Neuroendocrine Effects of Perfluorooctane Sulfonate in Rats

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Perfluorooctane sulfonate (PFOS) is a degradation product of sulfonyl-based fluorochemicals that are used extensively in industrial and household applications. Humans and wildlife are exposed to this class of compounds from several sources. Toxicity tests in rodents have raised concerns about potential developmental, reproductive, and systemic effects of PFOS. However, the effect of PFOS on the neuroendocrine system has not been investigated thus far. In this study, adult female rats were injected intraperitoneally with 0, 1, or 10 mg PFOS/kg body weight (BW) for 2 weeks. Food and water intake, BW, and estrous cycles were monitored daily. At the end of treatment, PFOS levels in tissues were measured by high-performance liquid chromatography (HPLC) interfaced with electrospray mass spectrometry. Changes in brain monoamines were measured by HPLC with electrochemical detection, and serum corticosterone and leptin were monitored using radioimmunoassay. Treatment with PFOS produced a dose-dependent accumulation of this chemical in various body tissues, including the brain. PFOS exposure decreased food intake and BW in a dose-dependent manner. Treatment with PFOS affected estrous cyclicity and increased serum corticosterone levels while decreasing serum leptin concentrations. PFOS treatment also increased norepinephrine concentrations in the paraventricular nucleus of the hypothalamus. These results indicate that exposure to PFOS can affect the neuroendocrine system in rats. *Key words:* **corticosterone, hypothalamus, leptin, neuroendocrine, norepinephrine, PFOS, reproduction, stress.** *Environ Health Perspect* **111:1485–1489 (2003). doi:10.1289/ehp.6128 available via** *http://dx.doi.org/* **[Online 3 April 2003]**

During the last three decades, there has been a steady increase in the use of perfluorinated compounds as components of adhesives, cosmetics, paper coatings, fire retardants, surfactants, lubricants, and insecticides. These compounds are used extensively in the apparel, leather, and upholstery industries. As surface treatment agents, they provide soil, oil, and water resistance to personal apparel and leather, fabric, upholstery, and carpets (Kannan et al. 2002; Seacat et al. 2002; Starkov and Wallace 2002). Perfluorinated chemicals are also produced for paper protection, including food contact applications (plates, food containers, bags, and wraps) and non-food-contact applications (folding cartons, carbonless forms, and masking papers; Kannan et al. 2002; Seacat et al. 2002). Release of perfluorinated compounds into the environment can occur from product manufacturing processes, supply chains, product use, and disposal. Perfluorooctane sulfonate (PFOS) is a primary degradative product of several sulfonyl-based organofluorine compounds (Figure 1).

PFOS and related fluorochemicals are persistent and bioaccumulative pollutants (Giesy and Kannan 2001; Kannan et al. 2001a, 2001b). PFOS has been detected in the liver and blood plasma of wildlife on a global scale (Giesy and Kannan 2001). Exposure to PFOS affects the liver primarily and causes vacuolation and hypertrophy of hepatic cells (Borges et al. 1978; Butenhoff and Seacat 2001; Pastoor et al. 1987). PFOS also decreases body weight (BW), serum cholesterol, and triglycerides and increases liver weight (Butenhoff and Seacat 2001; Seacat et al. 2002).

Many of the effects observed in animals after exposure to PFOS suggest the involvement of the central nervous system, specifically, the neuroendocrine system. However, no studies to date have assessed the effects of PFOS on the neuroendocrine system. In the present study we focused on the effects of PFOS on two axes of the neuroendocrine system: the reproductive axis and the stress axis. For this purpose, we measured the accumulation of PFOS in several body tissues, including brain, and we studied the effects of PFOS on neurotransmitters and the hormones that they regulate. We also examined the effects of PFOS on other parameters such as food intake and BW.

Materials and Methods

Animals. Adult female Sprague-Dawley rats were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, IN) and were housed individually in temperature (23 ± 2°C) and light-controlled (lights on from 0500 to 1900 hr) rooms. They were provided food and water *ad libitum*. Two weeks after acclimatization, rats were exposed to potassium salt of PFOS (> 96% purity; Tokyo Chemical Industries, Tokyo, Japan). All protocols were approved by the University Committee for Animal Care and Use at Michigan State University.

