S. FWS) Patuxent Research Refuge (Laurel, MD, USA) using Sherman traps (H.B. Sherman Traps, Tallahassee, FL, USA) baited with rolled oats and peanut butter, wet cat food, or crimped oats. Traps were checked 0.5 h prior to sundown, at noon, and 0.5 h after sunrise. Captured shrews were housed in aluminum cages (91 × 46 × 46 cm3) filled with 15 cm of dampened peat moss and covered with hardware cloth or a perforated stainless steel lid. Water was provided in bottles with sipper tubes and in small, 4-cm-deep dishes. A short polyvinyl chloride tube (2.5 cm diameter) provided cover, and a small piece of hardware cloth (10 cm2) enabled tunneling in the peat moss. Shrews were fed one frozen sucking white-footed mouse (*Peromyscus leucopus*) and one 3- to 5-g ration of horsemeat (Nebraska Bird of Prey Diet, Animal Spectrum, North Platte, NE, USA) each day. Mice are a preferred food item and were fed to *B. brevicauda* when first brought into captivity, but discontinued before dosing began (PCBs mixed into horsemeat; see below). Water was provided ad libitum.

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Trapping and husbandry procedures were approved by the Animal Care and Use Committee at the U.S. Geological Survey Patuxent Wildlife Research Center.

Prototype inducers of CYP isozymes were administered to determine the inducibility of monoxygenases and the optimal conditions for detecting enzyme products in short-tailed shrews. Six shrews were assigned randomly to each of the following four treatments: 50 ppm β-naphthoflavone (βNF, Sigma Chemical, St. Louis, MO, USA) suspended in corn oil, 50 ppm phenobarbital (PB), Sigma Chemical) dissolved in 0.9% sodium chloride (0.9% saline, w/v), and control groups receiving the vehicles alone. Shrews received intraperitoneal injections of 10-ml treatment solution/kg body weight. The βNF treatments and corn oil controls were administered once, 3 d prior to sacrifice. The PB treatments and saline controls were administered on each of the 3 d prior to sacrifice. Optimal conditions for monoxygenase assays were determined using microsomes prepared from livers pooled within dose groups; half of the liver from each shrew was added to the pooled sample. Microsomes were prepared from livers of individual shrews to determine the activity of hepatic monoxygenases BROD, EROD, MROD, and PROD.

Experimental shrews were assigned randomly to a control diet (n = 5) or diets containing PCBs at 0.6 ppm (n = 5), 9.6 ppm (n = 5), and 150 ppm (n = 6). Each group was fed two 5-g meals per day for 31 d. Experimental diets were prepared by dissolving a 1:2 wet weight mixture of Aroclors 1254:1242 (Accu Standard, New Haven, CT, USA) in corn oil, then mixing the PCB solution (1.0% w/v) into horsemeat (Nebraska Bird of Prey Diet, Animal Spectrum). Meat was frozen in portions of approximately 5 g. Cage rooms were kept at 24°C and a 12:12 h light:dark cycle. Seven B. breviceuda trapped in Carbondale (IL, USA) and two B. breviceuda captured on the U.S. FWS Patuxent Research Refuge were killed and dissected within 10 min after removal from traps; carcasses and livers were processed and monoxygenase activity determined as described above.

Microsome preparation and protein assay

Microsomes were prepared from liver tissue following methods of Melancon [13]. All microsomes were assayed on the same day they were prepared. Microsomal protein concentrations were measured following a reduced volume Lowry method [13,14] using an ultraviolet/visible wavelength spectrophotometer or by the bicinchoninic acid–based colorimetric protein assay [15], and modified for use with a Spectromax ultraviolet/visible microwell plate reader (Molecular Devices, Sunnyvale, CA, USA). Both methods used bovine serum albumin as a standard. Protein quantities determined using the Lowry method exceeded those measured using the bicinchoninic acid method by 16% in those samples evaluated by both methods. To facilitate comparison of data from both methods, results from the Lowry method were adjusted to the lower range of the bicinchoninic acid method and reported as such.

