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Elevated Blood Pressure in Offspring of Rats Exposed to Diverse Chemicals During Pregnancy

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Adverse intrauterine environments have been associated with increased risk of later cardiovascular disease and hypertension. In an animal model using diverse developmental toxicants, we measured blood pressure (BP), renal nephron endowment, renal glucocorticoid receptor (GR) gene expression, and serum aldosterone in offspring of pregnant Sprague Dawley rats exposed to dexamethasone (Dex), perfluorooctane sulfonate (PFOS), atrazine, perfluorononanoic acid (PFNA), arsenic, or nicotine. BP was assessed by tail cuff photoplethysmography, nephron endowment by confocal microscopy, and renal GR mRNA by qPCR. BP was also measured by telemetry, and corticosterone (CORT) was measured in resting or restrained Dex and atrazine offspring. Treated dams gained less weight during treatment in all groups except arsenic. There were chemical- and sex-specific effects on birth weight, but offspring body weights were similar by weaning. BP was higher in Dex, PFOS, atrazine, and PFNA male offspring by 7–10 weeks. Female offspring exhibited elevated BP at 10 weeks for PFNA and arsenic, and at 37 weeks for Dex, PFOS, and atrazine. Dex, PFOS, and atrazine offspring still exhibited elevated BP at 52–65 weeks of age; others did not. Elevated BP was associated with lower nephron counts. Dex, PFOS, and atrazine offspring had elevated renal GR gene expression. Elevations in BP were also observed in Dex and atrazine offspring by radiotelemetry. Atrazine offspring exhibited enhanced CORT response to restraint. Elevated offspring BP was induced by maternal exposure to toxicants. Because all treatments affected maternal gestational weight gain, maternal stress may be a common underlying factor in these observations.

Key Words: **DOHaD; fetal programming; fetal physiology; maternal toxicity; maternal stress.**

The Developmental Origins of Health and Disease (DOHaD) hypothesis states that environmental factors in early life, including *in utero*, can alter disease susceptibility and affect physiology later in life (McMillen and Robinson, 2005). Numerous epidemiological studies have demonstrated an inverse relationship between birth weight and risk of developing cardiovascular disease and components of the metabolic syndrome in adult life, including glucose intolerance, insulin resistance, hypertension, and obesity (Lau *et al.*, 2011). Birth weight, rather than being causal, has been viewed as a surrogate for a suboptimal intrauterine environment. Maternal nutrition during pregnancy has been the environmental factor of interest in most studies of the DOHaD hypothesis, including numerous studies on offspring of women pregnant during the "Dutch Famine" of World War II (Roseboom *et al.*, 2011).

Animal models have confirmed and extended the majority of the DOHaD epidemiological findings. Although research in the sheep, guinea pig, monkey, and mouse has recapitulated aspects of the epidemiological findings (Armitage *et al.*, 2004), the rat has been used most extensively. Several rat models have been used to investigate prenatal programming of adult health parameters; these models induce intrauterine growth retardation (IUGR) through the restriction of maternal dietary intake of food (Ellis-Hutchings *et al.*, 2010), protein (Langley-Evans and Nwagwu, 1998), or specific micronutrients (Lewis *et al.*, 2001), altered uteroplacental blood flow by vascular clamping (Simmons *et al.*, 2001), or administration of the synthetic glucocorticoid, dexamethasone (Dex) (Ain *et al.*, 2005). Higher offspring systolic blood pressure (BP) has been observed following maternal under nutrition during pregnancy (Ellis-Hutchings *et al.*, 2010), and glucose intolerance and insulin resistance have been reported in adult offspring of Sprague Dawley dams treated with Dex (Buhl

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et al., 2007), and Wistar dams fed a low protein diet during pregnancy (Zambrano *et al.*, 2006). Enhanced weight gain in rats during adulthood has been reported following maternal diet–induced IUGR in some studies (Desai *et al.*, 2007), but not others (Chernoff *et al.*, 2009; Ellis-Hutchings *et al.*, 2010). We have previously reported that under nutrition during pregnancy (ie, 50% of control intake per day) during gestation days (GD) 1–15 or 10–21 in Sprague Dawley rats, or GD 10–21 in Wistar rats, resulted in elevated BP in offspring (Ellis-Hutchings *et al.*, 2010).

Protecting against later-life outcomes of prenatal and early postnatal chemical exposures has been recognized as an important goal for environmental health (Boekelheide *et al.*, 2012). Extension of the DOHaD hypothesis to prenatal toxicity is reasonable, as fetal weight deficit is the most common finding in standard developmental toxicity bioassays, and is frequently the most sensitive developmental finding (Chernoff *et al.*, 2008). Thus, based on our prior experience and literature review, we chose diverse chemical dosages, routes, and exposure periods expected to marginally affect offspring birth weight, including Dex as a positive control known to affect offspring BP (Ortiz *et al.*, 2003). The focus of the study was not on the individual chemicals but on the general ability of developmental toxicants to affect offspring BP. Although our dosing was targeted to have a marginal effect on birth weight, lower birth weight is not necessary for programmed hypertension to occur (Ortiz *et al.*, 2003).

Chemicals chosen for study in addition to Dex include the perfluoroalkyl acids perfluorooctane sulfonate (PFOS) and perfluorononanoic acid (PFNA)—commercial chemicals with wide usages, the developmental toxicity of which we have studied extensively (Lau *et al.*, 2007); the herbicide atrazine, the reproductive toxicology of which has been studied by Ralph Cooper's group here at the Environmental Protection Agency (Cooper *et al.*, 2007); arsenic trioxide (DeSesso *et al.*, 1998); and nicotine (Somm *et al.*, 2008). These chemicals are developmental or reproductive toxicants at dosages at or below those used in the present studies (see above citations).

In addition to assessing offspring BP longitudinally, we investigated several of the factors known to affect BP, including renal nephron endowment (Luyckx and Brenner, 2005), renal glucocorticoid receptor (GR) expression (Whitworth *et al.*, 2001), and plasma aldosterone concentration (Ferrario, 1990).