Treatment. Animals were housed individually. They were injected intraperitoneally with either 1 or 10 mg/kg BW of PFOS dissolved in dimethyl sulfoxide (DMSO) daily for 14 days (n = 8 for both groups). The control animals (n = 8) were injected on the same schedule with the vehicle (DMSO) only. Food intake and BW of all animals was measured daily between 0800 and 1000 hr for 14 days. Estrous cyclicity was also monitored daily using vaginal cytology (MohanKumar PS et al. 1994). Animals that had repeated cycles of proestrus, estrus, and diestrus I and II, in that order, were called "regular cyclers." Those that missed a particular stage(s) repeatedly were called "irregular cyclers." Those that had prolonged periods of diestrus smears were said to be in "persistent diestrus."

Brain microdissection. At the end of treatment, the animals were sacrificed when they were in diestrus, by rapid decapitation between 1000 and 1100 hr. Their brains were removed quickly and frozen by placing them on dry ice. Trunk blood was collected and serum was separated by centrifugation and stored at –20°C until analyzed for corticosterone and leptin by radioimmunoassay (RIA). Serial sections (300 µm thick) of the brain were obtained using a cryostat, which was maintained at –10°C. The sections were transferred to cover slips, which were placed on a cold stage set at –10°C. The paraventricular nucleus (PVN) and the medial preoptic area (MPA) of the hypothalamus were located with the help of a stereotaxic atlas (Paxinos and Watson 1987) and were microdissected using the Palkovits's micropunch technique (MohanKumar SMJ et al. 1998; Palkovits 1973). The PVN regulates the stress axis, and the MPA regulates the reproductive axis. Tissue samples were obtained bilaterally and included

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all subdivisions of the nuclei. They were stored at –70°C until analyzed for norepinephrine (NE) and dopamine (DA) concentrations by high-performance liquid chromatography (HPLC) with electrochemical detection.

Neurotransmitter analysis. The concentrations of NE and DA were measured using HPLC with electrochemical detection, which has been described before (MohanKumar PS et al. 1994; MohanKumar SMJ et al. 1998). Briefly, the apparatus consists of an LC-4C amperometric detector (Bioanalytical Systems, West Lafayette, IN); a phase II, 5-µm ODS reverse-phase C-18 column (Phenomenex, Torrance, CA); a glassy carbon electrode, a model CTO-10 AT/VP column oven, and an LC-10 AT/VP pump (Shimadzu, Columbia, MD). The mobile phase was made with pyrogen-free water and contained monochloroacetic acid (14.14 g/L), sodium hydroxide (4.675 g/L), octanesulfonic acid disodium salt (0.3 g/L), ethylenediaminetetraacetic acid (0.25 g/L), acetonitrile (3.5%), and tetrahydrofuran (1.4%). The mobile phase was then filtered and degassed through the Milli-Q purification system (Millipore Co., Bedford, MA) and pumped at a flow rate of 1.8 mL/min. The sensitivity of the detector was 1 nA full scale, and the potential of the working electrode was 0.65 V. The temperature of the column was maintained at 37°C. At the time of HPLC analysis, tissue samples were homogenized in 150 µL 0.1 M $HClO_4$ and centrifuged at 10,000 × *g* for 10 min; 50 µL of the supernatant along with 25 µL of the internal standard (0.05 M dihydroxybenzylamine) was injected into the HPLC system.

