Endocrine, *vol. 26, no. 2, 161–168, March 2005* 0969–711X/05/26:161–168/$30.00

Neonatally Administered *tert*-Octylphenol Affects Onset of Puberty and Reproductive Development in Female Rats

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There now is evidence that many of the synthetic chemicals released into the environment can impact on the function of the endocrine system of many organisms. One group of chemicals, the alkylphenols, used in paints, pesticides, herbicides, detergents, and plastics, has been found to have the ability to bind estrogen receptors. This estrogenic property makes these compounds potentially hazardous to the developing reproductive system and neuroendocrine brain. In this study we determined the effects of exposure to the environmental toxins 4-nonylphenol (NP) and 4-*tert*-octylphenol (OP) and to synthetic estrogen diethylstilbesterol (DES) during the early postnatal period (d 0–10) on the development of reproductive function. The day of vaginal opening, ovulation, prepubertal LH levels, LH response to estradiol, estrous cyclicity, and ovarian histology were determined. In the OP- and DES-treated groups, the vaginal opening was observed to have occurred several days prior to that of the control group. The NP-treated group showed vaginal opening at ages similar to those of the control group. Treatment with OP prevented ovulation in a significant number of animals, as well as in all animals treated with DES, whereas the control and NP-treated animals ovulated normally. Animals treated with DES and OP had significantly lower ovarian weights and higher uterine weights than either control animals or NP-treated animals. Higher basal LH levels, as well as the absence of the prepubertal LH surge, were observed in both DES- and OP-treated animals. A significant number of OP-treated animals showed no LH response to the estradiol-17β challenge. NP-treated animals responded positively to the estradiol-17β challenge. Persistent estrus was also apparent in both OP- and DES-treated animals. Upon histological examination, the ovaries in OP-treated animals were found to have a decreased number of corpora lutea and an increased number of preantral and atretic follicles. These data suggest that exposure to OP during the critical period of sexual brain differentiation affects the onset of puberty and reproductive development.

Key Words: Octylphenol; nonylphenol; estrogen; puberty; ovulation; luteinizing hormone.

Introduction

Manmade by-products of the petrochemical industry have been found to have estrogenic effects that detrimentally affect reproduction in aquatic, wildlife, and human populations. A 400% increase in ectopic pregnancies in the United States between 1970 and 1987 and a 50% decrease in male sperm counts over the past 50 yr *(1)* give reason for concern and an impetus to investigate the actions of these petrochemical by-products that may be polluting the environment. *Tert*-octylphenol (OP) is a petrochemical by-product that belongs to the chemical group of alkylphenols. These compounds are found in paints, herbicides, pesticides, detergents, and plastics *(2)*. The metabolites of these compounds are often found in aquatic environments including river sediments and sewage effluent and found in low levels in the drinking water in New Jersey *(3)*. Epidemiological studies have provided correlative evidence for disruption of endocrine activities in aquatic and wild species and in humans *(4)*. Effects of estrogenic compounds include the stimulation of *vitellogenin* gene expression in trout hepatocytes, a decrease in fertility and hatching success in birds, and an increase in the growth of estrogen-responsive human breast tumor cells in cultured cell lines. These compounds have a high bioconcentration factor, are lipophilic, and are slow to biodegrade *(5)*.

Xenoestrogens present in the environment provide chronic, low-level exposure to the compound that may exert effects on estrogen-responsive tissues. A known estrogenic compound, diethylstilbestrol (DES), has produced decreased fertility as well as defeminization of the brain *(4,6)*. It is necessary to assess whether the effects of alkylphenols are similar to a known estrogenic compound such as DES. Proper development of the neuroendocrine axis is essential for the maintenance of reproductive functions; therefore, the

Received January 26, 2005; Revised February 14, 2005; Accepted February 28, 2005.

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effects of these compounds on the hypothalamic–pituitary–ovarian axis must be investigated. Once this information is obtained, it can be applied to various conditions affecting human fertility, and more significant correlations between cause and disease can be made.

Several previous studies have been conducted on the effects of OP and indicate that this compound does have an estrogenic effect *(7,8)*. Using the pig as an in vivo model, the effects of intrauterine exposure to OP on reproductive success over several generations has been studied. This treatment extended pregnancy length and induced basal cell proliferation in the cervical epithelium of the parental generation. The offspring of these sows, when treated with OP, showed an accelerated rate of the onset of puberty. All of these symptoms are associated with estrogen-treated animals *(7)*.