MATERIALS AND METHODS

Animal husbandry. All animal studies were conducted with approval by the U.S. EPA ORD/NHEERL Institutional Animal Care and Use Committee; procedures and facilities were consistent with the recommendations of the 1996 NRC "Guide for the Care and Use of Laboratory Animals," the Animal Welfare Act, and Public Health Service Policy on the Humane Care and Use of Laboratory Animals. Timed-pregnant Sprague Dawley rats were obtained from Charles River Laboratories (Raleigh, North Carolina). The rats were bred overnight, and the day of copulatory plug detection was designated as GD 0. Upon arrival at our facilities, animals were housed individually in hanging polypropylene cages with heat-treated pine shavings bedding and provided standard laboratory diet (LabDiet 5001, PMI Nutrition International, Brentwood, Missouri) and tap water *ad libitum*. Animal facilities were controlled for temperature (20°C–24°C) and relative humidity (40%–60%), and operated under a 12-h light-dark cycle. On GD 1, dams were weighed, randomized, and assigned to treatments as listed in Table 1. Additional female rats were mated 1 day before the rats assigned to treatment groups, and these additional dams served as foster mothers for the litters of the treated dams (see *Postnatal animal handling* below).

Chemical exposures. These experiments comprised 2 sets of chemicals and controls. Chemical set 1 included Dex, PFOS, and atrazine. Chemical set 2 included PFNA, arsenic trioxide (arsenic), and nicotine bitartrate (nicotine). Dex was selected as a positive control because treatment of pregnant animals with this synthetic glucocorticoid has been reported to result in hypertension in offspring in multiple species (Seckl, 2004). Other chemicals, dosages, and periods of administration were chosen based on our past experience with the chemicals and dosages predicted to produce marginally lower offspring birth weight although birth weight was not significantly affected in all groups. Dex was administered by SC injection in saline at a dosage of 0.6 mg/kg/day on GD 16–20. Atrazine (purity 97.1%), a gift from Syngenta Crop Protection (Greensboro, North Carolina), was prepared in 1.0% methyl cellulose (M-7140, Sigma, St Louis, Missouri) in deionized water and administered by oral gavage at 125 mg/kg/day on GD 16–20. PFOS was administered at a dosage of 18.75 mg/kg/day by oral gavage in 0.5% Tween-20 on GD 2–6. PFNA was administered at 5 mg/kg/day by oral gavage in water on GD 1–20. Arsenic was administered at 3 mg/kg/day by oral gavage in saline on GD 1–20. Nicotine was administered via osmotic minipump at an estimated 6 mg/kg/day on GD 2–20. Chemicals, dosages, routes of exposure, and treatment periods were chosen based on previous studies and are listed in Table 1. All dams were weighed daily during gestation. The numbers of dams/litters/offspring per group are provided in the figure legends.

Postnatal animal handling. Following parturition, designated as postnatal day (PND) 0, number and condition of neonates were noted. Litters from dams administered test chemicals during pregnancy were fostered to naïve control dams that had just delivered to assure adequate maternal care and to preclude postnatal chemical exposure. Control litters were cross-fostered within the control group. Litters were standardized to 10–12 pups per litter at birth. Litters were weighed by sex at birth and weekly until weaning on PND 21.

Tail cuff systolic BP measurements. Systolic BP was measured by tail cuff photoplethysmography (tail cuff) (IITC Life Science Inc., Woodland Hills, California) in offspring over time at ages ranging from 7 to 62 weeks. System calibration was performed daily prior to data acquisition. Rats were placed in a plexiglass restraining tube and acclimated in the testing chamber at 30°C for 10–15 min. Following acclimation, 6 consecutive BP readings were acquired per rat, and systolic BP was determined using the IITC model 31 NIBP software (v.1.32) module. If less than 3 of the 6 replicates were acceptable, the test was repeated with 5 additional replicates. During data analysis, nonreadable replicates (ie, due to excess movement) and replicates that varied by more than 20 mm Hg in systolic pressure for a single animal were excluded from the data set. One to 2 male and female offspring per litter were used for tail cuff BP measurements.

Surgery and radiotelemetry. Beginning on PND 55–60, 1 male per litter from controls and dams treated with Dex or atrazine was surgically implanted with a radio transmitter (Data Sciences Int., St Paul, Minnesota; model C50-PXT) according to the manufacturer's surgical instructions for real-time recording of aortic BP, heart rate, core temperature, and motor activity. Rats were anesthetized with isoflurane gas (4.5% initially followed

TABLE 1
Chemicals and Exposures Used in These Studies

Chemical	Dosage (mg/kg/day)	Vehicle	Route	Duration
Dexamethasone	0.6	Saline	SC injection	GD 16–20
Atrazine	125	1% methyl cellulose	Oral gavage	GD 16–20
PFOS	18.75	Water	Oral gavage	GD 2–6
PFNA	5	Water	Oral gavage	GD 1–20
Arsenic trioxide	3	Saline	Oral gavage	GD 1–20
Nicotine bitartrate	6	Saline	SC minipump	GD 2–20

with 2%–3% to maintain a surgical plane). The abdominal area was shaved and sterilized with several swabs of betadine followed with 70% alcohol. A midline incision was made in the abdomen, and the BP catheter was inserted into the abdominal aorta just above the iliac bifurcation. The catheter was held in place using Vetbond (3M, St Paul, Minnesota) adhesive and a cellulose patch (Data Sciences Int.). The 2 electrocardiogram leads were tunneled under the skin and positioned at the last left rib and below the right clavicle, in line with the right forelimb to detect the ECG. The body of the transmitter was sutured to the wall of the abdomen with 4-0 silk. The skin was closed with staples, and rats were administered an analgesic (buprenorphine; 0.03 mg/kg; SC) twice per day for 2 days. Rats were housed singly following surgery and allowed 10–14 days of recovery prior to handling or testing.

On approximately PND 70, telemetry data were recorded from rats in their home cages. Data were collected every 10 min, 24 h/day from telemetry receiver boards placed beneath each cage. Baseline recordings were collected for 4 days with minimal disturbance to the rats.

Response to restraint stress. To test BP response to stress, rats were restrained in plexiglas cones for 1 h and BP was recorded during and after restraint. Blood samples from a separate group of male offspring were taken from the tail vein at intervals during and after restraint (as depicted in Fig. 5) for measurement of serum corticosterone. Blood was collected in microcentrifuge tubes, allowed to clot, and centrifuged at 3000 rpm for 30 min. Serum was separated and frozen at −80°C. Corticosterone was measured in thawed serum samples by radioimmunoassay according to the manufacturer's instructions (Coat-A-Count; Siemens Healthcare Diagnostic, Deerfield, Illinois).