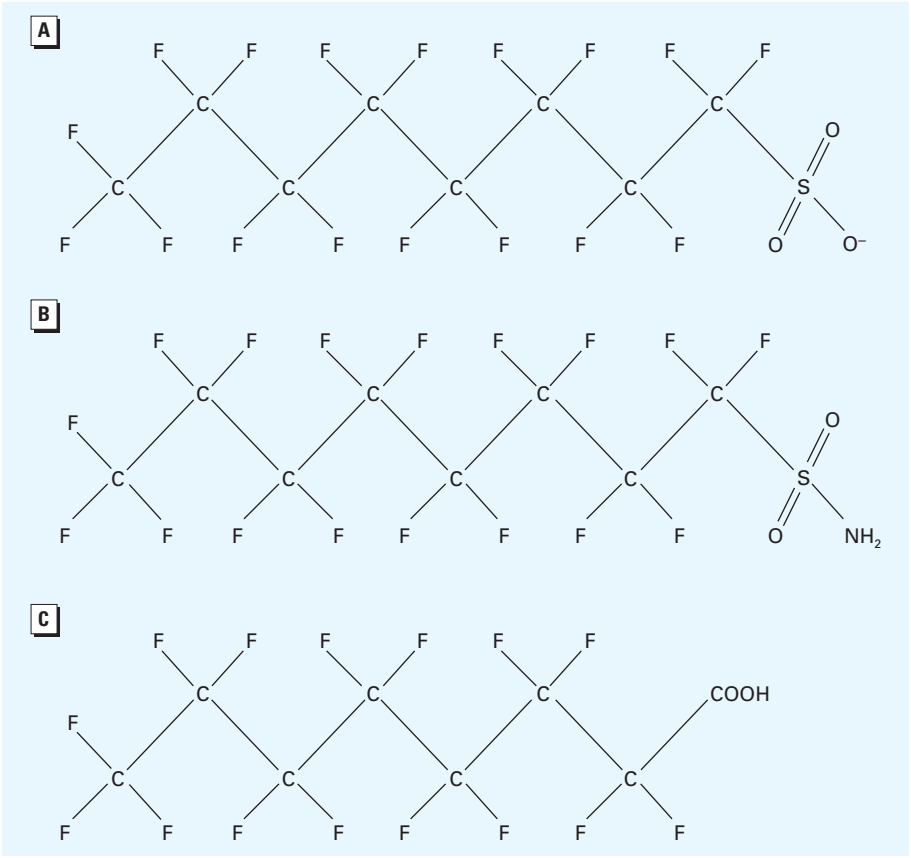

Figure 1. Structures of PFOS and related compounds. (*A*) PFOS. (*B*) Perfluorooctane sulfonylamide. (*C*) Perfluorooctanoic acid.

Table 1. PFOS concentrations in serum (ng/mL) and in various tissues (ng/g) on a wet weight basis.

Site	Untreated control	PFOS-treated groups	
		1 mg/kg BW	10 mg/kg BW
Serum	BDL	10,480 ± 1,428	45,446 ± 4,120*
Tissue			
Liver	BDL	26,617 ± 4,044	97,358 ± 25,668*
Heart	BDL	1,280 ± 697	23,490 ± 10,036*
Kidneys	BDL	9,581 ± 4,836	47,799 ± 29,512*
Spleen	BDL	76	15,873
Ovary	BDL	3,028	15,489
Adrenal	BDL	1,539	30,087
Brain			
Hypothalamus	BDL	< 50	15,706
Cortex	BDL	294	4,487
Hippocampus	BDL	115	8,966
Brain stem	BDL	363	5,346
Cerebellum	BDL	289	5,540
Rest of the brain	BDL	396	4,256

BDL, below detection limit (50 ng/g). Tissues from animals in each group were pooled for the measurement of PFOS concentrations in specific parts of the brain and in spleen, ovaries, and adrenals. $n = 4–5$ in each group of rats.
*$p < 0.05$ relative to the other groups.

PFOS analysis. Details of the analytical procedure to measure PFOS have been outlined previously (Giesy and Kannan 2001; Hansen et al. 2001; Kannan et al. 2001a, 2001b, 2002). Briefly, tetrabutylammonium hydrogen sulfate is added as an ion-pairing reagent to the homogenized tissues, and the analyte ion pair is partitioned into methyl-*tert*-butyl ether. The extract is dried under a nitrogen evaporator, and the solvent is reconstituted in 1.0 mL of methanol. The analyses were performed with an HPLC interfaced with an electrospray mass spectrometer, by monitoring the primary ion characteristic of PFOS. Molecular ion 499 was selected as the primary ion for PFOS ($C_8F_{17}SO_3$).

The following conditions were used for the analytical phase of this study: For liquid chromatograph, we used Hewlett-Packard Series 1100 Liquid Chromatograph System (Hewlett Packard, Palo Alto, CA) Analytical Column: Keystone Betasil C_{18} 2 × 50 mm (5 µm; Thermo Hypersil-Keystone, Bellefonte, PA). Column temperature was ambient. Of mobile phase components, component A was 2 mM ammonium acetate and component B was methanol; flow rate was 300 µL/min, and injection volume 10 µL. The mass spectrometer used was Micromass API/Mass Spectrometer Quadrupole System; software, Mass Lynx 3.4 (Waters, Milford, MA); cone voltage was 30–60 V; collision gas energy was 25–45 eV; mode was electrospray negative; and source block temperature was 150 ± 10°C.