Various effects of OP on female rats have already been documented. It has been shown that oral administration of the compound during early pregnancy has caused embryonic loss. This study involved sacrifice of the dams during pregnancy and examination of both the dam and her fetuses. Decreases in body weight gain during pregnancy and postimplantation loss were found to be significantly higher in the animals treated with OP; however, the pregnancy rates and body weights of fetuses were not significantly different *(9)*. Furthermore, it has been shown that rats treated with OP neonatally do not exhibit lordosis and have decreased displays of sexual receptive behavior in response to mounting stimuli delivered by stud male rats *(10)*, suggesting the possibility that OP-treated rats may have abnormality in female sexual behaviors.

During the critical development period, neuroendocrine brain cells differentiate to form a male or female phenotype *(11)*. The male brain is masculinized when estrogen is able to cross the blood–brain barrier and bind to estrogen receptors in the brain. In contrast, the female's blood contains systemic alpha-fetoprotein that has the ability to bind estrogen. The size of this binding protein prevents it from crossing the blood–brain barrier and affecting brain development. Alkylphenols may not bind to alpha-fetoprotein and are therefore able to cross the blood–brain barrier. Because of their estrogenic property, these compounds have the potential to masculinize the female neuroendocrine brain *(11)*. This defeminized female neuroendocrine brain will exhibit deleterious effects on the maturation of reproductive cycle that will become evident during the onset of puberty. Sexual brain differentiation reflects upon pituitary function. In re-sponse to circulating estrogen, the hormone gonadotropin–releasing hormone (GnRH) is released from the hypothalamus and travels to the pituitary gland where the gonadotropins (FSH and LH) are released. These hormones are necessary for new follicles to mature and for ovulation of these mature follicles to occur. Following ovulation, estrogen levels decrease, and therefore do not stimulate further release of GnRH *(12)*. This positive response of the hypothalamus to estrogen may be altered by the presence of synthetic estrogens in the body *(13)*. In this study, we determine the effect of neonatally administerd alkylphenols on the development of reproductive axis during the prepubertal and pubertal period.

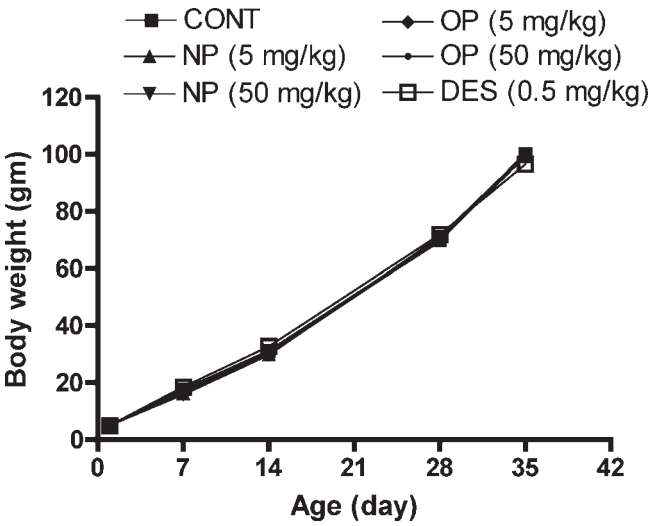


Fig. 1. Effects on body growth curve. The means ± SEM values of body weight at different times during the developmental period in animals treated neonatally with NP, OP, DES, or vehicle alone (control). Note that the overall growth of the animals is similar among all of the groups. $n = 6–8$.

Results

Effect on Puberty Onset

Body weights were determined weekly in animals treated with OP, NP, and DES and in control animals treated with vehicle. Comparison of the body growth curves identify no significant differences among the treatment groups ($p > 0.05$) (Fig. 1). The age of vaginal opening was significantly ($p < 0.001$) shorter in the rats being treated with 50 mg/kg of OP or 0.5 mg/kg of DES than in the control animals (Fig. 2). However, the low dose (5 mg/kg) of OP moderately, but not significantly, shortened the day of vaginal opening. Both doses of NP failed to significantly alter the timing of vaginal opening.