Kidney nephron endowment. Kidneys were isolated from male offspring on PND 22, an age at which nephrogenesis is complete in rats (Lelièvre-Pégorier *et al.*, 1998). Following decapsulation, kidneys were sliced sagittally and fixed overnight at 37°C in freshly prepared 4% paraformaldehyde. Fixed kidneys were dehydrated to 70% methanol, stained with 10μM Yo-Pro-1 (Molecular Probes, Eugene, Oregon), further dehydrated to 100% methanol, and cleared using a solution of benzyl alcohol and benzyl benzoate (BABB) as previously described (Zucker, 2006). Cleared kidneys in BABB were mounted between 2 glass cover slips sealed to aluminum microscope slides with a circular hole in the center. Confocal laser scanning microscopy was used to scan 1 mm^2 optical sections in the z-direction from the cortex to the medullary interface, with 3 locations per kidney scanned. All glomeruli within an optical z-series were counted using the spot detection feature in Imaris 6.0 (Bitplane Inc., St Paul, Minnesota). Whole-kidney nephron count was estimated as:

(mean z-series nephron count per 1 mm scanned)
× (mean z-series nephron counts per 1mm^2 scanned)
× (kidney surface area)

Renal GR mRNA. Male neonates were killed by decapitation and kidneys collected and frozen at −80°C. Measurement of GR mRNA was carried out by quantitative PCR (qPCR) of reverse-transcribed cDNAs. Total RNA was extracted from frozen/thawed samples using Tri reagent (Sigma T9424) and quantified using PicoGreen. RNA samples were then treated with DNase and quantified by Ribogreen Quantitation Kit (Invitrogen R11490). DNase-treated RNA was reverse transcribed (ABI cDNA Archive Kit 4322171), and cDNA was amplified using the ABI TaqMan Gene Expression Assay with the Rn00561369_m1Nr3c1 primer set and ABI Universal Master Mix 4304437. Amplification was performed on an ABI model 7900HT sequence detection system. All samples were run in duplicate. Glyceraldehyde 3-phosphate dehydrogenase was used as the endogenous control. Data were analyzed by the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008).

Basal serum aldosterone. Male offspring were fasted overnight (15–17 h) and euthanized between 0800 and 1130h by decapitation during postnatal week 28. Trunk blood was collected into vacutainer tubes and allowed to clot on ice for 30 min, followed by centrifugation at 5000 rpm. Basal aldosterone concentrations were determined in duplicate by enzyme immunoassay utilizing a mouse monoclonal antibody according to the manufacturer's protocol (10004377, Cayman Chemical Company, Ann Arbor, Michigan).

Statistics. Data were analyzed using SAS (v.8) software (Cary, North Carolina). All analyses were performed using the litter as the unit for comparison (ie, litter means were used when more than 1 pup per litter was assessed). Tail cuff BP measurements were analyzed using a repeated measures analysis (Proc Mixed). All other outcome variables were analyzed with 1-way analysis of variance (Proc GLM). Data were evaluated for homogeneity of variance across treatment groups using Bartlett's test, and where heterogeneity was observed, Welch's ANOVA was used (Proc ANOVA). Where there was a significant treatment effect ($p < .05$), *post hoc* analysis was conducted using Dunnett's test to determine whether individual treatment groups were significantly different from controls. For analysis of telemetric recordings, we used the telemetry system's foldagram routine to average data collected from offspring in their home cages. The foldagram averaged 4 days of data to represent a 24-h period for a single animal. Radiotelemetric data were analyzed using a repeated measures analysis of variance (RMANOVA, Sigma Plot, version 11.0).

RESULTS

Maternal Body Weight Gain

All chemical treatments except arsenic resulted in lower maternal weight gain beginning at the first day of treatment and persisting to lower maternal weights at term compared with controls (Fig. 1). Even though the period of treatment with PFOS was GD 2–6, this chemical has a long half-life in the body and would be expected to remain in the maternal liver and serum throughout pregnancy (Lau *et al.*, 2007). There were no maternal deaths or overt signs of toxicity in any of the dams during pregnancy.