RIA. Double antibody RIA was used to measure leptin and corticosterone levels in the serum. RIA kits from Linco Research Inc. (St. Charles, MO) and the Coat-A-Count kit from Diagnostic Products Inc. (Los Angeles, CA) were used to measure leptin and corticosterone levels, respectively, as described previously (Francis et al. 2000).

Statistical analysis. Changes in average daily food intake, BW, and water intake were analyzed by repeated measures analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD). Changes in PFOS

and neurotransmitter concentrations and in leptin and corticosterone levels were analyzed by ANOVA followed by Fisher's LSD test. Changes in estrous cyclicity was analyzed by Kruskal-Wallis nonparametric test.

Results

Accumulation of PFOS in tissues. Concentrations of PFOS in various tissues of rats exposed to either of two doses of PFOS are shown in Table 1. Although PFOS was not detectable in any of the tissues in control animals, there was a dose-dependent increase in the concentrations of PFOS in all of the tissues, including various parts of the brain from exposed rats. PFOS was also found in the brains of rats exposed to the lower dose. Among the various parts of the brain, the hypothalamus of rats exposed to the higher dose had greater accumulation, with at least a 3-fold increase compared with other brain areas. However, PFOS was not detected in the hypothalamus of the rats exposed to the lower doses. Among various body tissues, the liver contained the highest concentration, followed by the kidney and serum.

Body weight. There were no significant differences in BW among the treatment groups at the beginning of the experiment. BW (mean ± SE) in control animals on day 0 (pretreatment) was 233.5 ± 8.7 g and remained at about that level on day 14 (240.1 ± 8.2 g). In contrast, BW in animals treated with the high dose of PFOS (10 mg/kg of BW) was 235.9 ± 8 g on day 1 and decreased significantly to 208 ± 7.1 g by day 14 ($p = 0.0039$; $F = 7.306$; df = 2). Treatment with the low dose of PFOS did not produce any significant change in BW throughout the treatment period (Figure 2).

Food intake. Changes in food intake among the treatment groups are shown in Figure 3. The daily food intake (mean ± SE) of control animals on day 0 (pretreatment) was 16.3 ± 0.5 g, which did not vary significantly over the study period. In contrast, treatment with the high dose of PFOS (10 mg/kg of BW) decreased food intake significantly from 16.7 ± 0.7 g on day 1 to 9.1 ± 0.6 g on day 14 ($p < 0.0001$; $F = 60.979$, df = 2). Food intake in animals that were treated with the low dose of PFOS (1 mg/kg of BW) was similar to that in controls.

Estrous cycles. Figure 4 shows the changes in estrous cyclicity among the treatment groups. All the animals in the control group exhibited regular 4-day estrous cycles. In contrast, only about 66% of the animals were regular cyclers in the low-dose group. Treatment with the high dose of PFOS further reduced the number of regular cyclers to 42% and increased the number of animals in persistent diestrus from 8% in the low-dose group to 33%. There was a significant change in cyclicity between the control and high-dose group ($p = 0.0442$; df = 2).

Serum leptin. At the end of treatment, the leptin level (mean ± SE; Figure 5) in the sera of control animals was 10.5 ± 1.5 ng/mL. Treatment with the low dose of PFOS did not produce any significant changes in serum leptin levels. In contrast, treatment with the high dose of PFOS resulted in a marked decrease in serum leptin levels (1.5 ± 0.5 ng/mL; $p = 0.0183$; $F = 5.399$, df = 2).

Serum corticosterone. The corticosterone level (mean ± SE; Figure 6) in the sera of control animals at the time of sacrifice was 332.6 ± 62.8 ng/mL. Treatment with the low dose of PFOS did not affect serum corticosterone levels. In contrast, treatment with the high dose of PFOS significantly increased corticosterone levels by about 75% ($p = 0.0256$; $F = 4.814$, df = 2).

Monoamines in the hypothalamus. Catecholamine concentrations in the PVN and the MPA are shown in Figure 7. The NE level (mean ± SE protein) in the PVN of control animals was 11.2 ± 1.6 pg/µg. The level was significantly higher (19.7 ± 1.6 pg/µg) in animals treated with the high dose of PFOS ($p = 0.0413$; $F = 3.786$, df = 2). Treatment with the low dose of PFOS did not affect NE concentrations in the PVN. Treatment with either the low dose or high dose of PFOS did not affect NE concentrations in the MPA. PFOS treatment did not affect DA concentrations in either the PVN or the MPA.