Hepatic P450 monoxygenase assay

Monoxygenase activity was assayed in microsomes following techniques of Burke and Mayer [16,17] and Burke et al. [18,19] modified for a computer-coupled fluorescence microwell plate reader (Fluoroskan II, TiterTek Instrument, Huntsville, AL, USA [13]). Assays were conducted at a total volume of 260 μL at 37°C. A range of pHs and concentrations of substrate, microsomes, and nicotinamide adenine dinucleotide phosphate were evaluated for amount and linearity of activity. The following parameters were selected. Each well contained microsomes equivalent to 5.2 mg of liver (BROD, EROD, and MROD) (60–100 μg microsomal protein) or 0.33 mg of liver (PROD) (4–6 μg microsomal protein), nicotinamide adenine dinucleotide phosphate at 0.125 mM (BROD and PROD) or 0.25 mM (EROD and MROD), substrate at 2.5 μM (EROD) or 5 μM (EROD, MROD and PROD) brought to 260 μL total volume with 66 mM Tris buffer at pH 8.2 (BROD and EROD), pH 7.8 (MROD), or pH 8.6 (PROD). Rates of resorufin formation were calculated by comparing fluorescence against that produced by known amounts of resorufin.

Sodium dodecyl sulfate polycrylamide gel electrophoresis and western blot

Cytochrome P450 proteins in liver samples were separated following the sodium dodecyl sulfate polycrylamide gel electrophoresis (SDS-PAGE) method of Laemmli [20] using a Hoefer Model SE 250 Mighty Small II apparatus (Hoefer Scientific Instruments, San Francisco, CA, USA). A 4% (v/v) monomer stacking gel (pH 6.8) and a 7.5% (v/v) monomer separating gel (pH 8.8) were used in a Tris-glycine buffer system. Electrophoresis was performed at a constant voltage of 180 for approximately 1 h. Proteins separated using SDS-PAGE were electrophoretically transferred to nitrocellulose for 1 h at 100 volts using a 25 mM Tris, 192 mM glycine, 20% methanol, pH 8.3 transfer buffer in a Bio-Rad Mini Trans-Blot apparatus (Bio-Rad, Hercules, CA, USA). The nitrocellulose was blocked for 1 h with a 1.0% (w/v) solution of nonfat dry milk in Tris buffered saline with 0.05% tween ([TBST], Sigma Chemicals) for CYP1A1 blots or 5.0% (w/v) solution for CYP2B1 blots. After blocking, nitrocellulose blots were rinsed three times with TBST for 5 min, then incubated for 2 h with a 1:1000 dilution of antimallard βNF-induced CYP produced in rabbit (preparation was described by Brown et al. [21], or a 1:500 dilution of antimouse PB-induced CYP antibody produced in rat [Daiichi Pure Chemicals, Tokyo, Japan]). After rinsing with TBST, blots were incubated for 1 h in an antirabbit immunoglobulin G (IgG) conjugated to alkaline phosphatase (1:3,000 in TBST), or an antigoat IgG alkaline phosphatase conjugate (1:5,000 in TBST). After a final rinsing with TBST, blots were incubated in a 10-mM aqueous solution of Sigma Fast 5 bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium substrate (Sigma Chemicals) until protein bands appeared. Protein bands were quantified by comparison with bands of known amounts of protein in βNF-treated mallard and PB-treated rat microsomes. A single CYP1A1 reactive band with a mass of approximately 53 kDa was quantified. Three CYP2B-reactive bands with masses between 43 and 53 kDa were analyzed together as CYP2B.
Pentobarbital-induced sleeping time

On day 31 of the experimental diet, shrews were injected intraperitoneally with sodium pentobarbital (Abbot Laboratories, Chicago, IL, USA) at 60 mg/kg body weight, a dose chosen to produce approximately 1 h of sleep in control shrews. Immediately after injection, shrews were placed singly in cages with a light layer of insulating material. When movement and response to stimulus ceased, they were placed on their backs and observed, without interference, for righting behavior, which indicates recovery from the barbiturate [22]. Sleep time was defined as the time from injection with sodium pentobarbital until the animal stood on four feet. Sleep time varies inversely with the amount of monoxygenase available to metabolize the dose of pentobarbital.

