

# Effects of gestational and lactational exposure to Aroclor 1242 on sperm quality and in vitro fertility in early adult and middle-aged mice

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## Abstract

The objective of this study was to examine the effects of gestational and lactational exposure to Aroclor 1242 (0, 10, 25, 50, and 100 mg/kg-bw) on male fertility. Doses were administered to C57BL6 female mice orally every two days from two weeks before mating, during mating, and through gestation until postnatal day 21. Male B6D2F1 offspring were examined for anogenital distance, organ development, epididymal sperm count, sperm motility, and in vitro fertility at 16 and 45 weeks of age. Stomach samples of pups nursing from PCB-treated mothers in the 50 mg/kg dose group were analyzed for PCBs and chlorobiphenyls by high resolution gas chromatography coupled with low resolution mass spectrometry. It was estimated that the nursing pups were exposed to 0.2, 0.6, 1.2, and 2.4 mg/kg/day total PCBs in the 10, 25, 50, and 100 mg/kg dose groups, respectively. This exposure level approaches the maximum FDA recommended levels for PCBs in food and breast milk. The composition of the PCBs in the stomach samples was different from the parent mixture, as there was a higher proportion of heavily chlorinated congeners, as well as chlorobiphenyls. Anogenital distance at weaning, and liver, thymus, and testes weight at 16 and 45 weeks of age were not affected by PCB exposure. Epididymal sperm velocity and linearity were significantly increased in the 25 mg/kg dose group at 16 weeks of age. Sperm count was increased by 36% in this dose group ( $P = 0.06$ ). By 45 weeks of age, average sperm count in this dose group was similar to that of controls. With the exception of the 50 mg/kg dose group at 16 weeks of age, sperm fertilizing ability in vitro was significantly decreased in all PCB-exposed groups at 16 and 45 weeks of age. These results suggest that fertility in the adult mouse is susceptible to developmental exposure to Aroclor 1242 and is independent of testis weight or epididymal sperm count. © 2001 Elsevier Science Inc. All rights reserved.

**Keywords:** Aroclor 1242; In vitro fertilization; Polychlorinated biphenyls; Sperm count; Testis; Fertility; CASA

## 1. Introduction

Polychlorinated biphenyls (PCBs) are a class of lipophilic, persistent synthetic chemicals that exist as complex mixtures in environmental and human matrices, including blood, adipose tissue, breast milk, and fetal tissue [1]. PCBs are considered potential endocrine disruptors, among many other effects, due to their ability to act as estrogens,

antiestrogens and goitrogens (reviewed in Ref. 2). There is concern that exposure to PCBs and other organohalogenes may impair male fertility [3]. Recent epidemiologic evidence suggests that prenatal exposure to PCBs and polychlorinated dibenzofurans can cause adverse effects on semen quality [4]. There have also been numerous, but inconsistent, reports of adverse effects on male reproduction following prenatal or postnatal exposure to PCBs in laboratory rodents. These effects include alterations in testis weight, seminal vesicle weight, ventral prostate weight, reduced serum testosterone levels, and impaired fertility [5–11].

The effects of developmental exposure to PCBs on testis weight and fertility in laboratory rodents depend on the test

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congener or mixture, the dosage, the developmental stage during exposure, and the age of the animal at the time of examination, as well as species and strain. For example, Sager [5] previously showed that male Holtzman rats exposed to Aroclor 1254 through early lactation (from birth to day 9) exhibited decreased fertility at 18 weeks of age and increased testis weight at 23 weeks of age. The decreased fertility was not accompanied by a decrease in epididymal sperm count or changes in sperm morphology or motility, but rather a decline in the ability of sperm to fertilize eggs [6,8]. Cooke et al. [9] demonstrated that neonatal exposure (from birth to day 25) of Sprague-Dawley rats to Aroclor 1254 and 1242 increased testis weight and daily sperm production at 19 weeks of age. These effects appear to be due to PCB-induced hypothyroidism since thyroxine replacement attenuated the increase in testis weight and sperm production in Aroclor 1242-treated rats [9]. In contrast to the reduced fertility of Aroclor 1254-exposed pups reported by Sager et al. [5,6,8], all Aroclor 1242-treated pups successfully impregnated females [9]. These PCB-induced effects on sperm production and fertility also appear to be independent of changes in serum FSH or testosterone concentration, testicular histopathology, or sperm morphology or motility [8,9].

Previous studies investigating the reproductive effects of developmental exposure to PCBs in rodents have focused on effects in early adulthood, while few studies have addressed whether rodents at later stages of adulthood exhibit the same effects or recover to control levels. Previous studies have observed that neonatal exposure of male B6D2F1 mice to Aroclor 1254 does not adversely affect sperm fertilizing ability *in vitro* until 45 weeks of age [12]. Therefore, the objectives of this study were to determine if gestational and lactational exposure of B6D2F1 mice to Aroclor 1242 can cause alterations in organ development and sperm quality and fertility in young adult (16 weeks of age) male offspring and to determine if the effects persisted into middle age (45 weeks of age). To estimate lactational exposure, stomach samples from pups nursing on PCB-treated mothers in the 50 mg/kg dose group were analyzed for PCBs and chlorobiphenyls using high resolution gas chromatography coupled with low resolution mass spectrometry.

## 2. Materials and methods

### 2.1. Animals

C57BL6 female and DBA/2 proven breeder male mice were obtained from Charles River Laboratories (Raleigh, NC) and housed in polycarbonate cages with cellulose fiber chips (Aspen Chip Laboratory Bedding, Northeastern Products, Warrensburg, NY) as bedding and maintained in a humidity (30 to 40%) and temperature (23°C) controlled room on a 12-h light-dark cycle. All mice receiving PCB treatment were housed in a HEPA-filtered rack in the same

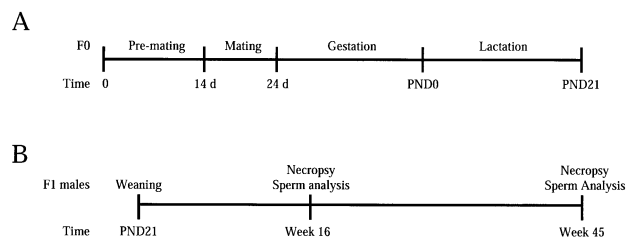


Fig. 1. Treatment schedule for F0 mice and necropsy schedule for F1 male offspring. A) F0 C57BL6 mice were dosed by gavage every other day with 0.1 ml of corn oil with or without Aroclor 1242 for a nominal dose of 0, 10, 25, 50 and 100 mg/kg of maternal body weight. F0 mice were treated for two-weeks prior to mating with a DBA/2. Treatment continued throughout a maximum ten-day mating period, and through gestation and lactation until PND 21 when the pups were weaned. B) Male offspring (B6D2F1) were weaned on PND 21 and housed with same-sex littermates. F1 males were split into two groups and necropsied at 16 or 45 weeks of age. Males were examined for testis weight, sperm count and motion analysis, and *in vitro* fertilizing ability (IVF).

room. All animals were given free access to deionized water and rodent feed (Harlen Teklad 22/5, Madison, WI).

### 2.2. Treatment

Fourteen-week-old C57BL6 female mice (F0) were randomly assigned to treatment groups, and treated for two weeks prior to mating, and throughout mating, gestation, and lactation until offspring were weaned on postnatal day (PND) 21 (Fig. 1A). Treatment was by gavage every other day with 0.1 ml corn oil (CPC International Inc., Englewood Cliffs, NJ) with or without Aroclor 1242 (S. Safe, Texas A&M, College Station, TX) for a nominal dose of 0 ( $n = 16$ ), 10 ( $n = 9$ ), 25 ( $n = 10$ ), 50 ( $n = 11$ ), and 100 ( $n = 11$ ) mg Aroclor 1242 per kg maternal body weight. There was a 1-day interruption of treatment on the day of parturition (PND 0). The dose of test chemical was adjusted to body weight for each mouse before daily dosing.

For mating, each female C57BL6 was paired for ten days with a DBA/2 proven breeder male mouse. Previous studies have shown that B6D2F1 pups are a suitable model system for *in vitro* fertilization (IVF) studies since B6D2F1 pups are very responsive to superovulation and control eggs exhibit high fertilization rates *in vitro* [10]. One or two females were housed per male per cage during the mating period. Females were housed individually for the duration of the study following the ten-day mating period. Females not conceiving or giving birth to live young were euthanized. Offspring were weaned on PND 21 and housed with same sex littermates. At this point, the F1 males from each litter were randomly split into two groups for assessment of sperm count and motion analysis and *in vitro* fertilizing ability (IVF) at either 16 or 45 weeks of age (Fig. 1B). F1 females were superovulated within a week of weaning and assessed for oocyte fertilizing ability *in vitro* (data not shown). The mothers were terminated on PND 21.