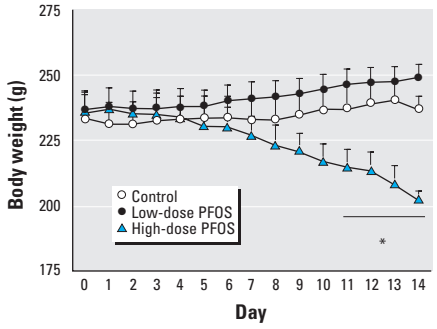

Figure 2. Changes in BW (mean ± SE) after treatment with DMSO (control) or with 1 or 10 mg PFOS/kg of BW/day. BW was measured daily after treatment of animals. $n = 8$ in each group.
*$p < 0.05$ compared by one-way repeated-measures ANOVA.

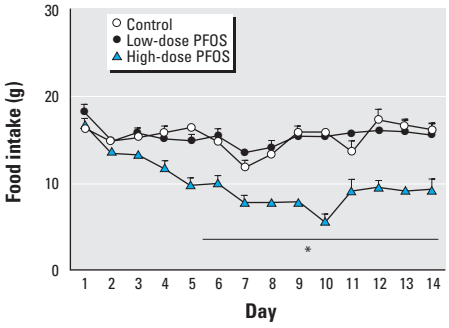

Figure 3. Changes in food intake (mean ± SE) after treatment with DMSO (control) or with 1 or 10 mg PFOS/kg of BW/day. Food intake was measured daily after treatment of animals. $n = 8$ in each group.
*$p < 0.05$ compared by one-way repeated measures ANOVA.

Discussion

In this study, PFOS was found in various body tissues, including the blood, liver, serum, kidney, heart, ovaries, adrenal, brain, and spleen (Table 1). The liver is the target organ of accumulation for PFOS (Borges et al. 1978; Butenhoff and Seacat 2001; Pastoor et al. 1987). The concentration of PFOS in the liver was 2–3-fold greater than that found in serum. Preferential accumulation of PFOS in the liver has been suggested because of its binding with specific proteins found in the liver (Luebker et al. 2002; Pastoor et al. 1987). Measured concentrations of PFOS in the liver of rats used in

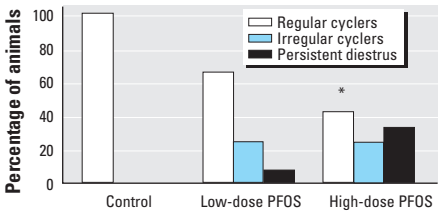

Figure 4. Changes in estrous cycles after treatment with DMSO (control) or with 1 or 10 mg PFOS/kg of BW/day. Estrous cycles were monitored by daily vaginal cytology. $n = 8$ in each group.

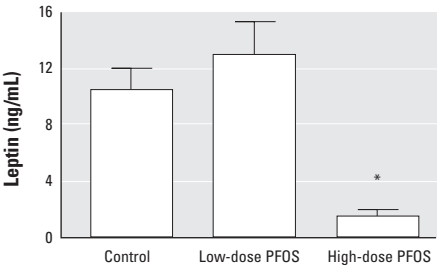

Figure 5. Changes in serum leptin levels (mean ± SE) after treatment with DMSO (control) or with 1 or 10 mg PFOS/kg of BW/day. Serum leptin levels were measured at the end of 2 weeks of treatment at the time of sacrifice. $n = 8$ in each group.
*$p < 0.05$ compared with control and low-dose groups.

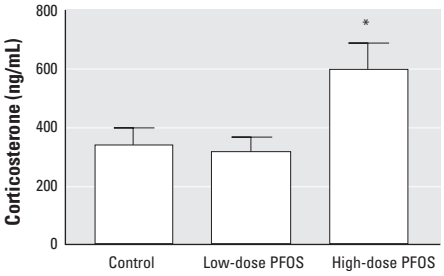

Figure 6. Changes in serum corticosterone (mean ± SE) levels after treatment with DMSO (control) or with 1 or 10 mg PFOS/kg of BW/day. Serum corticosterone levels were measured at the end of 2 weeks of treatment at the time of sacrifice. $n = 8$ in each group.
*$p < 0.05$ compared with control and low-dose groups.

this study were higher than the levels reported in wildlife (48–2630 ng/g; Giesy and Kannan 2001). However, PFOS concentrations measured in the serum of rats treated with the low dose of PFOS were similar to the levels reported in individuals occupationally exposed to this compound (9,930 and 12,830 ng/mL; Olsen et al. 1999). In addition, human blood contains several perfluorinated compounds such as perfluorooctanoic acid, which are thought to have effects similar to those of PFOS (Hansen et al. 2001; Olsen et al. 2000). Thus, the changes in neuroendocrine functions observed in our study may have implications for the populations that are affected by occupational exposure to these compounds.