Determination of the incidence of reproductive failure in these rats during puberty revealed that the DES-treated and the high-dose (50 mg/kg) OP-treated animals had 100% and 85% reproductive failure rates (Fig. 3A). The lower dose OP-treated animals had an approximate 36% reproductive failure rate. Only a small group (18%) of higher-dose NP-treated animals showed reproductive failure. Control and low-dose NP-treated animals showed no reproductive failure and minimal reproductive failure, respectively, as most

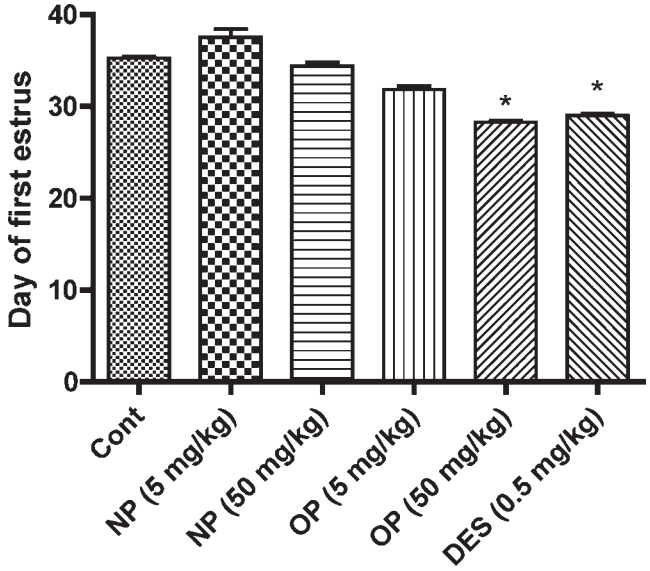


Fig. 2. Comparison of day of vaginal opening. Mean ± SEM values of the day of first estrus (sign of puberty) in animals treated neonatally with OP, NP, DES, or vehicle (control) are shown. *$p < 0.05$ compared to control group. $n = 6–8$.

of their ovaries had corpora luteua. Ovarian weights of the alkylphenol, DES, and control groups reflected the rate of reproductive failure; DES- and high-dose OP-treated groups showed lower ovarian weights than the control and NP-treated groups (Fig. 3B). The ovarian weights among control, NP-, and low-dose OP-treated groups were similar. The uterine weights of DES- and high-dose OP-treated groups were higher than the control and NP-treated groups (Fig. 3C), showing the estrogenic property of these agents. The uterine weights of control, NP-, and low-dose OP-treated treated groups were similar.

Pubertal Plasma LH Levels

Because DES treatments during the developmental period have been shown to induce reproductive failure in pubertal rats by preventing preovulatory release of LH *(6,13)*, we determined whether the pubertal LH release is altered in OP-treated animals. Determination of LH levels in plasma collected during 1300–1800 h on the day of the first proestrous (prior to vaginal opening) revealed that only the control group exhibited a prepubertal LH surge (Fig. 4; peak LH approx 19 ng/mL). In contrast, animals treated with a 50-mg/mL dose of OP did not exhibit a preovulatory LH surge (<5000 pg/mL), but did consistently have higher basal serum LH levels (2500 pg/mL vs 1000 pg/mL for the controls). Overall curves produced a difference of $p = 0.0033$.

Estradiol-Induced Plasma LH Levels

Estradiol treatment during early postnatal life has been shown to defeminize the reproductive neuroendocrine brain and to result in a loss of steroid positive feedback action on