### 2.3. Reproduction and necropsy

F0 body weight was recorded daily and liver and thymus weights were recorded on PND 21. The number of live pups born was recorded on the day of birth (PND 0). Litter weight was recorded on PND 1. Pup survival and sex ratio for each litter were recorded on PND 21. On PND 21, body weights were recorded and male anogenital distance (AGD; the length of the perineum from the base of the sex papilla to the proximal end of the anal opening) was measured using vernier calipers to an accuracy of 0.8 mm. Body, liver, thymus, and testis weights of F1 mice were measured at necropsy at 16 and 45 weeks of age. In addition, randomly selected female F1 mice were sacrificed on PND 4 or 5 from the control ( $n = 1$ ), 50 ( $n = 4$ ), and 100 ( $n = 1$ ) mg/kg dose groups. Whole stomachs were collected and individually frozen at  $-20^{\circ}\text{C}$  in amber vials for subsequent PCB congener analysis.

### 2.4. HRGC/LRMS analysis of PCBs and chlorobiphenyls in pup stomach samples

Individual stomach samples, consisting of both stomach contents and tissue, were transferred to glass culture tubes containing 6 ml of concentrated hydrochloric acid. The tube was capped and sonicated for 20 min and allowed to stand overnight to complete the digestion. The resulting digestate was extracted 3 times with hexane and the extracts combined. For PCB analysis, aliquots of the hexane extracts were spiked with mass-labeled ( $^{13}\text{C}_{12}$ ) PCBs and then washed with concentrated sulfuric acid (3 ml) followed by HPLC-grade water (3 ml) and finally 0.1 M aqueous potassium carbonate (0.5 ml). The hexane extracts were then concentrated to 100  $\mu\text{l}$  with solvent exchange into nonane and analyzed by high resolution gas chromatography coupled with low resolution mass spectrometry (HRGC/LRMS) as described below. For analysis of chlorobiphenyls (OH-PCBs), aliquots of the hexane extracts were first spiked with mass-labeled ( $^{13}\text{C}_{12}$ ) OH-PCBs and then washed with HPLC-grade water (3 ml). The hexane extracts were then concentrated to 100  $\mu\text{l}$  with solvent exchange into toluene and analyzed by HRGC/LRMS as described below.

Analysis of PCBs in the stomach extracts was performed using a Hewlett Packard 5890 high resolution gas chromatograph (HRGC) coupled to a Hewlett Packard 5970 mass selective detector (MSD). The MSD was operated in the selected ion monitoring (SIM) mode. The ions monitored were those in the molecular ion clusters of mono- through decachlorobiphenyl and their  $^{13}\text{C}_{12}$  analogues. The capillary column used was a 30 m DB-5 column (J&W Scientific; 0.25 mm i.d., 0.25  $\mu\text{m}$  film thickness) with splitless injection ( $250^{\circ}\text{C}$ ). The HRGC oven temperature program was as follows: initial temperature =  $100^{\circ}\text{C}$ ; initial time = 10 min; temperature program =  $10^{\circ}\text{C}/\text{min}$ ; final temperature =  $320^{\circ}\text{C}$ ; final time = 4 min.

Analysis of OH-PCBs in the stomach extracts was per-

formed using a Hewlett Packard 5890 series II HRGC coupled to a VG 70SE high resolution mass spectrometer (HRMS). The HRMS was operated in the EI/SIR mode at 10000 resolution. The ions monitored were those in the molecular ion clusters of the native and mass-labeled OH-PCBs from dichloro to pentachlorobiphenyls. The capillary column used was a 60 m DB-5 column (J&W Scientific) with the injector port at  $250^{\circ}\text{C}$  and source temperature at  $300^{\circ}\text{C}$ . The HRGC oven temperature program was as follows: initial temperature =  $100^{\circ}\text{C}$ ; initial time = 7 min; temperature program =  $10^{\circ}\text{C}/\text{min}$ ; final temperature =  $320^{\circ}\text{C}$ ; final time = 11.2 min.

The data were corrected for recovery of the  $^{13}\text{C}_{12}$ -labelled PCB and OH-PCB surrogates. Recoveries of the  $^{13}\text{C}_{12}$ -labelled PCB surrogates ranged from 64% to 123%. Recoveries of the  $^{13}\text{C}_{12}$ -labelled OH-PCB surrogates ranged from 54% to 125%. A 4-point calibration curve was used with the native PCBs (71 individual congeners) ranging in concentration from 0.01 ng/ $\mu\text{L}$  to 0.25 ng/ $\mu\text{L}$ . The  $^{13}\text{C}_{12}$ -labelled PCB surrogates were at 0.125 ng/ $\mu\text{L}$  in each calibration solution. The PCB surrogates used were PCB 3, 15, 28, 52, 70, 77, 101, 105, 118, 126, 138, 153, 156, 167, 169, 170, 178, 180, 189, 194, 206, 209. For the OH-PCBs, a 4-point calibration curve was used containing one OH-PCB per congener group ( $\text{Cl}_2$ – $\text{Cl}_5$ ) and one  $^{13}\text{C}_{12}$ -labelled OH-PCB surrogate per congener group. The native OH-PCBs in the calibration solutions ranged from 5 pg/ $\mu\text{L}$  to 500pg/ $\mu\text{L}$  with the surrogates at 50 pg/ $\mu\text{L}$  in each calibration solution. The  $^{13}\text{C}_{12}$ -labelled OH-PCB surrogates used were 3',4'-dichloro-4-[ $^{13}\text{C}_{12}$ ]biphenylol, 2',4',5'-trichloro-4-[ $^{13}\text{C}_{12}$ ]biphenylol, 2',3',4',5'-tetrachloro-4-[ $^{13}\text{C}_{12}$ ]biphenylol, and 2',3',4',5',5'-pentachloro-4-[ $^{13}\text{C}_{12}$ ]biphenylol.

### 2.5. Sperm count and motion analysis

Cauda epididymal sperm were collected from F1 males at 16 and 45 weeks of age by excising both epididymides and piercing with a 25 gauge needle in a 1-ml organ culture dish (Becton Dickinson, Franklin Lakes, NJ) containing 1 ml Brinster's BMOC-3 medium (Gibco/BRL, Grand Island, NY), a capacitation supporting medium, supplemented with 3  $\mu\text{g}/\text{mL}$  penicillin and 3  $\mu\text{g}/\text{mL}$  streptomycin (Gibco/BRL). Sperm suspensions were incubated at  $37^{\circ}\text{C}$  in a humidified 5%  $\text{CO}_2$  air environment for 30 min before sperm concentration and motion analysis and 60 min before insemination (see below). Sperm suspensions (20  $\mu\text{l}$ ) were placed on a 20  $\mu\text{m}$  deep counting chamber and analyzed using a CellSoft computer-assisted digital image analysis system (CASA; CRYO Resources Inc.). A minimum of 100 cells were analyzed from each animal to determine concentration, motility, velocity, linearity, mean amplitude of lateral head (ALH) displacement and beat/cross frequency. Motility is expressed as the percentage of sperm that move faster than 20  $\mu\text{m}/\text{s}$ . Velocity is defined as the average distance ( $\mu\text{m}$ ) traveled by motile sperm in 1 s. Linearity is the ratio of the straight to actual distance traveled, averaged

over all sperm. ALH displacement is a measure of the lateral movement of the sperm head from a computer-calculated mean of its track. The beat/cross frequency (Hz) is the numbers of beats (or crosses) per second. Every time the sperm cell crosses the computer-calculated curval mean, the computer counts that crossing as one beat. All measurements for each animal were performed in duplicates and the average recorded.

