Effects of Polychlorinated Biphenyls on Estrogen Receptor-β Expression in the Anteroventral Periventricular Nucleus

Jacklyn Salama, Tandra R. Chakraborty, Laurie Ng, and Andrea C. Gore

Kastor Neurobiology of Aging Laboratories, Fishberg Research Center for Neurobiology, and Brookdale Department of Geriatrics and Adult Development, Mount Sinai School of Medicine, New York, New York, USA

Polychlorinated biphenyls (PCBs) can disrupt the reproductive axis, particularly when the exposure occurs during the vulnerable developmental periods. Some effects of environmental endocrine disruptors such as PCBs may be exerted through binding to estrogen receptors (ERs). In this study we examined the endocrine-disrupting effects of Aroclor 1221 (a commercial PCB mixture), focusing on its actions on the ER-β, which has been implicated in mediating effects of endocrine-disrupting chemicals. A low, ecologically relevant dose of Aroclor 1221 or vehicle (ethanol) was administered three times each to rat dams, on gestational day 16 and on postpartum days 1 and 4, a developmental period during which steroid hormones have permanent effects on adult brain structure and function. Effects on ER-β cell number in the anteroventral periventricular nucleus (AVPV) were quantified; this sexually dimorphic nucleus of the brain is essential to female reproductive function. For comparison, we quantified ER-β cell number in another hypothalamic region, the supraoptic nucleus (SON). Using a stereologic approach, we found that Aroclor 1221 caused a highly significant down-regulation of the number of ER-β–expressing cells in the AVPV, but had no effect in the SON. Thus, PCB exposure has consequences for neural ER expression, and these findings have implications for wildlife and humans that have been exposed to environmental estrogens, particularly during the susceptible periods of early development. *Key words:* **anteroventral periventricular nucleus, Aroclor 1221, estrogen receptor, hypothalamus, PCB, puberty, supraoptic nucleus.** *Environ Health Perspect* **111:1278–1282 (2003). doi:10.1289/ehp.6126 available via** *http://dx.doi.org/* **[Online 10 April 2003]**

Endocrine-disrupting chemicals (EDCs) are substances found in the environment that interfere with the endocrine system by acting as agonists or antagonists to hormone receptors. Polychlorinated biphenyls (PCBs) can act as EDCs; depending upon their structures, they can act at estrogen receptors (ERs), androgen receptors, or thyroid hormone receptors (Conner et al. 1997; Jansen et al. 1993; Kester et al. 2000; Sager 1983; Zoeller 2002) and interfere with the enzymes involved in steroid hormone biosynthesis and metabolism (Hany et al. 1999; Kester et al. 2000). Although the production of PCBs has been outlawed in the United States since 1979, these chemicals persist in the air, water, and soil and biomagnify within the food chain (Evans et al. 1991; Safe et al. 1987; Tilson et al. 1998).

The brains of developing male rodents are naturally exposed to sex steroid hormones (testosterone and its metabolite estradiol) that are synthesized in the developing male testis (Barraclough 1961; Doecke et al. 1978; Ramaley 1979). The ovary of female rodents, by contrast, is essentially quiescent, resulting in relatively low exposure of the developing female brain to gonadal hormones (Barraclough 1961; Doecke et al. 1978; Ramaley 1979). These normal sex differences in perinatal steroid hormone exposure result in adulthood in normal male- and female-typical neuroendocrine function (e.g., secretion of gonadotropins), reproductive behavior, and other nonreproductive functions (Davis et al. 1996; Laessig et al. 1999; McEwen and Alves 1999; Simerly 2002). Therefore, exposure of the female brain to exogenous steroids, including estrogenic EDCs, during critically sensitive stages of development can have masculinizing or defeminizing effects (Atanassova et al. 2000; Barraclough 1961; Chung and Clemens 1999; Conner et al. 1997; Cooper and Kavlock 1997; Newland and Paletz 2000). For example, prenatal treatment of female rodents and other species with PCBs and other EDCs has been shown to alter the timing of puberty (Faqi et al. 1998; Lundkvist 1990), accelerate reproductive aging (Cooper and Kavlock 1997; Gellert 1978), affect gonadotropin release (Jansen et al. 1993; Khan and Thomas 1997), and interfere with sexual behavior (Chung and Clemens 1999).

In the present study, we used an environmentally relevant dose (Bush et al. 1990) of a commercial PCB mixture, Aroclor 1221, which is 21% chlorinated and exerts primarily estrogenic effects (Jansen et al. 1993; Webb and McCall 1972; Willis and Addison 1972). It is made up primarily of PCB congeners 1–4, 6–9, 13, and 15 and has chlorine substitutions primarily at positions 2 and 4, and to a lesser extent at position 3 (Frame et al. 1996; Webb and McCall 1972; Willis and Addison 1972). Our goal was to identify neurobiologic targets for actions of Aroclor 1221 and to correlate changes in these targets with other effects of Aroclor 1221 on reproductive and somatic growth and development. We focused our analysis on the ER-β because it has been reported to have a relatively high affinity for EDCs (Kuiper et al. 1998), and its activation results in transcriptional responses that are distinct from those mediated by the ER-α (Hall and Korach 2002). We performed our studies on the anteroventral periventricular nucleus (AVPV), a sexually dimorphic region of the brain that is critically involved in female reproductive physiology (Simerly 1989; Wiegand et al. 1978). For comparison, we studied the supraoptic nucleus (SON), a hypothalamic nucleus that expresses ER-β (Hrabovszky et al. 1998), and although it may be involved in some aspects of reproduction such as lactation and parturition, it is more strongly implicated in the control of water and electrolyte balance and maternal behavior [reviewed by Higuchi and Okere (2002)]. Our studies should provide novel information on the possible mechanisms and neural substrates at which PCBs may interfere with the sexual development of females.