PCB determinations

The PCB concentrations were determined in whole shrew carcasses (less liver) and in experimental diets by the U.S. FWS Patuxent Analytical Control Facility by gas chromatography, using the method of Cromartie et al. [23]. This basically involves Soxhlet extraction with hexane with sample clean up and separation via Florisil (60–100 mesh, standard grade, 1,200°F activation, U.S. Silica, Berkeley, WV, USA) and silica gel (100–200 American Society for Testing and Materials mesh, grade 923, Kontes, Vineland, NJ, USA) columns. Gas chromatographic analysis utilized a Ni® electron capture detector and a 30-m column coated with a 1.0-micron film of 7% cyanopropyl 7% phenyl polysiloxane. Spiked blank and duplicate samples were run every 20 samples for quality assurance and the identity of analytes in 10% of the samples was confirmed by gas chromatography–mass spectrometry. The nominal lower limit of detection was 0.01 mg/kg based on a 10-g aliquot (wt wt).

Statistical procedures

When monoxygenase assays were run on different days, or required more than one assay plate on a single day, activity values from different plates of microsomes were adjusted based on enzyme activity in reference microsomes included on every plate. Differences between mean enzyme activities were tested with Mann–Whitney U test and the correlations between enzyme activities, protein concentrations or sleep time, and PCB exposure were calculated using Spearman’s procedure based on ranks. The relationship between total liver weight and dose was tested with analysis of covariance using the method of Cromartie et al. [23]. This basically involves Soxhlet extraction with hexane with sample clean up and separation via Florisil (60–100 mesh, standard grade, 1,200°F activation, U.S. Silica, Berkeley, WV, USA) and silica gel (100–200 American Society for Testing and Materials mesh, grade 923, Kontes, Vineland, NJ, USA) columns. Gas chromatographic analysis utilized a Ni® electron capture detector and a 30-m column coated with a 1.0-micron film of 7% cyanopropyl 7% phenyl polysiloxane. Spiked blank and duplicate samples were run every 20 samples for quality assurance and the identity of analytes in 10% of the samples was confirmed by gas chromatography–mass spectrometry. The nominal lower limit of detection was 0.01 mg/kg based on a 10-g aliquot (wt wt).

RESULTS

Exposure to prototype inducers

The activities of BROD, EROD, and MROD, but not PROD, in liver microsome preparations from short-tailed shrews were induced significantly by treating the animals with βNF (Fig 1: BROD, p < 0.006; EROD, p < 0.006; MROD, p < 0.03; PROD, p = 0.47; Mann–Whitney U, for comparisons against control animals). None of the enzymes was induced by PB treatment (Fig 1: BROD, p = 0.52; EROD, p = 0.87; MROD, p = 0.52; PROD, p = 0.87; Mann–Whitney U, for comparisons against control animals). With βNF treatment, EROD was induced the most strongly (~5-fold over control levels), followed by BROD (~4-fold) and MROD (~2-fold). Because of difficulty in getting linear reaction rates in the PROD assay, the microsome concentration was very low, which probably contributed to the inability to find induction in this assay. Because of this PROD was not measured in subsequent experiments.