### 2.6. *In vitro* fertilization assay

The *in vitro* fertilizing ability of sperm from 16- and 45-week-old F1 male mice was assessed by inseminating oocytes collected from untreated 3-week-old B6D2F1 female mice that were superovulated with 10 IU pregnant mare's serum gonadotropin (PMSG) (Sigma, St. Louis, MO) followed 48 h later with 10 IU human chorionic gonadotropin (HCG; Sigma). Fourteen to 16 h later the oocytes from each female were collected from the proximal oviducts. Oocytes from each mouse were incubated in a 1-ml organ culture dish in Brinster's BMOC-3 medium supplemented with penicillin and streptomycin. Epididymal sperm from each F1 male was used to inseminate oocytes from two females with a final sperm concentration of  $3 \times 10^4$  sperm per dish. This concentration of sperm achieves slightly less than maximum fertilization in naive B6D2F1 mice, thus increasing the assay sensitivity in detecting changes in sperm fertilizing ability (unpublished data). Due to practical considerations, it was not possible to pool and distribute oocytes from all females to inseminate a fixed number of oocytes per male, since oocytes were contained with a cumulus mass and oocyte collection was coordinated with sperm collection and CASA analysis for consistency among inseminations. Following insemination, oocytes were incubated at 37°C under a humidified 5% CO<sub>2</sub> environment. Following a 24 h incubation, 50 µl of 35 µM bisbenzimidazole stain (Sigma) was added to the petri dish. The oocytes were incubated with stain for at least 30 min before being examined using a Nikon Optophot fluorescent microscope equipped with a 100-W mercury bulb, 365/10 nm excitation filter, 400 nm dichromic mirror and 400 nm barrier filter. Oocytes were counted and scored as fertilized if the eggs were at the 2-cell stage or at the 1-cell stage containing two pronuclei and a second polar body. Oocytes were also evaluated for fragmentation and degeneration.

### 2.7. Statistical analysis

All data analysis was performed using SAS version 7 (SAS Inc., Cary, NC). For the analysis of F1 data, the litter was considered the experimental unit. F0 data and F1 litter mean data were analyzed for normality using the Shapiro-Wilk test. Evidence for non-normality was declared at the  $P < 0.01$  level of significance. Non-normal data were analyzed by nonparametric one-way ANOVA using the NPAR1WAY procedure of SAS. Comparisons between

control and treated groups were made with the Kruskal-Wallis test. Normal data were analyzed using a one-way ANOVA as implemented in the MIXED procedure of SAS containing fixed effect of dose. When body and organ weights and anogenital distance of F1 mice were analyzed, litter size was included as a covariate. Organ weights were analyzed as both absolute weight and as a percentage of body weight. AGD was analyzed as absolute length and as a ratio to the cube root of body weight [13]. Comparisons between treatment groups were computed on least squares means. The effect of treatment on discrete data (fecundity, sperm IVF data) was analyzed using Generalized Estimating Equations as implemented in the GENMOD procedure of SAS. The level of significance was  $P < 0.05$ . *P* values less 0.1 are also reported.

## 3. Results

### 3.1. Analysis of lactational exposure to PCBs and chlorobiphenylol metabolites

The stomachs of 4 randomly selected F1 female mice in the 50 mg/kg dose group were dissected at 4 or 5 days of age and analyzed for PCBs, as well as di-, tri-, tetra-, and pentachlorobiphenylols (OH-PCBs) by HRGC/LRMS (Table 1–3). One stomach was also dissected from an F1 female in the corn oil control group to determine the level of background PCB and chlorobiphenylol contamination. Seventy individual PCB congeners and total PCBs were quantitated and compared to the congener and total PCB profile of Aroclor 1242 in order to estimate the level of lactational PCB exposure and to determine if the congener profile of PCBs was altered through maternal metabolism and lactational transfer. The 50-mg/kg dose group was selected in order to ensure detectable levels of PCBs and OH-PCBs in the stomach samples. Detection limits and quantitation of the 70 congeners was based on the height of individual peaks exceeding a 3:1 signal to noise threshold. Total PCBs and subtotals for each structural class were calculated from an integration of the chromatograph, rather than by summation of the 70 individual congeners that were examined (Table 1). The average total PCB content of the stomach samples from the 50-mg/kg dose group was  $8659 \pm 3312$  ng/g. Assuming that the milk made up approximately 90% of the weight of the stomach sample and nursing pups consume an estimated 0.5 g of milk per day (0.25 g milk/g body weight), it is estimated that the nursing pups were exposed to 0.24, 0.6, 1.2, and 2.4 mg/kg/day total PCBs in the 10, 25, 50, and 100 mg/kg dose groups, respectively. These estimates are based on linear extrapolations from the 50 mg/kg group. Indeed, analysis of total PCB concentration in one stomach sample from the 100 mg/kg dose group was approximately twice that of the 50 mg/kg sample (data not shown) and there were no detectable PCBs in the corn

Table 1  
Congener profile of Aroclor 1242 and stomach samples from mouse offspring nursing from Aroclor 1242-treated mothers in the 50 mg/kg/2-d dose group

| IUPAC # <sup>a</sup>  | Structure                     | Retention Time (min) | Aroclor 1242 (ng/g Aroclor) | 50 mg/kg/2-d <sup>b</sup> (ng/g stomach) |
|-----------------------|-------------------------------|----------------------|-----------------------------|--|
|                       | Monochlorobiphenyls           |                      |                             |  |
| 1                     | 2                             | 16.594               | 1.3                         | ND (4)                                   |
| 3                     | 4                             | 17.882               | 0.56                        | ND (4)                                   |
| Subtotal <sup>c</sup> |                               |                      | 1.98                        | ND (4)                                   |
|                       | Dichlorobiphenyls             |                      |                             |  |
| 4/10                  | 2,2'/2,6                      | 18.511               | 3.1                         | ND (4)                                   |
| 8                     | 2,4'                          | 19.641               | 9.0                         | 82 ± 3 (2)                               |
| 15                    | 4,4'                          | 20.763               | 2.9                         | ND (4)                                   |
| Subtotal              |                               |                      | 13.0                        | 82 ± 3 (2)                               |
|                       | Trichlorobiphenyls            |                      |                             |  |
| 16                    | 2,2',3                        | 21.161               | 7.7                         | ND (4)                                   |
| 18                    | 2,2',5                        | 20.712               | 13                          | ND (4)                                   |
| 19                    | 2,2',6                        | 20.153               | 0.95                        | ND (4)                                   |
| 22                    | 2,3,4'                        | 22.187               | 3.4                         | ND (4)                                   |
| 28                    | 2,4,4'                        | 21.799               | 16                          | 1800 ± 658 (4)                           |
| 33                    | 2',3,4                        | 22.015               | 7.1                         | ND (4)                                   |
| 37                    | 3,4,4'                        | 23.092               | 2.8                         | ND (4)                                   |
| Subtotal              |                               |                      | 56                          | 1800 ± 658 (4)                           |
|                       | Tetrachlorobiphenyls          |                      |                             |  |
| 44                    | 2,2',3,5'                     | 23.013               | 4.4                         | 235 ± 126 (4)                            |
| 49                    | 2,2',4,5'                     | 22.663               | 3.9                         | 355 ± 152 (4)                            |
| 52                    | 2,2',5,5'                     | 22.564               | 4.4                         | 748 ± 317 (4)                            |
| 54                    | 2,2',6,6'                     | 21.479               | ND                          | ND (4)                                   |
| 56                    | 2,3,3',4                      | 24.263               | 3.3                         | 510 ± 216 (4)                            |
| 64                    | 2,3,4',6                      | 23.283               | 4                           | 109 ± 65 (4)                             |
| 66                    | 2,3',4,4'                     | 23.916               | 4                           | 1473 ± 717 (4)                           |
| 70                    | 2,3',4',5                     | 23.835               | 4.6                         | 157 ± 90 (3)                             |
| 74                    | 2,4,4',5                      | 23.765               | 3.2                         | 1103 ± 503 (4)                           |
| 77                    | 3,3',4,4'                     | 25.148               | 0.44                        | 27 ± (1)                                 |
| 81                    | 3,4,4',5                      | 24.952               | ND                          | ND (4)                                   |
| Subtotal              |                               |                      | 35                          | 4550 ± 1907 (4)                          |
|                       | Pentachlorobiphenyls          |                      |                             |  |
| 87                    | 2,2',3,4,5'                   | 24.946               | 0.71                        | 122 ± 47 (4)                             |
| 95                    | 2,2',3,5',6                   | 23.933               | 0.94                        | 70 ± 19 (3)                              |
| 96                    | 2,2',3,6,6'                   | 23.388               | ND                          | ND (4)                                   |
| 99                    | 2,2',4,4',5                   | 24.512               | 0.62                        | 458 ± 202 (4)                            |
| 101                   | 2,2',4,5,5'                   | 24.4                 | 1.3                         | 81 ± 27 (4)                              |
| 104                   | 2,2',4,6,6'                   | 22.93                | ND                          | ND (4)                                   |
| 105                   | 2,3,3',4,4'                   | 26.186               | 0.50                        | 455 ± 159 (4)                            |
| 110                   | 2,3,3',4',6                   | 25.145               | 0.99                        | 45 ± 14 (4)                              |
| 114                   | 2,3,4,4',5                    | 25.89                | 0.08                        | 47 ± 3 (3)                               |
| 118                   | 2,3',4,4',5                   | 25.657               | 0.84                        | 668 ± 247 (4)                            |
| 119                   | 2,3',4,4',6                   | 24.647               | ND                          | ND (4)                                   |
| 123                   | 2',3,4,4',5                   | 25.616               | ND                          | 15 (1)                                   |
| 126                   | 3,3',4,4',5                   | 26.809               | ND                          | ND (4)                                   |
| Subtotal              |                               |                      | 5.3                         | 1919 ± 721 (4)                           |
|                       | Hexachlorobiphenyls           |                      |                             |  |
| 128/167               | 2,2',3,3',4,4'/2,3',4,4',5,5' | 27.174               | 0.04                        | 26 ± 7 (3)                               |
| 138                   | 2,2',3,4,4',5'                | 26.641               | 0.14                        | 103 ± 29 (4)                             |
| 149                   | 2,2',3,4',5',6                | 25.628               | 0.10                        | ND (4)                                   |
| 151                   | 2,2',3,5,5',6                 | 25.395               | 0.03                        | ND (4)                                   |
| 153                   | 2,2',4,4',5,5'                | 26.098               | 0.07                        | 74 ± 21 (4)                              |
| 155                   | 2,2',4,4',6,6'                | 24.221               | ND                          | ND (4)                                   |
| 156                   | 2,3,3',4,4',5                 | 27.615               | 0.01                        | 15 ± 5 (3)                               |
| 157                   | 2,3,3',4,4',5                 | 27.725               | 0.01                        | ND (4)                                   |
| 158                   | 2,3,3',4,4',6                 | 26.691               | ND                          | ND (4)                                   |
| 168                   | 2,3',4,4',5',6                | 26.156               | 0.05                        | ND (4)                                   |
| 169                   | 3,3',4,4',5,5'                | 28.318               | ND                          | ND (4)                                   |
| Subtotal              |                               |                      | 0.51                        | 208 ± 72 (4)                             |