PFOS exposure in our study produced marked changes in the BW and food intake of rats. The decreases in food intake and BW were followed by a significant reduction in serum leptin levels by the end of 2 weeks. In contrast, there was a significant increase in corticosterone and NE concentrations in the PVN after high-dose PFOS exposures. This indicates that PFOS has a stimulatory effect on the stress axis. Apart from affecting the stress axis, PFOS treatment also altered estrous cyclicity significantly. Taken together, these results indicate that PFOS has marked effects on the neuroendocrine system. Our study also indicates that these effects are probably mediated through an increase in the accumulation of PFOS in brain tissues, specifically, the hypothalamus.

A few studies have investigated the toxic effects of PFOS in rats, mice, guinea pigs, rabbits, and monkeys [reviewed by Starkov and Wallace (2002)]. Some of the signs of toxicity observed in these studies included liver abnormalities such as hepatomegaly, hepatocyte swelling and vacuolation, decreased food consumption, and loss of BW. Our results agree with these findings on food intake and BW. Exposure to the high dose of PFOS significantly decreased food intake and produced a corresponding decrease in BW. However, the gross and histologic examinations of the liver did not show any signs of abnormality. The discrepancy could be attributed to differences in doses (20 μg/kg to 30 mg/kg BW), duration (2–14 weeks), and the routes of administration (oral or intraperitoneal) among various studies.

The decrease in BW after treatment with the high dose of PFOS was accompanied by a significant decrease in serum leptin levels. Leptin is a protein hormone produced by white adipocytes and is involved in the regulation of various neuroendocrine functions, including food intake (Ahima 2000). Leptin levels are positively correlated with body fat. The increase in serum leptin levels is believed to decrease food intake, and vice versa (Ahima 2000). The low leptin levels observed in PFOS-exposed rats could be correlated with the reduced body fat stores observed during autopsy. However, this reduction in leptin levels failed to stimulate food intake in the treated animals, suggesting a possible derailment of the neurotransmitter activity that regulates feeding behavior. The inhibitory effect of PFOS exposure on food intake was therefore independent of the effect of PFOS on serum leptin levels. The low levels of leptin could possibly explain the reduction in cholesterol and triglycerides observed with PFOS treatment in other studies (Kannan et al. 2002; Seacat et al. 2002), and might be linked to the reduction in 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase that has been observed (Haughom and Spydevold 1992).

Our present observations indicate that PFOS exposure significantly alters estrous cyclicity in female rats. Adult female rats generally exhibit 4–5-day estrous cycles. Although all of the control animals were regular cyclers, treatment with the low dose of PFOS (1 mg/kg BW) altered the pattern of estrous cyclicity and reduced the number of regular cyclers in this group. Treatment with the high dose of PFOS (10 mg/kg BW) further affected the estrous cycles, rendering almost 60% of the treated animals irregular cyclers. Although PFOS has been shown to produce an increase in neonatal mortality (Case et al. 2001a, 2001b), its effects on reproductive cycles have not been reported before. This study shows for the first time that PFOS can affect estrous cyclicity and can therefore function as an endocrine disruptor.

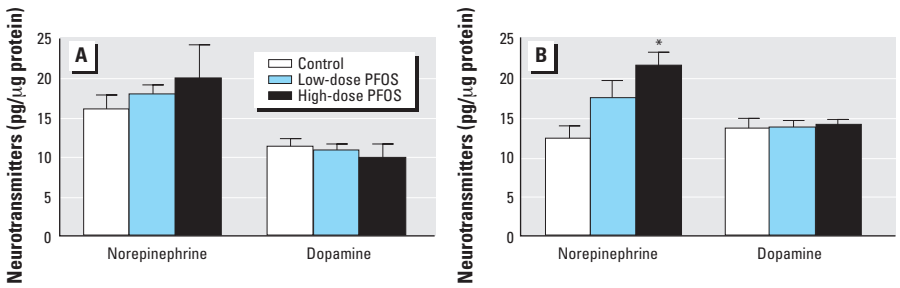

Figure 7. Changes in NE and DA levels (mean ± SE) in (*A*) MPA and (*B*) PVN in controls and in experimental animals treated with PFOS. NE and DA concentrations in specific areas were measured as described in "Materials and Methods." $n = 8$ in each group.
*$p < 0.05$ compared with the other groups.