Materials and Methods

Animals. Subjects were seven timed-pregnant female Sprague-Dawley rats, housed in shoebox cages in a 12:12-hr light:dark cycle (lights on at 0700 hr) and given Purina rat chow and water *ad libitum*. Animals were ordered from the vendor (Harlan Sprague-Dawley, Indianapolis, IN) and scheduled to arrive on their 14th day of pregnancy. Females were housed separately and were checked on gestational days 21 and 22 for parturition. After birth, female pups were cross-fostered on postnatal day 2 (P2) within respective treatment groups, and males were culled. For this experiment, we used six control pups (derived from four litters) and five experimental pups (derived from three experimental litters). All animal protocols were approved by the Institutional Animal Care

Address correspondence to A.C. Gore, University of Texas at Austin, Pharmacology/Toxicology A1915, 1 University Station, Austin, TX 78712 USA. Telephone: (512) 471-3669. Fax: (512) 475-6088. E-mail: andrea.gore@mail.utexas.edu

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and Use Committee of Mount Sinai School of Medicine, following *The Guide for the Care and Use of Experimental Animals* (National Research Council 1996).

Experimental procedure. Three intraperitoneal injections were administered to each dam. Dams in the experimental group (n = 3) were injected with Aroclor 1221 (1 mg/kg) dissolved in 100% ethanol, in a volume of 0.1 mL. Dams of the control group (n = 4) were injected with 0.1 mL of 100% ethanol. The dosage and volume were based on pilot studies from our laboratory (Gore 2001). Based on estimates from Chung and Clemens (1999), the transfer of PCBs from the dams to the pups was estimated at approximately 2 µg (0.34 mg/kg), although we were not able to verify PCB concentrations in our pups. The first injection was on gestational day 16, the second was on P1, and the third was on P4. The ages of treatment were chosen to span the critical period of sexual differentiation of the rat brain (Barraclough 1961; Doecke et al. 1978; Ramaley 1979) and were similar to those used by Chung and Clemens (1999), although we gave our injections at slightly closer intervals to better approximate the critical period. Pups self-weaned at approximately P22 (no more nursing was observed and pups were eating chow), although they were kept with their respective litter and mother until perfusion. Age at eye opening was noted for each animal. Pups' ears were punched for identification purposes on P20, and anogenital distance was measured on P25. Body weight was recorded every 2–3 days from P12 until P42, the latter being the age of perfusion. The day of vaginal opening (VO) was checked beginning on P32, and vaginal smears were taken from the day of VO until perfusion (P42).

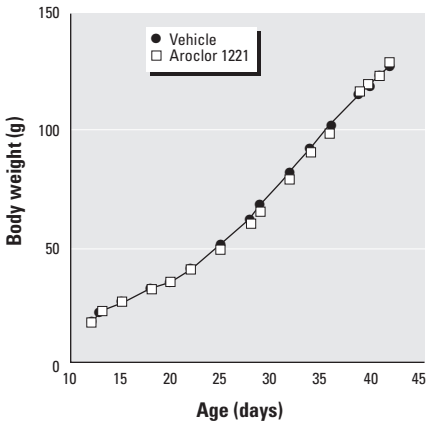

Figure 1. Average body weight (g) of vehicle-treated and Aroclor 1221–treated rats. Average body weights are plotted from P12 through P42. No differences between groups were observed.

Perfusion. At P42, rats were deeply anesthetized (0.3 mL of 100 mg/mL ketamine; 0.3 mL of 20 mg/mL xylazine) and then perfused transcardially first with saline (25 mL), then with heparin (1,000 U/mL at 25 mL), then with acrolein in 4% paraformaldehyde (25 mL), and finally with 4% paraformaldehyde [500 mL; all buffers were dissolved in phosphate-buffered saline (PBS)]. During perfusion, the descending aorta was clamped with a hemostat so that the reproductive organs remained unfixed. After perfusion, brains were removed and postfixed in 4% paraformaldehyde for 2 hr and then transferred into PBS for storage. The uterus and ovaries were dissected out, and their combined wet weight was determined.