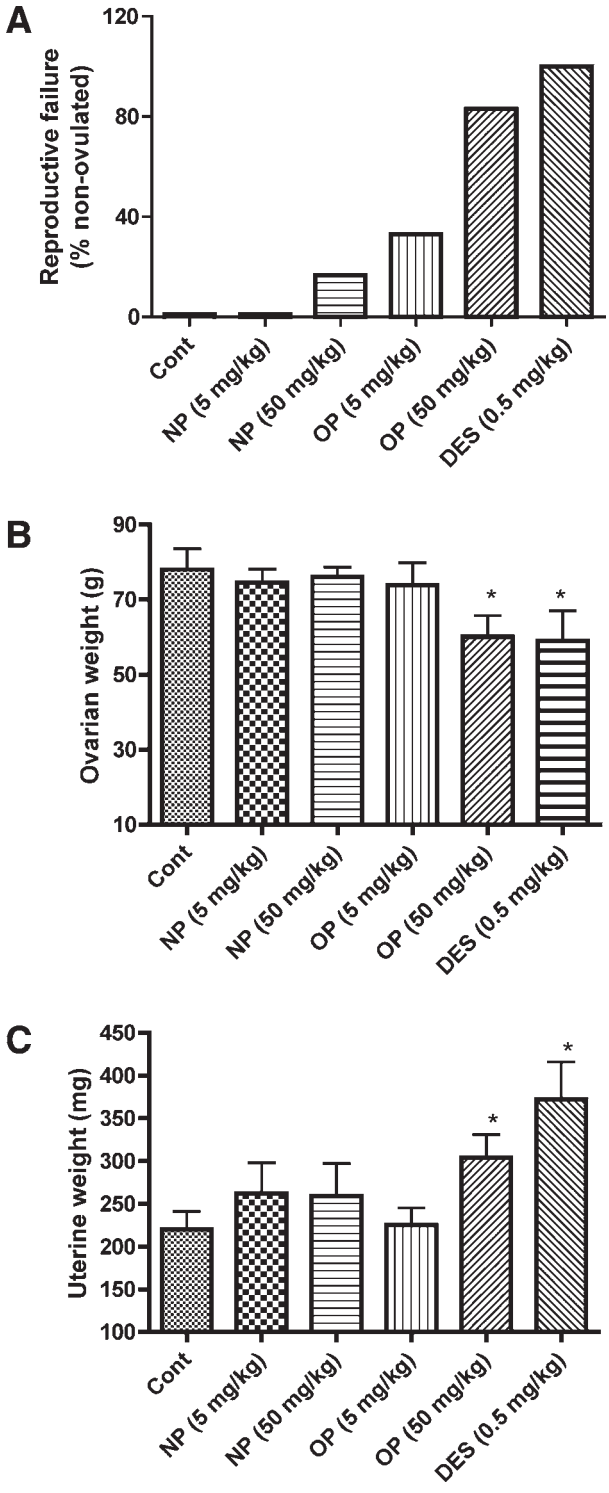


Fig. 3. Reproductive development during puberty. Demonstrates the effects of neonatal treatments with OP, NP, or DES on reproductive failure as determined by the percentage of rats that failed to ovulate (**A**). The percentage of animals showing no corpus luteum on the day of vaginal opening was calculated and used as the percentage of animals with reproductive failure. $n = 6–8$. Note that a large percentage of OP- and DES-treated rats had reproductive failure. The mean ± SEM values of ovarian weights (**B**) and uterine weights (**C**) of these animals are shown. *$p < 0.05$ compared to control group. $n = 6–8$.

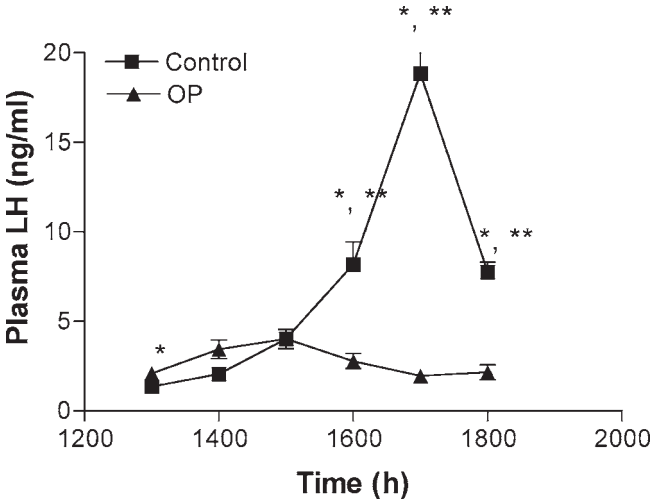


Fig. 4. Pubertal preovulatory LH release. Plasma levels of LH on the day before vaginal opening of animals treated neonatally with vehicle (control) or OP were determined. *$p < 0.05$ compared to LH levels of control animals at 1300 h. **$p < 0.05$ compared to the rest of the LH values of control animals at other time points. n = 4–7.

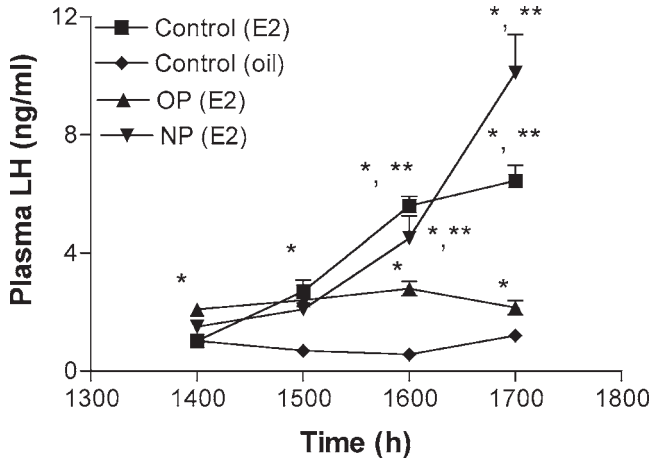


Fig. 5. Plasma LH response to estradiol. Juvenile female rats treated neonatally with control, OP (50 mg/kg), or NP (50 mg/kg) were sc implanted with an estradiol capsule to determine their LH response to the steroid. Some control animals were implanted with an empty capsule. n = 4–8. *$p < 0.05$ compared to LH levels, at the same time point, of control animals treated with oil. **$p < 0.05$ compared to LH values of similarly treated animals at 1400 h.