Exposure to dietary PCBs

Dietary PCB exposure for 31 d resulted in substantial concentrations of PCB in short-tailed shrews, with accumulation factors ranging from 3.4 at the lowest dietary level to 1.36 at the highest dietary level (Table 1). Total liver weights did not differ among the dose levels (analysis of covariance, p = 0.96 for dose, body wt was a significant covariate, p = 0.0004). The activities of BROD (r² = 0.88, p < 0.001), EROD (r² = 0.68, p < 0.002), and MROD (r² = 0.85, p < 0.001), were highly significantly correlated to the lipid concentrations of PCB in short-tailed shrews, indicating significant induction by PCBs. In addition, CYP1A (r² = 0.92, p < 0.001), but not CYP2B (r² = 0.32, p < 0.15), or barbiturate-induced sleep time (r² = 0.03, p < 0.91), was correlated significantly with PCB exposure measured on a lipid basis. The patterns of these relationships hold true even when shrews in the highest dose group were omitted from the analyses, thus representing a more environmentally realistic dose range. The strength of the correlations between enzyme activities and PCB exposure decreased slightly, but still were highly significant, when high doses were ignored, except for EROD, which increased slight-
In shrews sacriﬁced the day they were trapped (Fig. 4; BROD significantly higher in our experimental control shrews than (BROD, oxygenase activities correlated highly with PCB exposure (relationship between CYP2B and PCB exposure (enzyme activity the ﬁt evaluated with a regression of the linearized relationship dose group). A logarithmic function was ﬁt to the points and activity of each enzyme is plotted in Figure 2 (omitting the high other in PCB-exposed shrews, but none signiﬁcantly correlated ly. In addition, the activity of each of the three enzymes and the production of CYP1A all signiﬁcantly correlated with each other in PCB-exposed shrews, but none signiﬁcantly correlated with CYP2B or barbiturate-induced sleep time (Table 2).

The relationship between PCB exposure in shrews and activity of each enzyme is plotted in Figure 2 (omitting the high dose group). A logarithmic function was ﬁt to the points and the ﬁt evaluated with a regression of the linearized relationship (enzyme activity = b0 + b1 ln[exposure]). All three mono-oxygenase activities correlated highly with PCB exposure (BROD, 5 = 0.0003; EROD, 5 = 0.0001; MROD, 5 = 0.0013). Despite substantial enzyme activity in a few control animals, the intercept (b0) did not differ from zero except for BROD (5 = 0.035). The CYP1A also correlated highly with PCB exposure (5 = 0.78, p > 0.0001; Fig. 3), but there was no relationship between CYP2B and PCB exposure (5 = 0.02, p > 0.60).

The activities of BROD and EROD, but not MROD, were signiﬁcantly higher in our experimental control shrews than in shrews sacriﬁced the day they were trapped (Fig. 4; BROD p = 0.006, EROD p = 0.003, MROD, p = 0.32; Mann–Whitney U), indicating that the baseline activities of BROD and EROD in our experimental control shrews were elevated over those of shrews in the wild.

DISCUSSION

We examined short-tailed shrews for the inducibility of two major families of cytochromes P450, CYP1A, and CYP2B. Treatment with βNF, a classic inducer of CYP1A in rodents, resulted in signiﬁcantly increased dealkylation of a few alkoxylesorusufins (BROD, MROD, and EROD) by liver microsomes. The increases in EROD and MROD activities by βNF were typical of CYP1A induction, while the additional induction of BROD suggested that shrew CYP1A was different than that of laboratory rodent CYP1A. The association of rodent CYP2B speciﬁc dealkylases associated with CYP1A has been reported for other wildlife. Many avian species exhibit increases in BROD, and sometimes PROD, after administration of CYP1A inducers, with the increase (ratio of treated/control) for BROD typically as great or greater than that for EROD or MROD [25]. In the present study no increase in any of these three dealkylases resulted from treatment with PB, a typical inducer of CYP2B enzymes. Both BROD and PROD typically are associated with CYP2B in rats and mice [26,27], so the lack of induction of these enzymes in PB-treated shrews indicates the CYP2B family is not induced by PB in B. breviceps, at least under the conditions of this study. We also have observed low responsiveness of CYP and associated monoxygenase activities to PB in birds [13]. Two of these avian species consume insects for at least part of their diets, and of these, European starlings (Sturnus vulgaris) exhibited such induction though Redwinged blackbirds (Agelaius phoenicius) did not. Dosing routes and concentrations of prototype inducers varies widely among studies; however, the dosing regiment that we used is effective in other responsive species. Thus, at the very least, B. breviceps was less inducible by PB than laboratory rodents.