Tissue preparation and diaminobenzidine immunocytochemistry. Brains were cut on a vibratome (Ted Pella, Inc., Redding, CA) in PBS at 40 µm, and sections were stored in PBS plus 0.1% sodium azide until time of immunocytochemistry. During immunocytochemistry for ER-β, sections were incubated in 1% sodium borohydride in PBS and were washed with PBS until any evidence of sodium borohydride was removed (indicated by the presence of bubbles; Milner et al. 2001). Sections were then incubated in a 3:1 volume ratio of methanol: 3% H_2O_2 (in PBS) for 20 min to eliminate any endogenous peroxidase activity. After another wash in PBS, sections were incubated for 5 days in primary antibody to ER-β (Zymed catalog no. Z8P; Zymed Laboratories, Inc., San Francisco, CA; 1 µg/mL), with 10% normal horse serum and 10% normal goat serum, in PBS. The sections were then washed again in PBS and incubated for 1 hr in secondary biotinylated goat anti-rabbit immunoglobulin G (IgG; H + L) antibody (Vector Laboratories, Inc., Burlingame, CA; 1:300 in PBS) and 2% normal horse serum. After washing in PBS, the sections were incubated for 1 hr in a Vectastain ABC Kit solution (mouse IgG, Vector Laboratories, Inc.). After another wash with PBS, the tissue was subjected to a diaminobenzidine (DAB) reaction on ice. Immediately after the DAB reaction, sections were washed thoroughly in PBS and mounted on 0.1% gelatin-subbed slides. Counterstaining was performed using cresyl violet. Control tissues were incubated in the absence of primary antibody, although all other conditions were kept identical to those used for tissues treated with the antibody to ER-β, and no specific staining was seen in these control tissues.

Stereologic tissue analysis of ER-β. Stereologic analysis was done as described in a recent report (Chakraborty et al. 2003) and following stereologic principles described by Schmitz and Hof (2000). Numbers of ER-β–immunoreactive cells were quantified in the AVPV and SON. The AVPV was chosen because it is a sexually dimorphic region of the rat brain, expressing both ER-α and ER-β (Shughrue et al. 1997; Shughrue and Merchenthaler 2001), and it is essential to normal reproductive function (Simerly and Swanson 1987; Wiegand et al. 1978). We also studied the SON as another hypothalamic region that expresses ER-β (Hrabovszky et al. 1998) but is not sexually dimorphic [its size is proportionally larger in male than in female rats, but this difference is entirely attributable to the body weight difference (Madeira et al. 1993)]. The volumes of the AVPV and SON were also calculated by stereologic methods. For the AVPV, 8–12 consecutive sections were analyzed per rat. For the SON, six consecutive sections were analyzed.

Each section was analyzed using a computer-assisted morphometry system consisting of a Zeiss Axioplan 2 photomicroscope (Zeiss, Thornwood, NY) equipped with an Applied Scientific Instrumentation MS-2000XYZ computer-controlled motorized stage (Applied Scientific Instrumentation, Eugene, OR), a DAGE-MTI DC 330 video camera (DAGE-MTI, Michigan City, IN), a Dell microcomputer (Dell, Austin, TX), and MicroBrightfield morphometry and stereology software (MicroBrightfield, Colchester, VT). Standard accepted stereologic formulas using the built-in point counting, optical fractionator, and planar rotator protocols in MicroBrightfield were used for all analyses (West et al. 1991). The range of error for the number of ER-β nuclei counted in the Aroclor 1221 and vehicle rats was 0.05–0.07 and 0.04–0.05, respectively (Gundersen coefficient of error, m = 1).

In each section, the boundary of the AVPV or SON was contoured at low magnification (10×) with the help of a rat brain atlas (Swanson 1998), and the area was outlined on a live computer image. The AVPV and SON cross-sectional areas were measured, allowing the program to create a three-dimensional counting frame height. Dimensions (length × weight) of the counting frame were 50 µm × 50 µm. Disector height was kept at 2 µm, and a stack of five such disectors was studied,

Table 1. Effects of Aroclor 1221 on developmental parameters in female rats.

Parameter	Treatment		p-Value
	Vehicle (n = 6)	Aroclor 1221 (n = 5)	
Age at eye opening (days)	16.5 ± 0.7	15.8 ± 0.7	0.49
Age at vaginal opening (days)	38.5 ± 0.5	38.3 ± 0.6	0.78
Age at first estrus (days)	40.0 ± 0.3	38.6 ± 0.9	0.07
Anogenital distance (mm)	8.1 ± 0.2	8.7 ± 0.1	0.06
Combined uterine and ovarian weight (g)	0.39 ± 0.03	0.49 ± 0.05	0.12

Data are expressed as mean ± SE.

accounting for a final *z*-axis depth of 10 μm in each sampled location.

Analysis and statistics. Effects of exposure to Aroclor 1221 were determined by analysis of variance (ANOVA) using Statview (SAS Institute, Cary, NC) software for Macintosh computer. The dependent variables were developmental body weight, age at eye opening, anogenital distance, age at VO, age at first diestrus, wet weight of uterus and ovaries, number of ER-β–positive cells in the AVPV or SON, and volume of the AVPV or SON. An effect was considered significant at $p < 0.05$.

Results

Effects of PCBs on developmental parameters. Body weight and age at eye opening were monitored in developing female rats. The body weight growth curve was similar between the two treatment groups, and no significant effect of Aroclor 1221 on body weight was observed (Figure 1). ANOVA demonstrated no effect of Aroclor 1221 on the age at eye opening ($p = 0.49$; Table 1).

Effects of Aroclor 1221 on several reproductive markers were assessed. As shown in Table 1, the animals in the Aroclor 1221 group did not show any difference in the age at VO (38.3 days) compared with the control group (38.5 days). The age at first vaginal estrus occurred an average of 1.4 days earlier in the Aroclor 1221–treated rats than the control rats (Table 1; 38.6 days vs. 40.0 days, respectively). Although this difference was not significant, ANOVA demonstrated a trend for this effect ($p = 0.07$). Vaginal smear data showed that Aroclor 1221 rats had fewer estrus and proestrus days than diestrus days relative to vehicle animals, suggesting some qualitative difference in estrous cyclicity. Anogenital distance, measured on P25, was slightly larger (0.6 mm) in the Aroclor 1221 treatment group than in the control group, but this did not attain significance (Table 1; $p = 0.06$). Although on average the combined wet weight of the uterus and ovaries was greater in the Aroclor 1221 than the vehicle group, this difference was not significant ($p = 0.12$; Table 1).