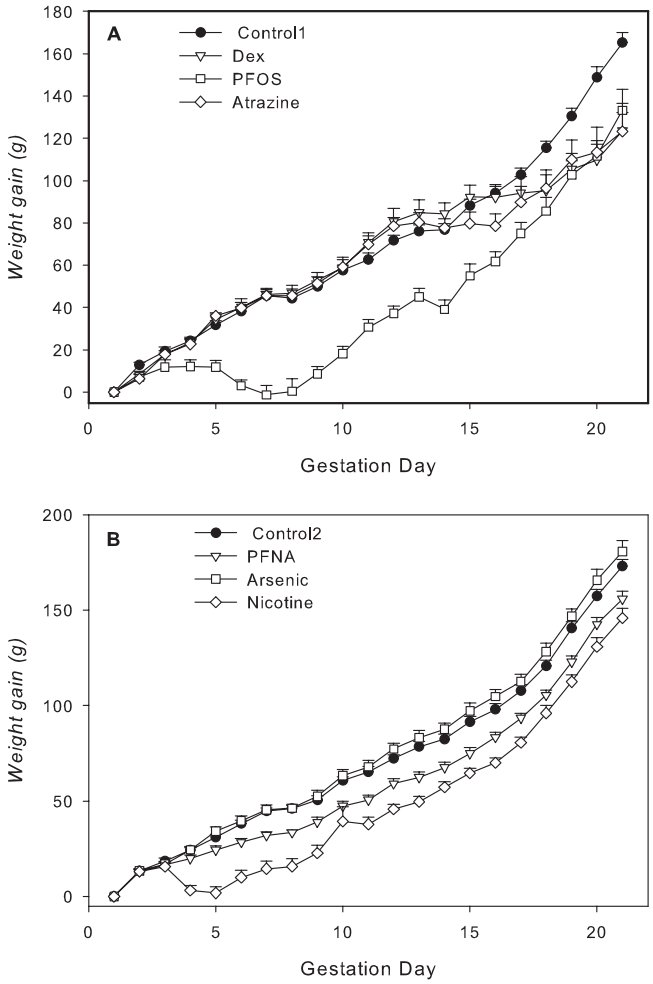

FIG. 1. Maternal weight gain during pregnancy. Dosing periods were (GD) chemical set 1—Control 1: 2–20, Dex: 16–20, PFOS: 2–6, atrazine: 16–20 and chemical set 2—Control 2: 1–20, PFNA: 1–20, arsenic: 1–20, nicotine: 2–20. See Table 1 for routes and dosages. A, Chemical set 1. Maternal weight gain was significantly ($p < .05$) lower than Control 1 in PFOS-treated dams on GD 4–20, in Dex-treated dams on GD 19–21, and in atrazine-treated dams on GD 16–21. B, Chemical set 2. Maternal weight gain was significantly ($p < .05$) lower than Control2 in nicotine-treated dams on GD 4–21 and in PFNA-treated dams on GD 4–19. Data are means ± SEM. Dams per group were Control1 (21), Dex (22), PFOS (21), and atrazine (12). Abbreviations: Dex, dexamethasone; GD, gestation days; PFNA, perfluorononanoic acid; PFOS, perfluorooctane sulfonate.

Birth Weight and Postnatal Growth

We selected maternal dosages predicted to result in marginally lower birth weights than controls. The Dex and PFNA groups had birth weights significantly lower than controls in both males and females, while only females were significantly lower in birth weight than concurrent controls for PFOS and atrazine (Fig. 2). All treated litters exhibited postnatal growth such that there were no significant differences in body weight among the groups at weaning on PND 21 or for the remainder of the study (data not shown).

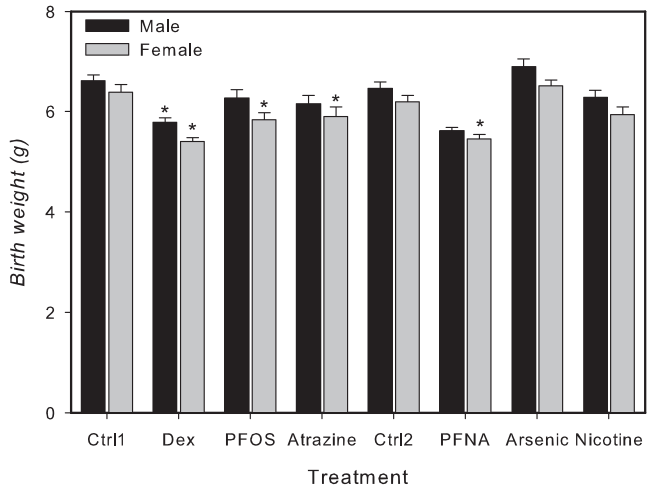

FIG. 2. Offspring birth weights. Dex, PFOS, and atrazine were compared to Ctrl 1; PFNA, arsenic, and nicotine were compared with Ctrl 2. *$p < .05$ compared with appropriate control and sex. Data are means ± SEM. Litters per group were Control 1 (21), Dex (22), PFOS (21), Atrazine (12), Control 2 (19), PFNA (18), Arsenic (17), Nicotine (17). Abbreviations: Ctrl 1, control 1; Ctrl 2, control 2; Dex, dexamethasone; PFNA, perfluorononanoic acid; PFOS, perfluorooctane sulfonate.

BP Testing by Tail Cuff and Radiotelemetry

Systolic BP in offspring, measured by tail cuff at different ages, is presented in Figure 3. Male offspring in the Dex, PFOS, and atrazine groups exhibited systolic BP higher than controls at 7 weeks of age in chemical set 1, as did PFNA male offspring at 10 weeks of age in chemical set 2. Elevated BP persisted to 52 weeks of age in male offspring of dams treated with Dex, PFOS, or atrazine. Male offspring systolic BP was not significantly different from controls in the PFNA group at 26 or 56 weeks of age or in the nicotine or arsenic groups at any age. BPs of female offspring were similar to controls for the Dex, PFOS, and atrazine groups at 7 weeks of age, but these groups exhibited elevated systolic BP compared with controls at 37 and 65 weeks of age. Systolic BP readings were generally lower across groups in female offspring at 37 weeks of age compared with earlier or later ages in chemical set 1, and readings were more variable; the reasons for this are unknown, but this downward shift did not occur at the intermediate time point in females or males of chemical set 2. Female offspring in the PFNA and arsenic groups had elevated BP at 10 weeks of age but were similar to controls at later ages. Collectively, these results suggest that prenatal chemical exposure can program increased offspring BP in juvenile and adult life.

Measuring BP telemetrically can provide a true measure of baseline BP, as the animals are undisturbed in their cages during assessment. Although more limited in the number of animals that can be tested, we undertook these assessments in male offspring of control, Dex-treated, and atrazine-treated dams as a replicate of our tail cuff experiments. All rats tested exhibited a nocturnal rise in mean arterial BP with an abrupt rise at the onset of the dark cycle (Fig. 4A). Atrazine- and