(Continued)

Table 1

Congener profile of Aroclor 1242 and stomach samples from mouse offspring nursing from Aroclor 1242-treated mothers in the 50 mg/kg/2-d dose group

| IUPAC # <sup>a</sup> | Structure                | Retention Time (min) | Aroclor 1242 (ng/g Aroclor) | 50 mg/kg/2-d <sup>b</sup> (ng/g stomach) |
|----------------------|--------------------------|----------------------|-----------------------------|--|
| Heptachlorobiphenyls |                          |                      |                             |  |
| 170                  | 2,2',3,3',4,4',5         | 28.483               | 0.01                        | ND (4)                                   |
| 171                  | 2,2',3,3',4,4',6         | 27.607               | 0.01                        | ND (4)                                   |
| 174                  | 2,2',3,3',4,5,6'         | 27.413               | 0.01                        | ND (4)                                   |
| 177                  | 2,2',3,3',4',5,6         | 27.519               | 0.01                        | ND (4)                                   |
| 178                  | 2,2',3,3',5,5',6         | 26.804               | ND                          | ND (4)                                   |
| 179                  | 2,2',3,3',5,6,6'         | 26.404               | ND                          | ND (4)                                   |
| 180                  | 2,2',3,4,4',5,5'         | 27.924               | 0.01                        | 14.5 ± 1 (2)                             |
| 183                  | 2,2',3,4,4',5',6         | 27.061               | 0.01                        | ND (4)                                   |
| 187                  | 2,2',3,4',5,6,6'         | 26.966               | 0.01                        | ND (4)                                   |
| 188                  | 2,2',3,4',5,6,6'         | 25.987               | ND                          | ND (4)                                   |
| 189                  | 2,3,3',4,4',5,5'         | 29.003               | ND                          | ND (4)                                   |
| 191                  | 2,3,3',4,4',5',6         | 28.058               | ND                          | ND (4)                                   |
| Subtotal             |                          |                      | 0.05                        | 14.5 ± 1 (2)                             |
| Octachlorobiphenyls  |                          |                      |                             |  |
| 194                  | 2,2',3,3',4,4',5,5'      | 29.643               | ND                          | ND (4)                                   |
| 195                  | 2,2',3,3',4,4',5,6       | 29.303               | ND                          | ND (4)                                   |
| 199                  | 2,2',3,3',4,5,5',6'      | 28.644               | 0.01                        | ND (4)                                   |
| 200                  | 2,2',3,3',4,5,6,6'       | 28.18                | ND                          | ND (4)                                   |
| 201                  | 2,2',3,3',4,5',6,6'      | 27.737               | ND                          | ND (4)                                   |
| 202                  | 2,2',3,3',5,5',6,6'      | 27.589               | ND                          | ND (4)                                   |
| 203                  | 2,2',3,4,4',5,5',6       | 28.743               | ND                          | ND (4)                                   |
| 205                  | 2,3,3',4,4',5,5',6       | 29.752               | ND                          | ND (4)                                   |
| Subtotal             |                          |                      | 0.01                        | ND (4)                                   |
| Nonachlorobiphenyls  |                          |                      |                             |  |
| 206                  | 2,2',3,3',4,4',5,5',6    | 29.278               | ND                          | ND (4)                                   |
| 208                  | 2,2',3,3',4,5,5',6,6'    | 30.328               | ND                          | ND (4)                                   |
| Subtotal             |                          |                      | ND                          | ND (4)                                   |
| Decachlorobiphenyls  |                          |                      |                             |  |
| 209                  | 2,2',3,3',4,4',5,5',6,6' | 30.88                | ND                          | ND (4)                                   |
| Total                |                          |                      |                             | 8659 ± 3312 (4)                          |

<sup>a</sup> IUPAC number based on Ballschmiter et al. [28] and revised by Guitart et al. [29].

<sup>b</sup> Based on average of 4 stomach samples collected from 4 or 5 day old pups. Values in mean ± SD (detected in n samples).

<sup>c</sup> Total PCBs and subtotal concentrations are based on integration of the chromatograph and not on the summation of the individual congeners listed.

ND - non detectable. Detection limits and quantitation of the 70 congeners was based on the height of individual peaks exceeding a 3:1 signal to noise threshold.

oil control stomach sample. The limit of detection for the corn oil control sample ranged from 3 to 20 ng/g.

There was very little di-CBs and no detectable mono-CBs in the stomach samples, which is similar to human breast milk [14]. The detection limit for the stomach samples ranged from 5 to 20 ng/g for the mono-CBs and 10 to 50 ng/g for the di-CBs. The total amount of each PCB structural class was calculated as a percentage of the total PCBs in the sample based on the average congener concentrations in Table 1. The relative proportion of structural classes of PCBs within the stomach samples was slightly different than of the parent Aroclor mixture and of PCBs found in human breast milk (Table 2; Ref. 12). For example, the predominant structural class of PCBs in the parent mixture was 50.9% tri-CBs, whereas the stomach sample consisted of 20.8% tri-CBs, which more closely resembles the percent of tri-CBs in human breast milk (14.6%) [14]. The predominant structural class of PCBs in the stomach sample was tetra-CBs (55.1%), followed by penta (22.2%)

and tri-CBs (20.7%). There was considerably less di-CBs in the stomach sample (0.9%) in comparison to the parent mixture (11.8%). Both the stomach samples and parent mixture had relatively low to no detectable levels of hexa- to deca-CBs. In contrast, the human breast milk sample consisted of primarily hexa-CBs (34%) and roughly equivalent amounts of tri-, tetra-, penta-, and hepta-CBs (14.6 to 18.9%). Human breast milk also had no detectable mono- or di-CBs [14].

The relative abundance of OH-PCBs parallels the relative abundance of the non-hydroxylated congeners, however the total amount of OH-PCBs represents only about 5% of the total PCBs + OH-PCBs in the 50 mg/kg sample (Table 3). The predominate OH-PCBs were tri- and tetra-chlorobiphenyls, which represented at least 74% of the total OH-PCBs detected. Penta-CBs represented about 20% of the total OH-PCBs in the stomach samples. The identities of the OH-PCBs were not determined, however, there were on average 33.8 ± 10 different OH-PCBs species per sam-

Table 2

Percent abundance of PCB congeners detected (based on ng PCB/g sample) within each structural class in Aroclor 1242 as a fraction of total PCBs in Aroclor 1242, stomach samples from mouse pups nursing on Aroclor 1242-treated mothers in the 50 mg/kg dose group, and human breast milk

| PCB Structural Class | Aroclor 1242 | 50 mg/kg <sup>a</sup> | Human Breast Milk <sup>b</sup> |
|----------------------|--------------|-----------------------|--------------------------------|
| Mono                 | 1.8          | ND                    | ND                             |
| Di                   | 11.8         | 0.9                   | ND                             |
| Tri                  | 50.9         | 20.8                  | 14.6                           |
| Tetra                | 31.8         | 55.1                  | 18.9                           |
| Penta                | 4.8          | 22.2                  | 15.3                           |
| Hexa                 | <1.0         | 2.4                   | 34.4                           |
| Hepta                | <0.1         | 0.2                   | 18.8                           |
| Octa                 | <0.1         | ND                    | 3.0                            |
| Nona                 | ND           | ND                    | 0.2                            |
| Deca                 | ND           | ND                    | 0.1                            |

<sup>a</sup> Based on average of 4 stomach samples collected from 4 or 5-d old pups.