Shrews readily accumulated PCBs at all three dietary exposure levels. On both wet weight and lipid weight bases tissue PCBs were much higher at the highest dietary level than at the lower exposure levels, but the bioaccumulation factor at the highest dietary level was lower, perhaps due to limitations in movements of the greater mass of PCBs. Liver enzyme induction was an effective measure of PCB exposure in B. breviceps. Both total CYP1A protein and the activity of speciﬁc enzymes were related signiﬁcantly to PCB concentration in shrews. However, our measurements of induction varied in sensitivity: In shrews exposed to PCBs, enzyme activity increased four- to six-fold over controls, and CYP1A concentration increased about ten-fold. The absence of any relation-

<table>
<thead>
<tr>
<th>PCB in food</th>
<th>PCB in carcasses</th>
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<tbody>
<tr>
<td>Nominal</td>
<td>Measured</td>
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<tr>
<td>0</td>
<td>ND*</td>
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<tr>
<td>0.6</td>
<td>0.55 ± 0.17</td>
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<tr>
<td>9.6</td>
<td>9.78 ± 7.00</td>
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<tr>
<td>150.0</td>
<td>214.25 ± 8.06</td>
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</tbody>
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* ND = Not detectable.

Bioaccumulation factor = (µg PCB/g in shrew [wet])/(µg PCB/g in food [measured]).

Table 1. Concentrations of polychlorinated biphenyls ([PCB], ppm) in food and carcasses (ppm; mean ± standard deviation) of short-tailed shrews following 31 d dietary exposure to an Aroclor 1254:1242 (1:2, w/w) mixture

Table 2. Correlations (Spearman’s rho; below diagonal) and p-values (H0: rho = 0; above diagonal) among different measures of induction of hepatic monoxygenase for short-tailed shrews experimentally exposed to polychlorinated biphenyls (PCB)

<table>
<thead>
<tr>
<th>EROD*</th>
<th>BROD</th>
<th>MROD</th>
<th>CYP1A</th>
<th>CYP2B</th>
<th>Sleep</th>
</tr>
</thead>
<tbody>
<tr>
<td>EROD</td>
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<td>0.001</td>
<td>0.005</td>
<td>0.728</td>
<td>0.515</td>
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<td>BROD</td>
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<td>0.0006</td>
<td>0.002</td>
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<td>MROD</td>
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<td>0.918</td>
<td>0.003</td>
<td>0.496</td>
<td>0.472</td>
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<tr>
<td>CYP1A</td>
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<td>0.821</td>
<td>0.790</td>
<td>0.912</td>
<td>0.746</td>
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<tr>
<td>CYP2B</td>
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<td>0.200</td>
<td>0.182</td>
<td>0.029</td>
<td>0.289</td>
</tr>
<tr>
<td>Sleep</td>
<td>−0.188</td>
<td>−0.293</td>
<td>−0.207</td>
<td>0.093</td>
<td>−0.306</td>
</tr>
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</table>

* EROD, BROD, and MROD represent the ethoxy-, benzyloxy-, and methoxy-resorufin-O-dealkylases, respectively, and CYP1A and CYP2B represent the cytochrome P4501A and 2B families, respectively.
Cytochrome P450 induction in short-tailed shrews

**Fig. 2.** The activities of benzyloxyresorufin-O-dealkylase (BROD), ethoxyresorufin-O-dealkylase (EROD), and methoxyresorufin-O-dealkylase (MROD) as a function of carcass lipid polychlorinated biphenyl (PCB) levels in short-tailed shrews fed PCBs for 31 d ($n = 21$). Curves are fit as a logarithmic function of exposure. All three of these monooxygenase activities are significantly related to PCBs in lipid ($p < 0.002$).