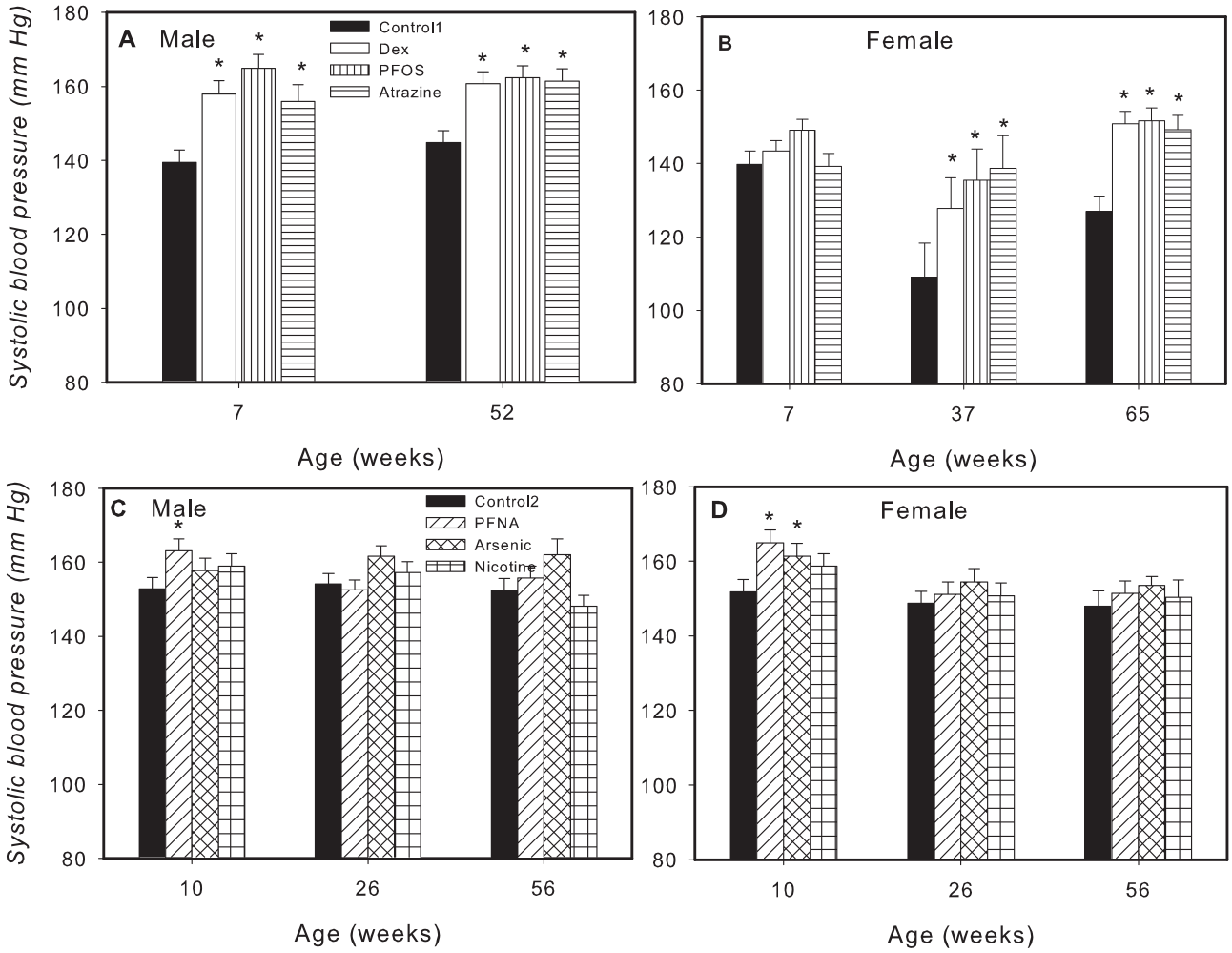

FIG. 3. Systolic BP in male and female offspring by tail cuff photoplethysmography at different ages. A, Males, chemical set 1. B, Females, chemical set 1. C, Males, chemical set 2. D, Females, chemical set 2. *$p < .05$ compared with same sex and age controls. Data are means ± SEM. One to 2 male and female offspring per litter were used for BP, measured at the ages indicated. Abbreviations: BP, blood pressure; Dex, dexamethasone; PFNA, perfluorononanoic acid; PFOS, perfluorooctane sulfonate.

Dex-exposed rats exhibited higher BP compared with controls that approached statistical significance ($p = .058$; Fig. 4A), maintaining a 5–10 mm Hg elevation in BP over the 4 consecutive 24-h periods tested. Although the telemetric data are mean arterial BP rather than systolic BP as measured by tail cuff, results generally agree with the tail cuff findings under the same treatment conditions.

Response to Restraint Stress

Upon restraint, BP in approximately 70-day-old offspring rose rapidly by about 25 mm Hg and returned to baseline within 1 h following restraint (Fig. 4B). Although the Dex-treated and atrazine-treated animals had nominally higher BP than controls during and following restraint, there were no significant differences between the groups at any time during restraint. In separate offspring from the same litters, measures of serum corticosterone during and after restraint showed a significantly higher corticosterone response to restraint stress in the offspring of atrazine-treated dams compared with control offspring (Fig. 5).

Nephron Endowment

Using confocal microscopy and 3D visualization to count renal glomeruli at 22 days of age in male offspring, we found that nephron endowment was significantly lower than controls in offspring of dams treated with Dex, PFOS, atrazine, or PFNA during pregnancy (Fig. 6A). Results shown are whole kidney nephron estimates from an average of 3 representative z-series acquired per kidney. It is important to point out that neither body weights nor kidney weights were significantly different from controls in any treatment group at the time kidneys were harvested (data not shown). There was good correlation between lower nephron endowment and elevated BP early in life, as the male offspring of the Dex, PFOS, PFNA, and atrazine groups exhibited both of these traits while the nicotine offspring exhibited neither. PFNA offspring did not show elevated