Stereologic analysis of effects of PCBs on ER-β in the AVPV. Photomicrographs showing expression of ER-β in the AVPV in a representative control and Aroclor 1221–treated rat are shown in Figure 2A and B. ER-β immunoreactivity was expressed intensely in cell nuclei of the AVPV, although in some cases there was faint cytoplasmic labeling (Figure 2E). The number of ER-β–positive nuclei in the AVPV was quantified by unbiased stereologic methods in each rat. ANOVA indicated a highly significant effect of treatment on the number of ER-β–positive nuclei, which was significantly lower in Aroclor 1221–treated than vehicle-treated rats ($p < 0.001$; Figure 3A). We also noted a qualitative difference in the distribution of ER-β–positive cells, which were more concentrated in medial parts of the AVPV in control rats but were more diffusely expressed in the AVPV of Aroclor 1221–treated rats (Figure 2, A vs. B). Although it may be somewhat difficult to discern in these low-power micrographs, this difference in distribution was extremely clear under the microscope and on the computer during stereologic analyses. No significant effect of Aroclor 1221 or vehicle treatment on AVPV volume was found by ANOVA (Figure 4A; $p = 0.24$).

Stereologic analysis of effects of PCBs on ER-β in the SON. Photomicrographs of ER-β expression in the SON of a representative control and Aroclor 1221–treated rat are shown in Figure 2C and D. ER-β immunoreactivity was strongly expressed in cell nuclei, with very low cytosolic expression (Figure 2F). The number of ER-β–immunoreactive nuclei in the SON of female rats was quantified using stereologic methodology. Figure 3B shows that administration of Aroclor 1221 did not significantly affect the number of ER-β–positive nuclei compared with vehicle control ($p = 0.49$), nor did it change SON volume in female rats (Figure 4B). In comparing results of the SON with those in the AVPV, although the volumes of the SON and AVPV were comparable (Figure 4, A vs. B), the number of ER-β–positive cells was substantially greater in the AVPV than in the SON (e.g., Figure 2, E vs. F; Figure 3, A vs. B).

Discussion

Early exposure to endogenous or exogenous hormones can have long-term and profound consequences on adult physiologic functions. We hypothesized that perinatal exposure of female rats to PCBs, which can act as agonists or antagonists of steroid hormone receptors, would have permanent effects on the brain, particularly in those regions that express these steroid hormone receptors and are sexually dimorphic. In support of our hypothesis, we observed region-specific effects of neonatal treatment with a low and ecologically relevant dose of an estrogenic PCB mixture, Aroclor 1221 (Bush et al. 1990). Aroclor 1221 caused a substantial down-regulation of numbers of ER-β–positive cells in the AVPV, a key brain area controlling reproductive function (Simerly 1998; Wiegand et al. 1978), but had no effect on the SON, a region involved in osmoregulation, parturition, and lactation (Higuchi and Okere 2002). Although this low dose of Aroclor 1221 was not sufficient to induce gross physiologic changes, it caused subtle alterations that would be expected to compromise