Estrous cyclicity is regulated by the MPA of the hypothalamus. The MPA houses a large number of luteinizing hormone (LH)–releasing hormone (LHRH) neurons (MohanKumar PS et al. 1994; Ramirez et al. 1984). This hormone is very important for the induction of the preovulatory LH surge from the pituitary during the afternoon of proestrus of each estrous cycle. This surge is responsible for ovulation and is critical for maintaining estrous cyclicity in animals. Increase in noradrenergic activity in the MPA is very important for the stimulation of LHRH neurons, and thereby the regulation of estrous cycles (MohanKumar PS et al. 1994; Ramirez et al. 1984). In the present study, although PFOS treatment disrupted estrous cycles, it did not result in changes in NE concentrations in the MPA. This could be attributed to several causes. First, NE levels in the MPA were measured after the animals had been sacrificed on the day of diestrus. The day of diestrus is marked by the absence of fluctuations in NE, LHRH, and LH. Therefore, it is not surprising that we did not see any difference in NE on this day, between control and experimental animals. Monitoring the changes in neurotransmitters and hormones simultaneously at various time points through the day during proestrus will help us to determine whether PFOS-induced alterations in estrous cycles are accompanied by changes in the levels of LH and NE. Nevertheless, results from the present study indicate that PFOS can affect estrous cyclicity. The mechanism for this alteration needs further investigation.

Besides affecting the reproductive axis, PFOS treatment produced marked changes in the stress axis. PFOS was able to increase serum corticosterone in treated rats, in a dose-dependent fashion. This effect could again be mediated through the hypothalamus. The PVN of the hypothalamus has a large number of corticotropin-releasing hormone neuronal cell bodies and receives rich noradrenergic innervation from the brainstem, through the ventral noradrenergic bundle (Petrusz and Merchenthaler 1992). Studies involving neurotoxic blockade of noradrenergic fibers and direct administration of NE into the PVN have demonstrated the importance of NE in the regulation of corticosterone secretion (e.g., Szafarczyk et al. 1988). In the present study, PFOS treatment significantly increased NE concentrations in the PVN. This effect was probably responsible for the increase in corticosterone observed in the PFOS-treated rats. The only other study that has examined the effects of PFOS on the stress axis showed no changes in cortisol levels, in monkeys, 5–9 weeks after cessation of treatment (Seacat et al. 2002). However, changes in the levels of cortisol during the treatment period were not assessed in that study. In the present study,

we measured corticosterone levels immediately after the end of treatment and found significant alterations, indicating that the stress axis was activated by PFOS exposure.

The mechanism by which PFOS affects brain neurotransmitters is not clear. It is possible that the neurons of the hypothalamus are exposed to higher levels of PFOS. Table 1 shows the levels of PFOS in various brain areas. We were able to detect significant levels of PFOS in several parts of the brain, including the hypothalamus. PFOS concentrations in the hypothalamus of rats exposed to high doses were at least 3-fold higher than in other brain areas. Occurrence of PFOS in the brain suggests that this compound can cross the blood–brain barrier. The increased levels of PFOS in the hypothalamus after treatment with the high dose of PFOS compared with the low-dose group indicates that the higher dose may cause an increase in the permeability of the blood–brain barrier to this compound. The increase in PFOS levels in the microenvironment around neurons could possibly contribute to the alterations in their activity. The exact mechanisms by which PFOS brings about these changes require further investigation. Nevertheless, our results provide the first evidence that peripheral administration of PFOS can result in its accumulation in the brain and this may be a possible route by which PFOS affects central and neuroendocrine functions.

In summary, exposure to PFOS was found to activate the stress axis while inhibiting the reproductive axis. PFOS was also found to decrease food consumption, BW, and serum leptin levels. Although the mechanism by which PFOS produces its neuroendocrine effects is still unclear, our study indicates that hypothalamic NE could play a role in this phenomenon. Further studies are needed to characterize the role and mechanism of hypothalamic NE in precipitating the neuroendocrine effects of PFOS.

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