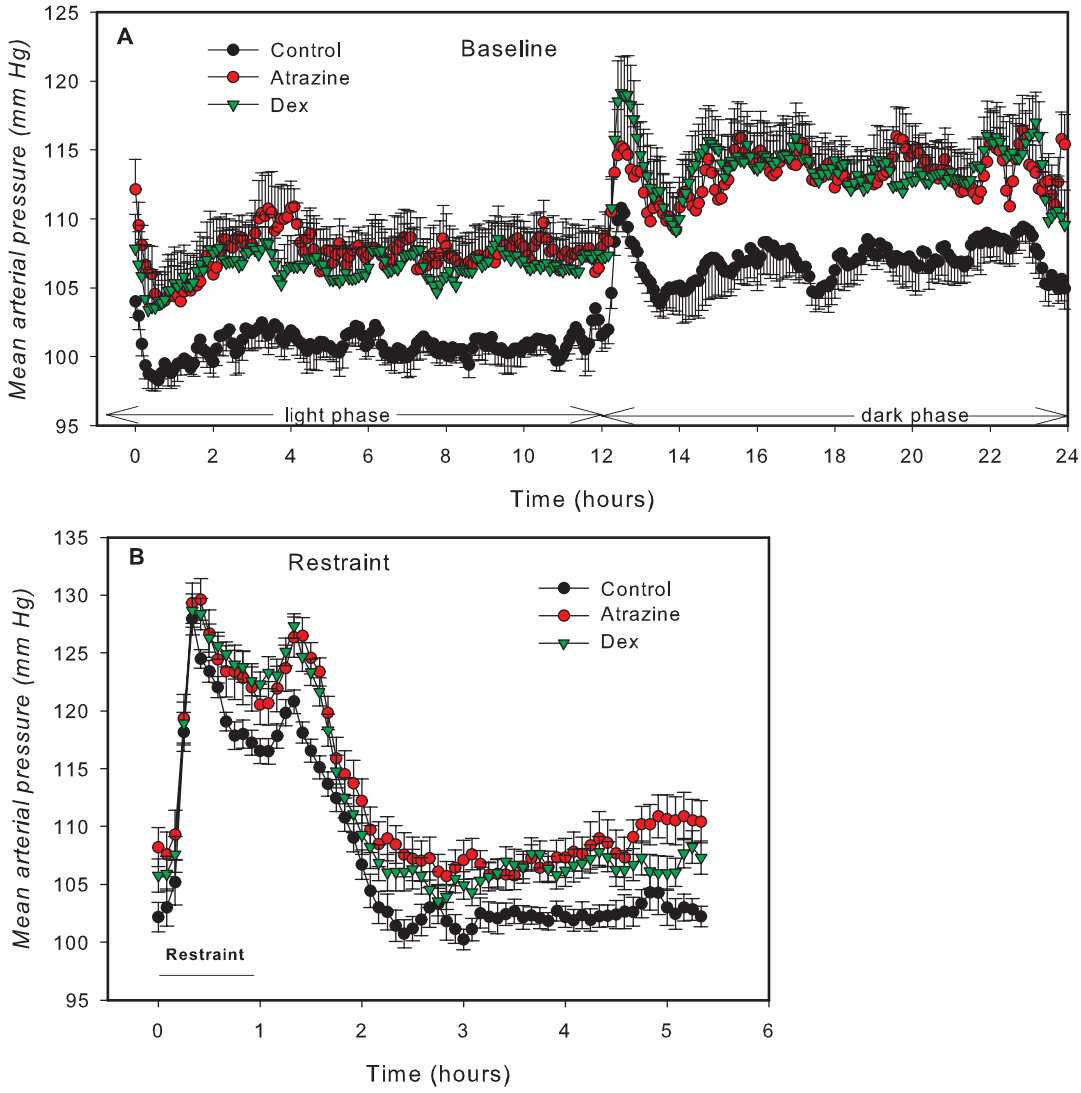

FIG. 4. A, Mean arterial BP measured by radiotelemetry in approximately 70-day-old male offspring of dams treated with atrazine or Dex, and controls. Clear circadian patterns were observed (compare light phase to dark phase); $p < .058$ for atrazine and Dex compared with controls. B, BP response to restraint. One hour of restraint induced markedly increased BP, but there were no significant differences between either atrazine- or Dex-treated groups and controls. BP returned to baseline by approximately 1 h after release from restraint. Data are means ± SEM. Five males from 5 different litters were tested in each group. Abbreviations: BP, blood pressure; Dex, dexamethasone.

BP later in life, despite the lower nephrons count that presumably persisted throughout life, and arsenic showed no change in nephron endowment with a mixed BP response.

Expression of Renal GR mRNA

Quantitation of GR message in kidneys of male neonates revealed increased relative expression in offspring of dams treated with Dex, PFOS, or atrazine during pregnancy, whereas offspring in the PFNA, arsenic, and nicotine groups were similar to controls (Fig. 6B). Although the upregulation of GR mRNA in the Dex group was robust (160% of control) as expected, the significant increases for PFOS and atrazine were somewhat smaller, on the order of 130%–135% of control.

Serum Aldosterone Concentrations

Serum aldosterone concentrations in fasted male offspring at 28 weeks of age were unaffected by treatment except for an increase in the Dex group, the positive control. Serum aldosterone was 128 ± 11 pg/ml in Dex offspring, 100 ± 4 pg/ml in controls, and 88–102 pg/ml in offspring of other treated groups.

DISCUSSION

The studies on which the DOHaD hypothesis is based examined relationships between birth weight and incidence of adult diseases including coronary heart disease, hypertension, diabetes, and obesity (Lau *et al.*, 2011). In animal models, IUGR

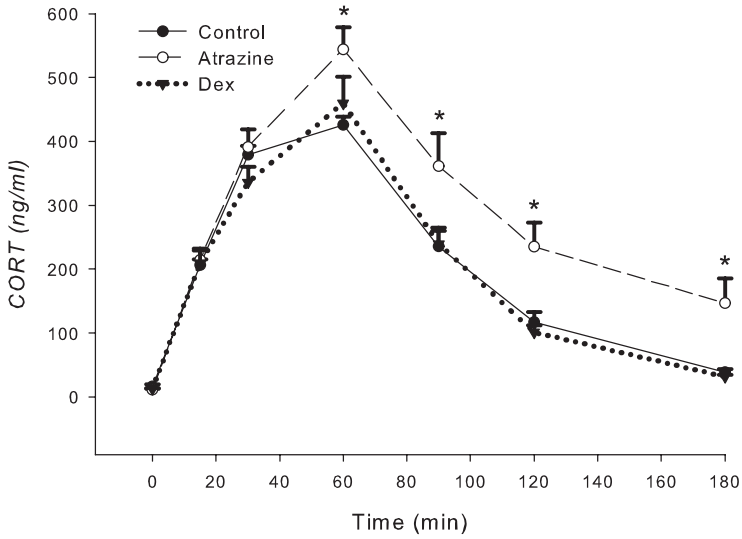

FIG. 5. Offspring serum CORT levels during and following 1 h restraint stress in approximately 70-day-old male offspring of dams treated with Atrazine or Dex, and controls. Atrazine-treated, but not Dex-treated, animals have increased CORT levels beginning at the end of the restraint period. *$p < .05$ for Atrazine compared with controls. Data are means ± SEM. Five males from 5 different litters were tested in each group. Abbreviations: CORT, corticosterone; Dex, dexamethasone.

has been induced by maternal under- or malnutrition, hypoxia, glucocorticoid treatment, or reduced uteroplacental blood flow (Armitage *et al.*, 2004; McMillen and Robinson, 2005). It is important to reiterate that birth weight or fetal growth is not hypothesized to be the cause of later disease but represents a marker of *in utero* distress or at least a suboptimal intrauterine environment. To determine whether chemical exposure could recapitulate the phenotype seen with these experimental manipulations, we selected diverse chemical exposures predicted to marginally affect fetal growth, although not all groups showed significantly lower birth weights.