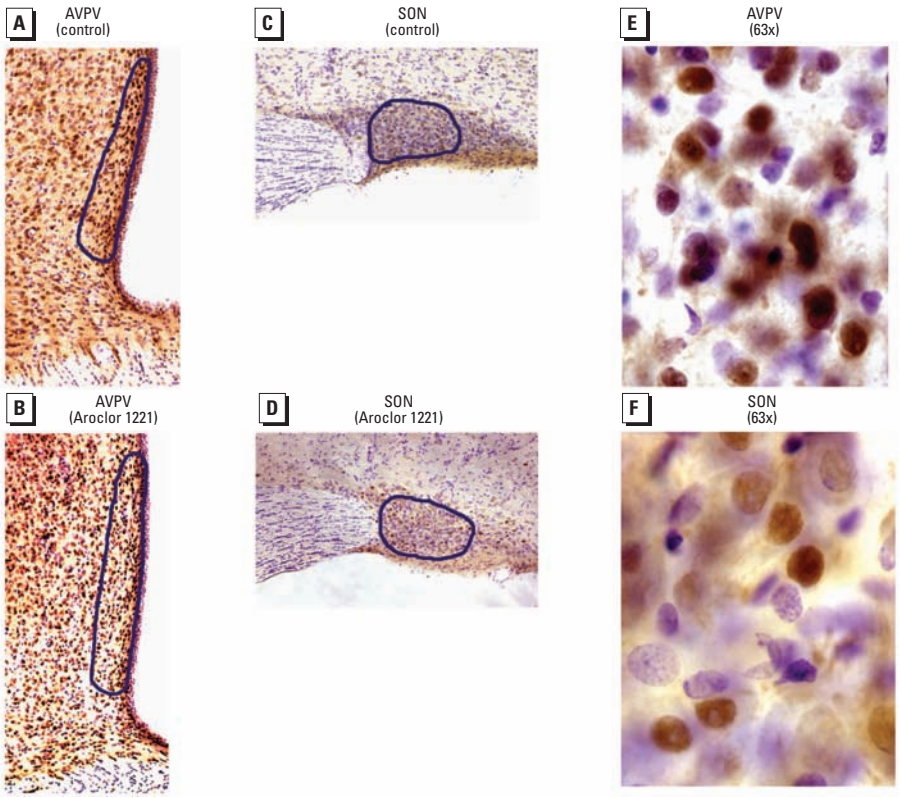

Figure 2. Photomicrographs showing ER-β immunoreactivity in the AVPV (*A* and *B*, 10×) and SON (*C* and *D*, 10×) of a representative vehicle-treated (*A* and *C*) and Aroclor 1221–treated (*B* and *D*) rat. *E* and *F* are high-power (63×) photomicrographs of ER-β immunoreactivity in the AVPV (*E*) and SON (*F*) of representative rats. ER-β nuclei are labeled with dark-brown diaminobenzidine product, and tissues are shown counterstained with cresyl violet (purple). The regions of the AVPV and SON are delineated with a blue contour.

the reproductive success of the organism, particularly in the challenges of the wild.

Effects of Aroclor 1221 on the AVPV. The AVPV of female rats is essential for the regulation of hypothalamic gonadotropin-releasing hormone neurons and the control of the estrogen-induced surge of gonadotropin-releasing hormone and luteinizing hormone (Gu and Simerly 1997; Le et al. 2001; Wiegand and Terasawa 1982; Wiegand et al. 1978). This preoptic brain region is larger in female than in male rats, and this sexual dimorphism develops in response to perinatal exposure to sex steroid hormones mediated at least in part by the ER-β (Orikasa et al. 2002; Simerly 1998, 2002; Wiegand et al. 1978). Within the AVPV, the number and distribution of ER-β–expressing cells also differ between the sexes: There are more ER-β mRNA–positive cells in the AVPV of females than in that of males, and the distribution of ER-β–positive cells in AVPV is concentrated more medially in females and is more diffusely distributed in males (Orikasa et al. 2002). Again, this sexually dimorphic pattern in ER-β expression in the AVPV is organized by exposure to neonatal steroid hormones (Orikasa et al. 2002).

In the present study, we found that perinatal Aroclor 1221 treatment to female rats resulted in a significant decrease in ER-β cell number in the AVPV. Our results are consistent with a masculinizing effect of Aroclor 1221 on this cell phenotype, similar to effects of exogenous estrogen, which also decreased ER-β cell number in the AVPV of neonatal female rats (Orikasa et al. 2002). Moreover, Aroclor 1221 caused a shift in the distribution of ER-β–expressing cells in the AVPV, again similar to effects of perinatal exogenous estrogen treatment in female rats (Orikasa et al. 2002). Therefore, both Aroclor 1221 and estrogen produced a more masculinized pattern of ER-β distribution in the AVPV. However, unlike estrogen, which decreased the size of the AVPV when given to neonatal female rats (Davis et al. 1996; Hutton et al. 1998), Aroclor 1221 treatment had no such effect in our study. It is possible that our low dose of Aroclor 1221, although sufficient to specifically decrease ER-β cell number, did not have such an effect on most cells in the AVPV, resulting in no net change in the volume of the AVPV in Aroclor 1221–treated rats. In fact, because ER-β cell number decreased in response to Aroclor 1221 but AVPV volume remained constant, other cell numbers may have increased to maintain overall AVPV size. These results suggest that the ER-β–expressing cells in the AVPV may be particularly vulnerable to EDCs such as Aroclor 1221. These results provide additional support for a role of the ER-β in the mediation of effects of EDCs (Hall and Korach 2002; Kuiper et al. 1998).

Effects of Aroclor 1221 on the SON. The SON is involved in regulating diverse physiologic functions including osmoregulation, blood pressure regulation, parturition, and lactation [reviewed by Higuchi and Okere (2002)]. In the present study, we observed abundant expression of ER-β in this brain region, in support of other studies (Alves et al. 1998; Hrabovszky et al. 1998). The presence of the ER-β in the SON probably underlies the estrogen sensitivity of this brain region with respect to $GABA_A$ receptor binding (Amico et al. 2000), prolactin mRNA levels (Torner et al. 1999), and other functions (Higuchi and Okere 2002), because ER-α is not expressed in the SON (Shughrue et al. 1997). Our stereologic analyses of numbers of ER-β–immunoreactive neurons in the SON demonstrated no effect of Aroclor 1221 on this parameter, nor did we observe any effect of Aroclor 1221 on SON volume. This latter result is not surprising given that any sexual dimorphism in the volume of the SON is entirely attributable to body size, such that the larger SON of male rats is proportional to the larger body size of males, and the smaller SON and body size of females are similarly proportional (Madeira et al. 1993). In our study, we observed no effect of Aroclor 1221 on either body size or SON volume, and our result is therefore consistent with that of an earlier study by Madeira et al. (1993). To our knowledge, numbers of ER-β–expressing cells in the SON have never been quantified in previous reports, and whether estrogen is similarly ineffective as Aroclor 1221 in altering ER-β cell number in the SON remains to be tested.