<sup>b</sup> From Safe et al. [12].

ND - non detectable.

ple based on the number of unique peaks identified in the chromatograms. The majority of the peaks were found to be tri- ( $14 \pm 3.9$ ) and tetra-OH-CBs ( $16.8 \pm 5.2$  peaks).

### 3.2. Reproductive performance of F0 generation

Exposure of F0 female C57BL6 mice to Aroclor 1242 prior to mating and through gestation and lactation did not significantly affect body weight gain or liver weight (Table 4). Although body weights in the 25 mg/kg dose group were significantly different ( $P < 0.05$ ) from control mice prior to dosing, the difference was not apparent by the mating period. There was a decrease ( $P = 0.0523$ ) in average body weight in the 50 mg/kg dose group on PND 21, however weight gain was not affected. There was approximately a 50% increase ( $P < 0.05$ ) in thymus weight and percent thymus weight in the 25 mg/kg maternal dose group ( $P < 0.05$ ) on PND 21. There were no other significant changes in thymus weight in the PCB-treated groups.

Only 5 of 11 (45.5%) females in the 100 mg/kg dose group gave birth to live young. Due to the low fecundity in the control group (10/16, 62.5%), there was no significant

difference in fecundity when compared to controls ( $P > 0.1$ ). However, when fecundity in the 100 mg/kg dose group was compared to the 10 mg/kg dose group, the decrease in fecundity was significant ( $P < 0.05$ ). Four mothers in the 100 mg/kg dose group showed no evidence of conception, while one mother appeared pregnant based on weight gain on day 16 after the initial pairing with a male, there was no parturition. However, uteri from these mice were not examined for implantation scars so it is unclear if conception had occurred. One mother in the 100 mg/kg group gave birth to stillborn pups. Two of three pups in one litter died on PND 1, while the third pup died on PND 6. The remaining 4 litters survived to PND 21; however, one mother died on PND 20 from unknown causes and the pups were subsequently removed from the study. Due to the small number of litters remaining in the 100 mg/kg dose group, no conclusions regarding treatment-related effects in F1 males could be determined with confidence, although the results are presented.

There was no significant difference between treatment groups in the number of days to parturition from the initiation of pairing with a male ( $P > 0.1$ ). There was a decrease in litter size ( $6.0 \pm 1.1$  vs  $7.6 \pm 0.3$ ,  $P < 0.01$ ) in the 100 mg/kg dose group, although no difference in litter weight was detected ( $P > 0.1$ ). There was a small decrease in average pup weight ( $P = 0.077$ ). There was no significant differences in pup survival, however there was a smaller proportion of females in the 100 mg/kg dose group when compared to controls ( $P < 0.01$ ). Due to the small sample size and the unknown sex of the pups that died on PND 1, the true sex ratio is unknown. There was no postweaning F1 mortality throughout the study.

### 3.3. Developmental effects on F1 mice

There were no significant effects on body weight, AGD, or AGD:cube root body weight ratio on PND 21; however there was a slight increase ( $P = 0.092$ ) in average AGD:cube root body weight ratio in the 25 mg/kg dose group (Table 5). There was no effects on body, liver, thymus, or testis weight at 16 or 45 weeks of age ( $P > 0.1$ , Table 5).

Table 3

Chlorobiphenylols in stomach samples from pups nursing from Aroclor 1242-treated mothers in the 50 mg/kg/2-d dose group. Values in ng OH-PCB/g stomach  $\pm$  SD (detected in n samples) [% of total]

| OH-PCB                            | 50 mg/kg/2 day <sup>a</sup> | Average number of peaks |
|-----------------------------------|-----------------------------|-------------------------|
| Total Dichlorobiphenylol          | $37 \pm 34$ (2) [7.1%]      | $2.0 \pm 1.4$ (2)       |
| Total Trichlorobiphenylol         | $199 \pm 118$ (4) [38%]     | $14 \pm 3.9$ (4)        |
| Total Tetrachlorobiphenylol       | $197 \pm 109$ (4) [37.7%]   | $16.8 \pm 5.2$ (4)      |
| Total Pentachlorobiphenylol       | $45 \pm 17$ (3) [17.2%]     | $2.7 \pm 0.6$ (3)       |
| Total Di to Pentachlorobiphenylol | $448 \pm 237$ (4) [100%]    | $33.8 \pm 10$ (4)       |

<sup>a</sup> Based on average of 4 stomach samples collected from 4 or 5 day old pups.

Table 4  
Body and organ weights and reproductive performance of F0 female C57BL6 mice treated with Aroclor 1242 through gestation and lactation<sup>a</sup>

|                                  | Dose of Aroclor 1242 (mg/kg/2-d) |                   |                    |                   |                    |
|----------------------------------|----------------------------------|-------------------|--------------------|-------------------|--------------------|
|                                  | 0                                | 10                | 25                 | 50                | 100                |
| Body weight, g                   |                                  |                   |                    |                   |                    |
| Predosing                        | 20.74 ± 0.41 (10)                | 19.76 ± 0.40 (8)* | 22.02 ± 0.09 (7)** | 20.59 ± 0.39 (8)  | 20.93 ± 0.33 (7)   |
| Premating                        | 21.73 ± 0.23 (10)                | 21.25 ± 0.36 (8)  | 21.74 ± 0.20 (7)   | 21.64 ± 0.39 (8)  | 21.76 ± 0.27 (7)   |
| PND 21                           | 30.93 ± 0.56 (9)                 | 29.68 ± 0.77 (8)  | 30.56 ± 0.62 (7)   | 28.96 ± 0.93 (7)* | 30.27 ± 0.33 (3)   |
| Weight gain <sup>b</sup>         | 10.17 ± 0.93 (9)                 | 9.90 ± 1.01 (8)   | 8.42 ± 0.64 (7)    | 8.27 ± 0.82 (7)   | 9.36 ± 0.58 (3)    |
| Liver weight, g                  | 2.22 ± 0.12 (9)                  | 2.16 ± 0.11 (8)   | 2.33 ± 0.13 (7)    | 2.17 ± 0.08 (7)   | 2.35 ± 0.04 (3)    |
| Liver:body weight ratio*100      | 7.16 ± 0.35 (9)                  | 7.27 ± 0.24 (9)   | 7.64 ± 0.35 (7)    | 7.49 ± 0.21 (7)   | 7.76 ± 0.22 (3)    |
| Thymus weight, mg                | 30.83 ± 4.70 (9)                 | 34.23 ± 5.88 (8)  | 46.89 ± 4.11 (7)** | 31.69 ± 5.46 (7)  | 29.43 ± 6.35 (3)   |
| Thymus:body weight ratio*100     | 0.10 ± 0.01 (9)                  | 0.12 ± 0.02 (8)   | 0.15 ± 0.01 (7)**  | 0.11 ± 0.02 (7)   | 0.10 ± 0.02 (3)    |
| Fecundity                        |                                  |                   |                    |                   |                    |
| # of breeding pairs              | 16                               | 9                 | 10                 | 11                | 11                 |
| # with live births               | 10                               | 8                 | 7                  | 8                 | 5                  |
| % Fecundity                      | 62.5                             | 88.9              | 70.0               | 72.7              | 45.5 <sup>c</sup>  |
| Days to parturition <sup>c</sup> | 21.6 ± 1.8 (10)                  | 21.0 ± 1.4 (8)    | 20.7 ± 1.1 (7)     | 20.9 ± 1.1 (7)    | 21.2 ± 1.1 (5)     |
| Litter size on PND 0             | 7.6 ± 0.3 (10)                   | 7.5 ± 0.6 (8)     | 7.9 ± 0.4 (7)      | 8.1 ± 0.6 (8)     | 6.0 ± 1.1 (5)*     |
| Litter weight on PND 1, g        | 12.17 ± 0.48 (10)                | 12.17 ± 1.66 (8)  | 11.29 ± 0.49 (7)   | 13.24 ± 0.94 (7)  | 10.45 ± 1.27 (4)   |
| Average pup weight on PND 1, g   | 1.60 ± 0.02 (10)                 | 1.62 ± 0.08 (8)   | 1.45 ± 0.08 (7)*   | 1.54 ± 0.05 (7)   | 1.54 ± 0.10 (4)    |
| Sex ratio, % male                | 0.44 ± 0.04 (10)                 | 0.51 ± 0.06 (8)   | 0.55 ± 0.09 (7)    | 0.44 ± 0.06 (7)   | 0.71 ± 0.04 (4)*** |
| Survival <sup>d</sup>            | 90 ± 10 (10)                     | 100 ± 0 (8)       | 91.2 ± 3.6 (7)     | 84.5 ± 12.2 (8)   | 80.0 ± 20.0 (5)    |

<sup>a</sup> F0 female C57BL6 mice were dosed orally every other day for two weeks prior to being paired with an untreated male DBA/2 and were continually dosed every other day until postnatal day (PND) 21.