Elevations in BP resulting from maternal Dex, PFOS, or atrazine exposure were significant at all ages examined in male offspring, starting at 7 weeks, whereas in female offspring of dams treated with these chemicals, elevated BP was not observed until 37 weeks. Elevated BPs in these groups persisted in both males and females through final assessments at 52–65 weeks of age. Studies in rats using maternal dietary restriction, maternal protein restriction, high dietary sodium, or placental insufficiency similarly found that female offspring were less sensitive to elevated BP (for review, see Gilbert and Nijland, 2008). Conversely, adding lard to the maternal pregnancy diet resulted in higher BP in female offspring but not males (Khan *et al.*, 2003). In our studies, female offspring were as sensitive as males to early elevations in BP from maternal treatment with PFNA. Elevations in BP were more robust and consistent in chemical set 1 (Dex, PFOS, and atrazine) than in chemical set 2 (PFNA, arsenic, and nicotine). BPs in chemical set 2 were higher in all groups by about 10 mm Hg compared with chemical set 1, and elevations by chemical exposure were less pronounced. The 2 sets of chemicals were tested at different times by different handlers, so there may have been differences in stress, known to affect BP (Kurtz *et al.*, 2005; Whitesall *et al.*, 2004). In agreement with our results, a recent study in Wistar rats reported systolic BP of 120–140 mm Hg in control offspring at 8 weeks of age by tail cuff (Harrison and Langley-Evans, 2009).

Although there were chemical- and age-dependent findings of higher BP in both male and female offspring by tail cuff, subsequent telemetry and mechanistic studies were limited to male offspring due to constraints of time and resources. However, our choice to limit telemetry and mechanistic studies to males should not be taken to diminish the importance of findings of higher BP in females for some of the chemical tested. Additional studies in females to examine BP by telemetry and effects on the kidney are needed to test whether effects similar to those seen in male offspring are observed. Readers interested in sex differences in developmental programming of hypertension are referred to the excellent review and synthesis by Gilbert and Nijland (2008).

Measuring BP by tail cuff is noninvasive and allows testing of more animals than does radiotelemetry, but disadvantages are that it requires restraint and warming of the animal and can be prone to artifact due to stress and movement of the animal during testing. We tested 1 chemical consistently positive by tail cuff (atrazine) and Dex using radiotelemetry, which allowed assessment of a true resting baseline mean BP (Van Vliet *et al.*, 2000). Both Dex and atrazine resulted in resting mean BP of 5–10 mm Hg higher than controls. This difference did not reach statistical significance with a conservative 2-sided test ($p < .058$), in part due to the low numbers of offspring we could test with this method (5 per group). BP measured by radiotelemetry represents mean arterial pressure rather than the systolic pressure measured by tail cuff, which is one reason why BP values by radiotelemetry were lower than by tail cuff. The higher BPs measured by tail cuff also lends support to the idea that animals

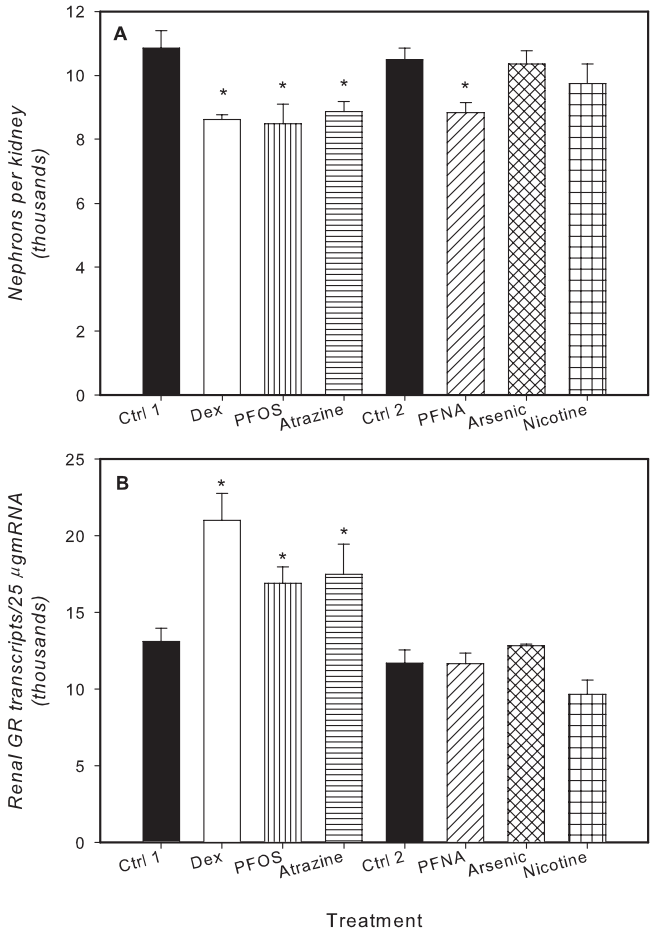

FIG. 6. A, Estimated nephron endowment at 22 days of age in male offspring. Five males from 5 different litters were tested in each group. B, Renal glucocorticoid receptor mRNA levels in newborn male offspring. *$p < .05$ compared with controls. Five males from 5 different litters were tested in each group. Abbreviations: Ctrl 1, control 1; Ctrl 2, control 2; Dex, dexamethasone; PFNA, perfluorononanoic acid; PFOS, perfluorooctane sulfonate.

restrained for tail cuff assessment are stressed (Augustyniak *et al.*, 2010; McMillen and Robinson, 2005). Although several studies using tail cuff measured increases of about 20 mm Hg in systolic BP in offspring of dams fed low protein during pregnancy (Gardner *et al.*, 1997; Langley and Jackson, 1994; McMullen *et al.*, 2004), others using radiotelemetry have reported smaller increases (Tonkiss *et al.*, 1998) or no difference from control (Augustyniak *et al.*, 2010). In contrast to our findings and those of others, a recent study using radiotelemetry reported resting hypotension, but a stress-induced hypertension in Dex-exposed Wistar rat offspring (O'Regan *et al.*, 2008).