Effects of Aroclor 1221 on reproductive and somatic development. In our experiment we examined the effects of Aroclor 1221 on a number of reproductive end points: the timing of puberty, measured by VO and first estrus; reproductive tract weight; and estrous cyclicity. We did not find any significant effects of Aroclor 1221 treatment on any of these parameters, although we noted several trends. The timing of puberty (day of first estrus) on average was slightly accelerated by Aroclor 1221, and average anogenital distance and reproductive organ weight were somewhat larger in rats exposed to Aroclor 1221. The literature shows that effects of PCBs on these end points can vary depending on the mixture, dosage, and time of exposure and that, in general, effects of PCBs on these parameters are modest (Brezner et al. 1984; Chung and Clemens 1999; Lundkvist 1990). Nevertheless, the direction of these trends in our present study (i.e., an earlier onset of puberty and larger reproductive organ weight) is consistent with other published stimulatory effects of Aroclor 1221 on the timing of puberty (Ecobichon and MacKenzie 1974; Gellert 1978) and are also consistent with effects of exogenous estrogens given during development [reviewed by Gore (2002)]. Thus, although these effects of Aroclor 1221 were modest, they could still potentially impact reproductive success.

Previously, Chung and Clemens (1999) reported that perinatal exposure of female rats to Aroclor 1221 significantly altered female-typical sexual behavior in adulthood (Chung and Clemens 1999). Taken together with this previous study, our results indicate that Aroclor 1221 can substantially affect levels of a molecule in the brain that is important for

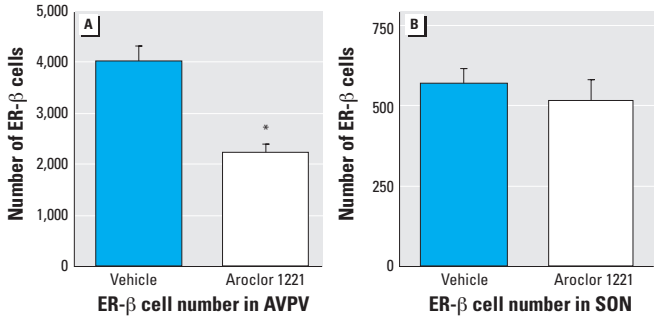

Figure 3. Quantification of the number of ER-β–positive cells in the AVPV (*A*) and SON (*B*). The dark bar shows data for vehicle-treated rats, and the light bar shows data for Aroclor 1221–treated rats (mean ± SE). In AVPV, Aroclor 1221–treated rats had significantly fewer ER-β–expressing cells than did vehicle controls. In SON, there was no effect of Aroclor 1221 on ER-β cell number.
*$p < 0.001$ versus vehicle.

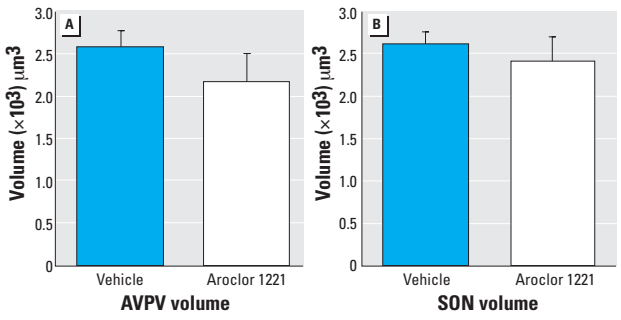

Figure 4. Average volume of the AVPV (*A*) and SON (*B*) in control and Aroclor 1221–treated rats. The dark bar shows data for vehicle-treated rats, and the light bar shows data for Aroclor 1221–treated rats (mean ± SE). Aroclor 1221 did not affect volume of either the AVPV or the SON.

reproduction (the ER-β in the AVPV) without causing other gross morphologic or physiologic changes. Future studies will examine the effects of Aroclor 1221 on the fertility and fecundity of treated animals, as well as investigate the role of the ER-α in the AVPV, in an effort to further elucidate the mechanisms for these endocrine-disrupting effects of this environmental contaminant on reproductive success.

References

Alves SE, Lopez V, McEwen BS, Weiland NG. 1998. Differential colocalization of estrogen receptor β (ER β) with oxytocin and vasopressin in the paraventricular and supraoptic nuclei of the female rat brain: an immunocytochemical study. Proc Natl Acad Sci USA 95:3281–3286.

Amico JA, Davis AM, McCarthy MM. 2000. An ovarian steroid hormone regimen that increases hypothalamic oxytocin expression alters [3H] muscimol binding in the hypothalamic supraoptic nucleus of the female rat. Brain Res 857:279–282.

Atanassova N, McKinnell C, Turner KJ, Fisher JS, Morley M, Millar MR, et al. 2000. Comparative effects of neonatal exposure of male rats to potent and weak (environmental) estrogens on spermatogenesis at puberty and the relationship to adult testis size and fertility: evidence for stimulatory effects of low estrogen levels. Endocrinology 141:3898–3907.

Barraclough CA. 1961. Production of anovulatory sterile rats by single injections of testosterone propionate. Endocrinology 68:122–129.

Brezner E, Terkel J, Perry AS. 1984. The effect of Aroclor 1254 (PCB) on the physiology of reproduction in the female rat-I. Comp Biochem Physiol 77C:65–70.