<sup>b</sup> Difference between initial body weight and body weight on postnatal day 21.

<sup>c</sup> Average number of days between pairing of female with male and parturition.

<sup>d</sup> (Number of pups alive on PND 21/number of pups alive on PND 0) × 100, %

<sup>e</sup> Significantly less ( $P < 0.0347$ ) than 10 mg/kg dose group.

All values are means ± SEM of (n) mice. Different from control group at the following level of significance. \* $0.1 < P < 0.05$ ; \*\* $0.05 < P < 0.01$ ; \*\*\* $P < 0.01$ .

### 3.4. Sperm count and motion analysis

Cauda epididymal sperm from F1 males were analyzed on a computer-assisted sperm analysis system for sperm count and various motion parameters (Table 6). Comparison of the sperm counts among 16 week old F1 mice indicated that mice from the 25 mg/kg maternal dose group had a 36% greater average sperm count that was close to statistical significance ( $P = 0.064$ ). Sperm count was not affected in other treatment groups ( $P > 0.1$ ). There was also a significant increase in sperm velocity ( $P < 0.05$ ) and linearity ( $P < 0.01$ ) in the 25 mg/kg dose group. All other motion parameters were unaffected by PCB exposure except for an increase ( $P = 0.098$ ) in sperm linearity in the 50 mg/kg dose group. By 45 weeks of age, average sperm count of F1 mice in the 25 mg/kg dose group was similar to that of control mice ( $P > 0.1$ ). Again, the other dose groups were not affected. There were no significant effects on sperm motion parameters except for an increase in average ALH displacement in the 10 ( $P = 0.067$ ) and 50 mg/kg ( $P = 0.090$ ) dose groups.

### 3.5. In vitro fertilizing ability

Oocytes from untreated female B6D2F1 mice were inseminated in vitro with cauda epididymal sperm (30,000

sperm cells/mL) from F1 males and evaluated for fertilization 24 h later. Sperm from PCB-exposed F1 mice fertilized significantly ( $P < 0.001$ ) fewer eggs than sperm from control F1 mice at both 16 and 45 weeks of age, with the exception of the 50 mg/kg dose group at 16 weeks of age (Table 7). At 16 weeks of age, sperm from the 10, 25, and 100 mg/kg maternal dose groups fertilized 10%, 29%, and 15% fewer oocytes. At 45 weeks of age, sperm from the 10, 25, 50, and 100 mg/kg maternal dose group fertilized 14%, 28%, 17%, and 27% fewer oocytes than control sperm, respectively. There was a significant ( $P < 0.05$ ) decrease in the percent of fragmented oocytes in the 50 mg/kg dose group at 16 weeks of age and in the 25 and 50 mg/kg dose group at 45 weeks of age, however, the magnitude of effects were small. There was a significant increase ( $P < 0.05$ ) in the percent of 1 cell fertilized eggs in the 10 mg/kg dose group at 16 weeks of age. There was no difference in the percent of 1 cell fertilized eggs at 45 weeks of age (Table 7).

## 4. Discussion

Due to the ability of Aroclor 1242 to disrupt normal signaling of both estrogen and thyroid hormones in vivo (reviewed in Ref. 2), we hypothesized that Aroclor 1242 may impact spermatogenesis and reproductive development

Table 5

Body and organ weights and anogenital distance (AGD) of B6D2-F1 male offspring from dams exposed to Aroclor 1242 from pre-mating through lactation

| Male   | Dose of Aroclor 1242 (mg/kg/2-d) |                  |                  |                  |                   |
|--|----------------------------------|------------------|------------------|------------------|-------------------|
|  | 0                                | 10               | 25               | 50               | 100 <sup>a</sup>  |
| AGD, mm  | 6.56 ± 0.29 (9)                  | 6.31 ± 0.44 (8)  | 6.99 ± 0.23 (7)  | 6.33 ± 0.35 (6)  | 7.67 ± 0.53 (3)   |
| AGD:cube root body weight ratio, mm/g <sup>1/3</sup> | 3.29 ± 0.11 (9)                  | 3.19 ± 0.17 (8)  | 3.52 ± 0.10 (7)* | 3.22 ± 0.14 (6)  | 3.66 ± 0.18 (3)   |
| Body weight, g                                       |                                  |                  |                  |                  |                   |
| PND 21   | 7.94 ± 0.39 (9)                  | 7.66 ± 0.49 (8)  | 7.81 ± 0.20 (7)  | 7.55 ± 0.30 (6)  | 9.20 ± 0.69 (3)   |
| 16 weeks   | 27.24 ± 0.59 (8)                 | 28.24 ± 0.80 (8) | 28.19 ± 1.05 (7) | 27.52 ± 0.49 (7) | 28.72 ± 1.21 (3)  |
| 45 weeks   | 40.60 ± 1.18 (8)                 | 40.82 ± 1.35 (8) | 42.65 ± 0.32 (6) | 39.70 ± 1.75 (6) | 43.26 ± 2.64 (3)  |
| Liver weight, g                                      |                                  |                  |                  |                  |                   |
| 16 weeks   | 1.50 ± 0.08 (8)                  | 1.48 ± 0.04 (8)  | 1.48 ± 0.07 (7)  | 1.42 ± 0.06 (7)  | 1.47 ± 0.19 (3)   |
| 45 weeks   | 1.82 ± 0.05 (8)                  | 1.92 ± 0.07 (8)  | 1.85 ± 0.02 (6)  | 1.88 ± 0.08 (6)  | 1.98 ± 0.19 (3)   |
| Liver:body weight ratio*100                          |                                  |                  |                  |                  |                   |
| 16 weeks   | 5.49 ± 0.24 (8)                  | 5.24 ± 0.14 (8)  | 5.43 ± 0.29 (7)  | 5.14 ± 0.13 (7)* | 5.08 ± 0.49 (3)   |
| 45 weeks   | 4.51 ± 0.12 (8)                  | 4.72 ± 0.11 (8)  | 4.33 ± 0.06 (6)  | 4.79 ± 0.10 (6)  | 4.57 ± 0.38 (3)   |
| Thymus weight, mg                                    |                                  |                  |                  |                  |                   |
| 16 weeks   | 37.2 ± 2.9 (9)                   | 36.8 ± 3.8 (8)   | 41.1 ± 1.1 (7)   | 36.8 ± 2.3 (7)   | 19.6 ± 9.1 (3)**  |
| 45 weeks   | 43.1 ± 3.7 (8)                   | 42.0 ± 4.2 (8)   | 48.1 ± 2.0 (6)   | 46.7 ± 4.4 (6)   | 50.5 ± 9.3 (3)    |
| Thymus:body weight ratio*100                         |                                  |                  |                  |                  |                   |
| 16 weeks   | 0.14 ± 0.01 (9)                  | 0.13 ± 0.01 (8)  | 0.15 ± 0.004 (7) | 0.13 ± 0.1 (7)   | 0.07 ± 0.03 (3)** |
| 45 weeks   | 0.11 ± 0.01 (8)                  | 0.10 ± 0.01 (6)  | 0.11 ± 0.004 (6) | 0.12 ± 0.01 (6)  | 0.12 ± 0.02 (3)   |
| Testes weight, mg                                    |                                  |                  |                  |                  |                   |
| 16 weeks   | 204.9 ± 8.9 (8)                  | 204.3 ± 7.3 (8)  | 213.4 ± 12.4 (7) | 220.1 ± 5.6 (7)  | 216.2 ± 6.4 (3)   |
| 45 weeks   | 222.1 ± 6.5 (8)                  | 221.1 ± 8.8 (8)  | 207.6 ± 13.4 (6) | 220.3 ± 10.0 (6) | 251.3 ± 10.4 (3)  |
| Testes:body weight ratio*100                         |                                  |                  |                  |                  |                   |
| 16 weeks   | 0.75 ± 0.02 (8)                  | 0.73 ± 0.03 (8)  | 0.78 ± 0.03 (7)  | 0.80 ± 0.02 (7)  | 0.75 ± 0.02 (3)   |
| 45 weeks   | 0.55 ± 0.02 (8)                  | 0.54 ± 0.02 (8)  | 0.49 ± 0.03 (6)  | 0.57 ± 0.03 (6)  | 0.58 ± 0.01 (3)   |

<sup>a</sup> Due to the small number of litters remaining in the 100 mg/kg dose group, no conclusions regarding treatment-related effects in F1 males could be determined with certainty, although the results are presented.