**Fig. 3.** Cytochrome protein concentrations as a function of carcass lipid polychlorinated biphenyls (PCB) in short-tailed shrews fed PCBs for 31 d ($n = 21$). The amount of cytochrome P4501A (CYP1A) is related significantly to exposure ($r^2 = 0.78$, $p < 0.0001$), but CYP2B is not ($r^2 = 0.02$, $p > 0.60$).

**Fig. 4.** The activities of benzyloxyresorufin-O-dealkylase (BROD), ethoxyresorufin-O-dealkylase (EROD), and methoxyresorufin-O-dealkylase (MROD) (mean, standard deviation) from experimental short-tailed shrews fed control meatballs for 31 d ($n = 5$), compared to enzyme activities in shrews sacrificed on the day of capture from the wild ($n = 9$).

...ship between PCB exposure and enzyme activity, CYP2B protein concentration, or pentobarbital-induced anesthesia, is consistent with the lack of induction of monooxygenase activity in PB-exposed shrews. Note that *B. brevicauda* showed no increase in CYP2B protein concentration, hence the lack of measured enzyme activity is not due to poor substrate specificity in enzymes that were induced, as suggested for cotton rat (*Sigmodon hispidus*) [28]. Aroclors, mixtures of PCB congeners, usually induce both CYP1A and CYP2B in wild [28] and laboratory rodents [29], so the lack of CYP2B response is *B. brevicauda* is noteworthy.

The sensitivity of *B. brevicauda* to induction of CYP1A enzymes by PCBs should be compared to that of other wild mammals to help evaluate the usefulness of *B. brevicauda* as...
a biomonitor for PCBs. The shape of dose-enzyme activity curves in *B. brevicauda* was similar to that for EROD activity in *S. hispidus* ([28], Fig 1.; [30], Fig. 6., sharp increase in induction with dose, then a plateau). The EROD induction in *B. brevicauda* was similar or slightly less sensitive to PCB exposure than in *S. hispidus*, assuming that our measure of exposure (lipid concentration) is roughly ten times greater than that of Henneman et al. ([30], Fig. 6., concentration in liver).

The order of dealkylase activities in the present study

The EROD > MROD > BROD > PROD agreed with the results of several experiments with white-toothed shrew (*Sorex araneus*) by Bosveld et al. [31], which in general had the same order of activity. In addition, our experimental controls in the PCB feeding study showed significantly more EROD activity than in shrews tested immediately after capture, raising the possibility that true basal enzyme activity is lower than we observed in the PCB feeding experiment. Interestingly, Bosveld et al. [12] reported that shrews (*Crocidura russula*) that had been maintained in captivity for over a month had much higher EROD activity, 670 pmol/min/mg microsomal protein, than freshly field-captured shrews (*Sorex araneus*) at 158 pmol/min/mg microsomal protein. In a report of four additional experiments [31] where the shrews had been maintained in captivity for from over a month to over a year the control EROD values averaged 390, 170, 110, and 480 pmol/min/mg microsomal protein. Because the shrews handled differently were of different species, it is not possible to determine whether the difference is due to the species difference or the difference of the handling regimens (diets and shrew activity). In both species the control monooxygenase activity is much higher than what we found in *B. brevicauda*, regardless of the regimen.

We have shown that *B. brevicauda* cytochrome P450 and associated monooxygenases were inducible, at least by chemicals that stimulate CYP1A production, including PCBs. In addition, enzyme induction from PCB exposure in *B. brevicauda* roughly is similar in sensitivity to other small mammals, and because shrews readily concentrate PCBs from food and eat at high trophic levels, they are subject to biomagnification and could serve as a biomonitor of low-level PCB pollution in terrestrial habitats where *B. brevicauda* is found. We found no evidence of CYP2B induction, so measures that rely on that family of enzymes (such as pentobarbital-induced sleep time) would not be useful with *B. brevicauda*, nor would *B. brevicauda* be a useful monitor of contaminants primarily metabolized by CYP2B enzymes.

Acknowledgement—This study was supported in part by the Pennsylvania Game Commission.

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