Bush B, Streeter RW, Sloan RJ. 1990. Polychlorobiphenyl (PCB) congeners in striped bass (*Morone saxatilis*) from marine and estuarine waters of New York State determined by capillary gas chromatography. Arch Environ Contam Toxicol 19:49–61.

Chakraborty TR, Ng L, Gore AC. 2003. Colocalization and hormone regulation of estrogen receptor alpha and NMDA receptor in the hypothalamus of female rats. Endocrinology 144:299–305.

Chung Y-W, Clemens LG. 1999. Effects of perinatal exposure to polychlorinated biphenyls on development of female sexual behavior. Bull Environ Contam Toxicol 62:664–670.

Conner K, Ramamoorthy K, Moore M, Mustain M, Chen I, Safe S, et al. 1997. Hydroxylated polychlorinated biphenyls (PCBs) as estrogens and antiestrogens: Structure-activity relationships. Toxicol Appl Pharmacol 145:111–123.

Cooper RL, Kavlock RJ. 1997. Endocrine disruptors and reproductive development: a weight-of-evidence overview. J Endocrinology 152:159–166.

Davis EC, Shryne JE, Gorski RA. 1996. Structural sexual dimorphisms in the anteroventral periventricular nucleus of the rat hypothalamus are sensitive to gonadal steroids perinatally, but develop peripubertally. Neuroendocrinology 63:142–148.

Doecke F, Rohde W, Smollich A, Dorner G. 1978. Hormones and brain maturation in the control of female puberty. In: Hormones and Brain Development (Dorner G, Kawakami M, eds). Amsterdam:Elsevier, 327–340.

Ecobichon DJ, MacKenzie DO. 1974. The uterotropic activity of commercial and isomerically-pure chlorobiphenyls in the rat. Res Commun Chem Pathol Pharmacol 9:85–95.

Evans MS, Noguchi GE, Rice CP. 1991. The biomagnification of polychlorinated biphenyls, toxaphene and DDT compounds in a Lake Michigan offshore food web. Arch Environ Contam Toxicol 20:87–93.

Faqi AS, Dalsenter PR, Merker HJ, Chahoud I. 1998. Effects on developmental landmarks and reproductive capability of 3,3′,4,4′-tetrachlorobiphenyl and 3,3′4,4′,5-pentachlorobiphenyl in offspring of rats exposed during pregnancy. Hum Exp Toxicol 17:365–372.

Frame GM, Cochran JW, Bowadt SS. 1996. Complete PCB congener distributions for 17 Aroclor mixtures determined by 3 HRGC systems optimized for comprehensive, quantitative, congener-specific analysis. J High Resolut Chromatogr 19:657–668.

Gellert RJ. 1978. Uterotrophic activity of polychlorinated biphenyls (PCB) and induction of precocious reproductive aging in neonatally treated female rats. Environ Res 16:123–130.

Gore AC. 2001. Environmental toxicant effects on neuroendocrine function. Endocrine 14:235–246.

———. 2002. Gonadotropin-releasing hormone (GnRH) neurons: gene expression and anatomical studies. Prog Brain Res 141:193–208.

Gu GB, Simerly RB. 1997. Projections of the sexually dimorphic anteroventral periventricular nucleus in the female rat. J Comp Neurol 384:142–164.

Hall JM, Korach KS. 2002. Analysis of the molecular mechanisms of human estrogen receptors alpha and beta reveals differential specificity in target promoter regulation by xenoestrogens. J Biol Chem 277:44455–44461.

Hany J, Lilienthal H, Sarasin A, Roth-Harer A, Fastabend A, Dunemann L, et al. 1999. Developmental exposure of rats to a reconstituted PCB mixture of Aroclor 1254: effects on organ weights, aromatase activity, sex hormone levels, and sweet preference behavior. Toxicol Appl Pharmacol 158:231–243.

Higuchi T, Okere CO. 2002. Role of the supraoptic nucleus in regulation of parturition and milk ejection revisited. Microsc Res Tech 56:113–121.

Hrabovszky E, Kallo I, Hajszan T, Shughrue PJ, Merchenthaler I, Liposits Z. 1998. Expression of estrogen receptor-beta messenger ribonucleic acid in oxytocin and vasopressin neurons of the rat supraoptic and paraventricular nuclei. Endocrinology 139:2600–2604.

Hutton LA, Gu G, Simerly RB. 1998. Development of a sexually dimorphic projection from the bed nuclei of the stria terminalis to the anteroventral periventricular nucleus in the rat. J Neurosci 18:3003–3013.

Jansen HT, Cooke PS, Porcelli J, Liu T-C, Hansen LG. 1993. Estrogenic and antiestrogenic actions of PCBs in the female rat: *in vitro* and *in vivo* studies. Reprod Toxicol 7:237–248.

Kester MHA, Bulduk S, Tibboel D, Meinl W, Glatt H, Falany CN, et al. 2000. Potent inhibition of estrogen sulfotransferase by hydroxylated PCB metabolites: a novel pathway explaining the estrogenic activity of PCBs. Endocrinology 141:1897–1900.

Khan IA, Thomas P. 1997. Aroclor 1254-induced alterations in hypothalamic monoamine metabolism in the Atlantic croaker (*Micropogonias undulatas*): correlation with pituitary gonadotropin release. Neurotoxicology 18:553–560.