All values are means ± SEM of (n) litters. Different from control group at the following level of significance. \*0.1 < P < 0.05; \*\*0.05 < P < 0.01; \*\*\*P < 0.01.

Table 6

Epididymal sperm count and sperm motion of 16 and 45 week old F1 male offspring from dams exposed to Aroclor 1242 from pre-mating through lactation

|                                    | Dose of Aroclor 1242 (mg/kg/2-d) |                   |                     |                   |                    |
|------------------------------------|----------------------------------|-------------------|---------------------|-------------------|--------------------|
|                                    | 0                                | 10                | 25                  | 50                | 100 <sup>a</sup>   |
| 16 week old                        |                                  |                   |                     |                   |                    |
| Sperm count, × 10 <sup>6</sup> /mL | 36.56 ± 4.67 (8)                 | 32.27 ± 3.92 (8)  | 49.74 ± 4.44 (7)*   | 41.86 ± 8.82 (7)  | 38.99 ± 5.35 (3)   |
| Motility, % <sup>b</sup>           | 69.3 ± 4.5 (8)                   | 63.7 ± 3.6 (8)    | 71.1 ± 2.5 (7)      | 70.7 ± 4.3 (7)    | 69.5 ± 3.6 (3)     |
| Velocity, μm/s                     | 131.19 ± 5.39 (8)                | 127.25 ± 3.91 (8) | 145.95 ± 2.85 (7)** | 130.72 ± 5.47 (7) | 133.01 ± 5.99 (3)  |
| Linearity                          | 4.84 ± 0.13 (8)                  | 4.84 ± 0.17 (8)   | 5.56 ± 0.17 (8)***  | 5.17 ± 0.24 (7)*  | 5.14 ± 0.08 (3)    |
| ALH displacement                   | 4.96 ± 0.43 (8)                  | 4.98 ± 0.16 (8)   | 5.34 ± 0.4 (7)      | 4.99 ± 0.42 (7)   | 5.05 ± 0.38 (3)    |
| Tail cross frequency, Hz           | 11.84 ± 0.60 (8)                 | 10.86 ± 0.46 (8)  | 11.65 ± 0.79 (7)    | 11.78 ± 0.52 (7)  | 12.26 ± 0.71 (3)   |
| 45 week old                        |                                  |                   |                     |                   |                    |
| Sperm count, × 10 <sup>6</sup> /mL | 39.58 ± 4.42 (8)                 | 44.42 ± 1.92 (8)  | 31.63 ± 5.66 (6)    | 41.84 ± 5.66 (6)  | 44.31 ± 5.37 (3)   |
| Motility, %                        | 65.3 ± 3.4 (8)                   | 67.7 ± 2.8 (8)    | 63.6 ± 4.1 (8)      | 64.1 ± 2.9 (6)    | 62.3 ± 10.1 (3)    |
| Velocity, μm/s                     | 134.46 ± 2.94 (8)                | 141.99 ± 3.87 (8) | 128.62 ± 3.03 (6)   | 135.56 ± 2.80 (6) | 144.83 ± 5.16 (3)  |
| Linearity                          | 5.01 ± 0.17 (8)                  | 5.20 ± 0.20 (8)   | 4.77 ± 0.22 (6)     | 4.92 ± 0.17 (6)   | 5.42 ± 0.58 (3)    |
| ALH displacement                   | 4.78 ± 0.28 (8)                  | 5.55 ± 0.27 (8)*  | 4.77 ± 0.27 (6)     | 5.85 ± 0.44 (6)*  | 5.61 ± 0.83 (2)    |
| Tail cross frequency, Hz           | 11.58 ± 0.51 (8)                 | 12.17 ± 0.50 (8)  | 11.37 ± 0.73 (6)    | 12.17 ± 0.74 (6)  | 14.53 ± 1.30 (2)** |

<sup>a</sup> Due to the small number of litters remaining in the 100 mg/kg dose group, no conclusions regarding treatment-related effects in F1 males could be determined with certainty, although the results are presented.

All values are means ± SEM of (n) litters. Different from control group at the following level of significance. \*0.1 < P < 0.05; \*\*0.05 < P < 0.01; \*\*\*P < 0.01.

<sup>b</sup> Sperm motion parameters were measured after 30 min in capacitation medium.

Table 7

*In vitro* fertilizing ability of cauda epididymal sperm from 16 and 45 week old male offspring from dams exposed to Aroclor 1242 from prenatally through lactation<sup>a</sup>

|                           | Dose of Aroclor 1242 (mg/kg/2-d) |                   |                   |                   |                   |
|---------------------------|----------------------------------|-------------------|-------------------|-------------------|-------------------|
|                           | 0                                | 10                | 25                | 50                | 100 <sup>b</sup>  |
| 16 weeks old              |                                  |                   |                   |                   |                   |
| Percent fertilized        | 83.2 (583/701)                   | 75.0 (392/523)*** | 58.8 (332/566)*** | 84.3 (702/833)    | 70.6 (324/459)*** |
| Percent fragmented        | 4.1 (29/701)                     | 3.3 (17/523)      | 3.2 (18/566)      | 1.9 (16/833)**    | 1.7 (8/459)**     |
| Percent 1 cell fertilized | 0.6 (4/701)                      | 1.9 (10/523)**    | 0.7 (4/566)       | 1.0 (8/883)       | 0.4 (2/459)       |
| 45 week old               |                                  |                   |                   |                   |                   |
| Percent fertilized        | 76.7 (440/574)                   | 65.6 (548/835)*** | 55.3 (271/490)*** | 63.6 (419/659)*** | 55.8 (202/362)*** |
| Percent fragmented        | 2.4 (14/574)                     | 1.8 (15/835)      | 0.6 (3/490)**     | 0.8 (5/659)**     | 3.6 (13/362)      |
| Percent 1 cell fertilized | 0.4 (2/574)                      | 0.5 (4/835)       | 0.2 (1/490)       | 0.5 (2/659)       | 0.6 (2/362)       |

<sup>a</sup> Eggs were considered fertilized if they were at the two-cell stage or the one-cell stage with two pronuclei. Degenerate eggs were not included in the analysis of percent eggs fertilized.

<sup>b</sup> Due to the small number of litters remaining in the 100 mg/kg dose group, no conclusions regarding treatment-related effects in F1 males could be determined with certainty, although the results are presented.

Different from control group at the following level of significance. \*0.1 < *P* < 0.05; \*\*0.05 < *P* < 0.01; \*\*\**P* < 0.01.

in males exposed through gestation and lactation. Decreases in adult testis size and sperm production following prenatal and neonatal exposure to estrogenic chemicals have been well documented [15,16]. In contrast, increases in adult testis size and sperm production in males exposed neonatally to goitrogenic compounds and Aroclor 1242 and 1254 have also been reported [9,17]. Based on previous studies [6,8,9], it was expected that male offspring exposed to Aroclor 1242 through gestation and lactation would exhibit an increase in adult testis size and sperm production, but reduced fertility. However, differences in species, timing of exposure, dose, and the age when males are examined may influence the observed effects.