The chemicals that caused elevated BP in offspring (other than Dex) have not previously been tested for this effect. Elevated BP may be due in part to nonspecific maternal stress, as all chemicals except arsenic affected maternal weight gain during pregnancy. Prenatal exposure to atrazine results in increased circulating ACTH and corticosterone in both male Wistar (Laws *et al.*, 2009) and female Long-Evans rats (Fraites *et al.*, 2009). Maternal exposure to excess glucocorticoids or inhibition of placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), which converts active to inactive glucocorticoids, causes elevated BP in offspring of several species (Dodic *et al.*, 2002; Drake *et al.*, 2007; Langley-Evans 1997; Ortiz *et al.*, 2003; Roghair *et al.*, 2005), including humans receiving antenatal glucocorticoid therapy (Doyle *et al.*, 2000; Kari *et al.*, 1994). Animal models of developmental programming of hypertension, including maternal low protein diet (Bertram *et al.*, 2001; Langley-Evans *et al.*, 1996), placental insufficiency (Baserga *et al.*, 2007), hypoxia (Hardy and Yang, 2002), and stress (Takahashi *et al.*, 1998) have reported elevations of glucocorticoids and/or decreased expression of 11β-HSD2. Maternal psychosocial stress *per se* has been associated with elevated offspring BP in humans (van Dijk *et al.*, 2012). The link between maternal chemical toxicity, stress, and elevated offspring BP merits further study.

The BP values for 37- week-old females in chemical set 1 were lower than other ages and sexes. The controls were particularly low, about 20% lower than other control readings, whereas the Dex and PFOS groups had readings about 10% lower than other time points and sexes. We can only speculate that the sensitivity of the apparatus might have been lower, causing an artifactual effect on readings, or that these animals were particularly calm. The group comparisons were done only with animals tested at this time, but nonetheless these values may be open to question.

The only chemical in this study without any effect on offspring BP was nicotine. Maternal smoking during pregnancy has been associated with elevated BP in young (Simonetti *et al.*, 2011), adolescent (Högberg *et al.*, 2012), and adult children (Cupul-Uicab *et al.*, 2012) although some studies did not find this association (Horta *et al.*, 2011). Adult offspring of pregnant rats receiving 6 mg/kg/day nicotine from GD 4 to lactation day 10 had elevated BP in response to angiotensin II, but their resting BP was unaffected (Xiao *et al.*, 2008). Female offspring of C57Bl/6J mice given 200 μg/ml nicotine from 2 weeks prebreeding though weaning had elevated BP (Fox *et al.*, 2012). Thus, studies of maternal nicotine exposure have been inconsistent.

O'Regan *et al.* (2008) measured BP telemetrically in offspring of rats treated with Dex during pregnancy and observed lower resting BP but stress-induced elevated BP. We conducted similar experiments with Dex- and atrazine-treated offspring and also measured the corticosterone response to restraint stress. In contrast to the findings of O'Regan and coworkers, both Dex and atrazine offspring exhibited elevated resting BP but no exacerbation of this treatment effect by restraint stress. Maternal atrazine but not Dex treatment caused an elevated corticosterone response to stress in adult offspring. Elevation of ACTH and corticosterone by atrazine in male (Laws *et al.*, 2009) and female rats (Fraites *et al.*, 2009) has been observed, but an altered stress response in offspring of rats treated with atrazine has not been reported.

Maternal treatments that caused elevated offspring BP at 7–10 weeks also resulted in lower offspring nephron endowments at 22 days of age, despite similar body and kidney weights at that time. Elevated BP in offspring has been associated with reduced nephron endowments following maternal protein restriction (Woods *et al.*, 2004), under nutrition (Ellis-Hutchings *et al.*, 2010) or glucocorticoid treatment (Ortiz *et al.*, 2003), and reduced nephron endowment is associated with low birth weight in humans (Hughson *et al.*, 2003). Fewer nephrons results in higher load on available nephrons, starting a cycle of sclerosis and pressure natiuresis that can raise BP. It is unclear why the kidney was a target organ for the disparate chemicals tested in this study, but the previous link between excess glucocorticoids and reduction in nephron number again points to maternal stress as a common factor. Nephrogenesis in the rat occurs from midgestation to PND 10 (Guron and Friberg, 2000). The chemical exposures used here would be expected to result in the chemicals being in the dam during late gestation, but the cross-fostering we used was intended to preclude postnatal exposure.

The GR mediates the action of glucocorticoids in most tissues of the body, including the kidney. We observed robust elevation of renal GR gene expression in Dex offspring and less robust but significant renal GR elevation in PFOS and atrazine offspring. Our results with Dex are similar to those of Wyrwoll *et al.* (2007), who reported a doubling of renal GR gene expression in male offspring of dams treated with Dex during pregnancy. Maternal low protein diet has been shown to increase GR expression in offspring liver (Bertram *et al.*, 2001; Lillycrop *et al.*, 2007), lung, and brain (Bertram *et al.*, 2001). Although enhanced glucocorticoid activity in the kidney can interact with angiotensin II to increase proximal tubule sodium reabsorption (Brem, 2001), it is unclear whether the increased GR gene expression observed in PFOS and atrazine offspring contributed to elevated BP.

Aldosterone is an adrenal mineralocorticoid that acts on its receptors in the kidney to increase sodium reabsorption and fluid retention. Aldosterone can start a cascade leading to nephron fibrosis. Serum aldosterone was elevated only in offspring of Dex-treated dams and does not appear to have played a role in BP elevations in other groups. Dex is the only chemical tested that can also bind to the mineralocorticoid receptor, but it is unknown whether this played a role in its unique ability among the chemicals tested to raise serum aldosterone.

This is among the first reports of elevated BP after prenatal chemical exposure. Elevated BP in offspring of dams exposed to chemicals was correlated with lower renal nephron endowments, but the underlying cause of this effect remains to be elucidated. Although maternal toxicity and stress likely represent a common etiology, there may also be chemical-specific mechanisms. Hypertension is one component of the metabolic syndrome, the incidence rate of which is rapidly rising in children and young adults. If prenatal chemical exposure is contributing to this increase, it will be important to understand the extent of this contribution and the mechanisms involved.

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