Kuiper GGJM, Lemmen JG, Carlsson B, Corton JC, Safe SH, Van der Saag PT, et al. 1998. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology 139:4252–4263.

Laessig SA, McCarthy MM, Silbergeld EK. 1999. Neurotoxic effects of endocrine disruptors. Curr Opin Neurol 12:745–751.

Le W-W, Wise PM, Murphy AZ, Coolen LM, Hoffman GE. 2001. Parallel declines in fos activation of the medial anteroventral periventricular nucleus and LHRH neurons in middle-aged rats. Endocrinology 142:4976–4982.

Lundkvist U. 1990. Clinical and reproductive effects of Clophen A50 (PCB) administered during gestation on pregnant guinea pigs and their offspring. Toxicology 61:249–257.

Madeira MD, Sousa N, Cadete-Leite A, Lieberman AR, Paula-Barbosa MM. 1993. The supraoptic nucleus of the adult rat hypothalamus displays marked sexual dimorphism which is dependent on body weight. Neuroscience 52:497–513.

McEwen BS, Alves SE. 1999. Estrogen actions in the central nervous system. Endocrine Rev 20:279–307.

Milner TA, McEwen BS, Hayashi S, Li CJ, Reagan LP, Alves SE. 2001. Ultrastructural evidence that hippocampal alpha estrogen receptors are located at extranuclear sites. J Comp Neurol 429:355–371.

National Research Council. 1996. The Guide for the Care and Use of Laboratory Animals. Washington, DC:National Academy Press.

Newland CM, Paletz E. 2000. Animal studies of methylmercury and PCBs: what do they tell us about expected effects in humans? Neurotoxicology 21:1003–1028.

Orikasa C, Kondo Y, Hayashi S, McEwen BS, Sakuma Y. 2002. Sexually dimorphic expression of estrogen receptor β in the anteroventral periventricular nucleus of the rat preoptic area: implication in luteinizing hormone surge. Proc Natl Acad Sci USA 99:3306–3311.

Ramaley JA. 1979. Development of gonadotropin regulation in the prepubertal mammal. Biol Reprod 20:1–31.

Safe S, Safe L, Mullin M. 1987. Polychlorinated biphenyls: environmental occurrence and analysis. In: Polychlorinated Biphenyls (PCBs): Mammalian and Environmental Toxicology (Safe S, Hutzinger O, eds). Berlin:Springer-Verlag, 1–13.

Sager DB. 1983. Effect of postnatal exposure to polychlorinated biphenyls on adult male reproductive function. Environ Res 31:76–94.

Schmitz C, Hof PR. 2000. Recommendations for straightforward and rigorous methods of counting neurons based on a computer simulation approach. J Chem Neuroanat 20:93–114.

Shughrue PJ, Lane MV, Merchenthaler I. 1997. Comparative distribution of estrogen receptor-alpha and -beta mRNA in the rat central nervous system. J Comp Neurol 388:507–525.

Shughrue PJ, Merchenthaler I. 2001. Distribution of estrogen receptor beta immunoreactivity in the rat central nervous system. J Comp Neurol 436:64–81.

Simerly RB. 1989. Hormonal control of the development and regulation of tyrosine hydroxylase expression within a sexually dimorphic population of dopaminergic cells in the hypothalamus. Mol Brain Res 6:297–310.

———. 1998. Organization and regulation of sexually dimorphic neuroendocrine pathways. Behav Brain Res 92:195–203.

———. 2002. Wired for reproduction: organization and development of sexually dimorphic circuits in the mammalian forebrain. Annu Rev Neurosci 25:507–536.

Simerly RB, Swanson LW. 1987. The distribution of neurotransmitter-specific cells and fibers in the anteroventral periventricular nucleus: implications for the control of gonadotropin secretion in the rat. Brain Res 400:11–34.

Swanson LW. 1998. Brain Maps: Structure of the Rat Brain. 2nd ed. Amsterdam:Elsevier.

Tilson HA, Kodavanti PR, Mundy WR, Bushnell PJ. 1998. Neurotoxicity of environmental chemicals and their mechanism of action. Toxicol Lett 102–103:631–635.

Torner L, Nava G, Duenas Z, Corbacho A, Mejia S, Lopez F, et al. 1999. Changes in the expression of neurohypophyseal prolactins during the estrous cycle and after estrogen treatment. J Endocrinol 161:423–432.

Webb RG, McCall AC. 1972. Industrial chemicals: identities of polychlorinated biphenyl isomers in Aroclors. J Assoc Anal Chem 55:746–752.

West MJ, Slomianka L, Gundersen HJ. 1991. Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator. Anat Rec 231:482–497.

Wiegand SJ, Terasawa E. 1982. Discrete lesions reveal functional heterogeneity of suprachiasmatic structures in regulation of gonadotropin secretion in the female rat. Neuroendocrinology 34:395–404.

Wiegand SJ, Terasawa E, Bridson WE. 1978. Persistent estrus and blockade of progesterone-induced LH release follows lesions which do not damage the suprachiasmatic nucleus. Endocrinology 102:1645–1648.

Willis DE, Addison RF. 1972. Identification and estimation of the major components of a commercial polychlorinated biphenyl mixture, Aroclor 1221. J Fish Res Bd Can 29:592–595.

Zoeller TR. 2002. Thyroid hormone, brain development, and the environment. Environ Health Perspect 110(suppl 3):355–361.