In this study, treatment of pregnant mice with doses of Aroclor 1242 up to 50 mg/kg/2-day did not seriously impact maternal health or reproduction. Body weight gain and organ weights of treated F0 mice were not significantly different from control F0 mice, indicating PCB-treatment had no general systemic toxicity. The significant increase in thymus weight in F0 mice in the 25 mg/kg dose group may have been due to the weak estrogenic activity of Aroclor 1242, since estrogen is known to play an important role in controlling thymus size [18]. However, the increase was not linearly related to dose. There was an apparent 17% decrease in average fecundity in the 100 mg/kg dose group, although the results were not statistically significant due to the abnormally low fecundity in the control group (Table 4). However, when the 100 mg/kg dose group was compared to the 10 mg/kg dose group, there was a 45% decrease in fecundity that was significant. The low fecundity in the controls was due to 6 of 16 females not conceiving, whereas 3 litters in the 100 mg/kg group were either stillborn, experienced early F1 death, or late F0 death. Since it is unknown whether the failure to give birth to live young was due to fetal abortion and/or resorption, it cannot be concluded that Aroclor 1242 caused reproductive failure at the

doses used in this study. However, treatment of mature female rats with 150 mg/kg/day Aroclor 1242, but not 75 mg/kg/day, has been shown to decrease serum progesterone and abolish reproductive success [19]. The ability of PCBs, such as Aroclor 1242 and selected non-coplanar *ortho*-substituted congeners, to stimulate oscillatory contractions of pregnant rat uterine muscle could contribute to the disruption of pregnancy, in addition to effects on progesterone levels [20]. In any event, effects on the offspring in the current study, at least in the 10, 25, and 50 mg/kg dose groups, are not likely to be due to compromised maternal health but rather are likely to be mediated through direct gestational and lactational exposure.

Gestational exposure was not measured in this study, however, placental transfer of PCBs is well documented. Lactational exposure was estimated based on the PCB concentration determined from stomach samples obtained from nursing pups. An estimate of the level of lactational exposure to PCBs was 0.2, 0.6, 1.2, and 2.4 mg/kg/day total PCBs in the 10, 25, 50, and 100 mg/kg dose groups, respectively. This level of exposure approaches the maximum FDA recommended level for food and breast milk (4 ppm). In addition to the chlorobiphenyls in the stomach samples, nursing pups were exposed to a mixture of PCBs containing a higher proportion of penta-, hexa-, hepta-CBs and a lower proportion of mono- and di-CBs in comparison to the parent Aroclor 1242 mixture. This exposure profile is more similar to human milk samples than to the parent mixture (Table 2 and 3). This difference is likely due to the amenable metabolism and excretion of the lower chlorinated biphenyls and retention of the more persistent highly chlorinated biphenyls by the mothers. This is evident from the higher proportion of trichlorobiphenyls found in the stomach samples (Table 3). A large number of PCBs found in the stomach samples were *ortho*-substituted PCBs containing at least one *para*-substituted chlorine (Table 6). This structure

has been found to be optimal for estrogen receptor binding and estrogenic activity in vitro [21,22]. *Ortho*-substituted congeners with lateral substitution have also been shown to bind to transthyretin, a thyroxine and retinol binding and transport protein [23]. *Para*-hydroxylation of *ortho*-substituted PCBs increases the estrogen receptor binding affinity [22], but decreases the affinity for transthyretin [23]. The extent of hydroxylation of the tri- and tetra-CBs is likely to be large, since there were on average 34 individual peaks in the chromatogram. Although the identity of the hydroxylated species was not determined, *para*-hydroxylation of PCBs is a preferred reaction in rat [24].

In this study, gestational and lactational exposure of mice to Aroclor 1242 increased average sperm count in 16 week old mice, although the magnitude of the effect was smaller than observed in rats [9], not linearly related to dose, and only close to being statistically significant ( $P = 0.064$ ) (Table 6). In contrast to the effects in rats, testis weight was not significantly increased at 16 weeks of age (Table 5). There is no evidence in this study that androgen status of F1 mice was affected since AGD was not decreased following PCB-exposure (Table 5). The observed effect of Aroclor 1242 on rat sperm production is thought to be due to a thyroid-dependent mechanism, since goitrogen (PTU)-induced hypothyroidism in the neonatal rat has also been shown to increase adult testis weight and daily sperm production [25]. Indeed, thyroxine replacement was found to eliminate or decrease the increased testis weight and sperm production in Aroclor 1242- and 1254-treated rats [9]. Hypothyroidism may be accounted for, in part, by increased thyroxine conjugation and excretion by UDP-glucuronosyltransferases (UDPGT) following enzyme induction by PCBs and Aroclor 1254 [2]. Consistent with this hypothesis is the observation that in utero exposure of mice to the coplanar congener 3,4,3',4'-tetrachlorobiphenyl (PCB77) increased testis size [10]. However, acute treatment of some noncoplanar congeners and Aroclor 1242 can decrease serum thyroxine concentration [2]. One hypothesis is that the PCBs and/or their hydroxylated metabolites compete with thyroxine for binding to transthyretin, which results in an increase in the free fraction of thyroxine, thus increasing conjugation and excretion [26]. There is no evidence in this study that thyroid status in PCB-exposed mice was affected since testes weight and sperm count was not significantly increased. Mice also appear to be less sensitive to chemically induced thyroid alterations when compared to rats [27]. These discrepancies may also be due to the timing and route of exposure, dose, or species differences.

Neonatal exposure to Aroclor 1254 has previously been observed to decrease sperm fertilizing ability in 45 week old mice [12]. In contrast to the trends observed in the 25 mg/kg maternal dose group at 16 weeks of age, 45 week old mice in this dose group had average testis weight and average sperm counts similar to the controls. Changes in spermatogenesis and testis size in hypothyroid rats increase to a maximum at 160 days of age [25]. It is unknown whether

these changes persist to middle age or older. Most studies examining the effects of developmental exposure to endocrine disrupting chemicals on male fertility have focused on younger adults (<45 weeks old), while the persistence and manifestation of effects at later stages of life have been relatively ignored [28,29].

Neonatal exposure of rats to Aroclor 1242 does not affect fertility in breeding studies [9]. However, one study has shown adverse effects on fertility in 18 week old rats following neonatal exposure to Aroclor 1254 [5]. These effects were not accompanied by a decrease in epididymal sperm count or changes in sperm morphology or motility, but rather a decline in the ability of sperm to fertilize eggs [6,8]. Therefore, a more sensitive assay to detect changes in the ability of sperm to fertilize eggs from Aroclor 1242 exposed males at 16 and 45 weeks of age was used. At 16 weeks of age, epididymal sperm from all but the 50 mg/kg maternal dose group showed a significant decline in the number of oocytes fertilized in vitro, with the 25 mg/kg dose group being affected most dramatically. At 45 weeks of age, epididymal sperm fertilizing ability was significantly decreased in all dose groups and the 25 mg/kg dose group was again most affected. These changes were not related to apparent changes in sperm motion. The fertilized ova were able to progress to the 2 cell stage greater than 98% of the time and there was no increase in oocyte fragmentation, but rather a small but significant decrease in the 25 and 50 mg/kg dose groups (Table 7). Adverse effects on human sperm have also been observed in young men exposed prenatally to PCBs and polychlorinated dibenzofurans (i.e. Yu-Cheng exposure), including sperm motility, velocity, beat cross frequency and hamster oocyte penetration ability [4]. However, sperm count and semen volume were not significantly affected. In the current study, testis size and epididymal sperm count were also not predictive of effects on in vitro fertility. Other reproductive parameters, such as hormone levels and testicular sperm count, have also been shown not to be predictive of fertility [29]. The advantage of the IVF assay is the increased sensitivity in detecting adverse effects on sperm fertilizing ability, since dramatic reductions (>80%) in sperm production are usually required prior to observing effects on fertility in breeding studies [29]. In vitro fertility studies, however, do not provide information on whether sperm function is affected as a result of testicular, sperm or epididymal malfunction. Considering the normal testes size, epididymal sperm count and sperm motility among treatment groups, the malfunction(s) responsible for the reduced fertility in vitro may be more subtle than gross structural lesions or germ cell differentiation. These malfunctions may involve biochemical, structural, or functional changes within the spermatozoa as a result of alterations in the expression of genes involved in chromosomal packaging, acrosome function, or other processes involved in fertilization. These changes could have occurred during spermiogenesis in the gonad or sperm maturation in the epididymis.

In this study, gestational and lactational exposure to Aroclor 1242 does not increase testes size and epididymal sperm count in 16 or 45 week old mice. Testis size, epididymal sperm count and motility were not predictive of the decline in fertility in vitro, which occurred in both young adult and middle age mice. Furthermore, these effects occurred in male offspring that were exposed in utero and through lactation at levels approaching the maximum FDA recommended level for food and breast milk (4 ppm). Based on these and other studies, it appears that adverse effects on fertility due to developmental exposure to PCBs can occur in both humans and rodents in the absence of significant changes in testis size or sperm production. This indicates that changes at the molecular or biochemical level may have occurred during testicular development and/or sperm maturation to negatively impact sperm fertilizing ability. Gene and protein expression profiling technologies may be useful for identifying these changes in the somatic and germ cells of the male gonad and reproductive tract.

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