LH release *(11,13)*. Whether the OP-treated animals had altered steroid feedback action was determined. As shown in Fig. 5, treatment with an estradiol capsule, during the juvenile period induced an increased afternoon release of LH in plasma of control rats. Control rats treated with an empty capsule did not have increased LH release. The 50-mg/kg dose of NP, which had no effect on ovulation, also showed a positive LH response to estradiol. However, estradiol treatment failed to increase LH levels in OP-treated animals.

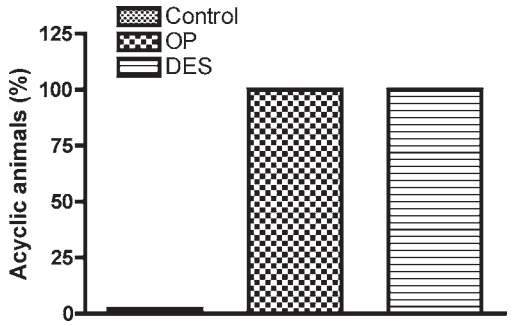


Fig. 6. Percentage showing abnormal estrus cycle. Vaginal smears revealed that 100% of the rats in the OP (50 mg/kg) and DES (0.5 mg/kg) groups exhibited a persistent estrus after vaginal opening, while 100% of the control animals had regular 4–5-d cycles. Persistent estrus was determined by the constant presence of nucleated epithelial and cornified cells. n = 5–14.

Estrous Cyclicity

Estrous cycles of the OP, DES, and control groups were inspected between d 40 and 60 after birth. Vaginal smears revealed that 100% of the rats in the 50-mg/kg OP (14/14) and 0.5-mg/kg DES (5/5) groups exhibited persistent estrus after vaginal opening while 100% of the control animals (11/11) all had regular 4–5-d cycles following puberty (Fig. 6). Persistent estrus was determined by the constant presence of nucleated epithelial and cornified cells.

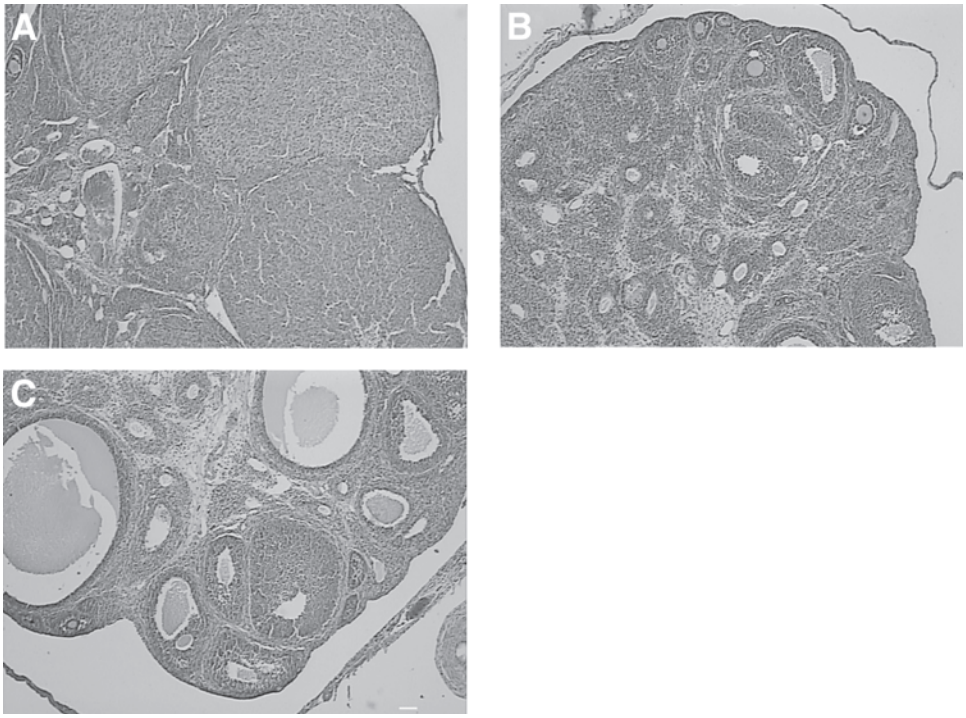
Ovarian Histology

Histologically, the ovaries of both the OP- and DES-treated rats contained significantly more preantral ($p < 0.05$) and atretic ($p < 0.01$) follicles than did those of the control group (Fig. 7). In addition, the OP and DES groups showed a significantly reduced number of corpora lutea ($p < 0.001$). No significant differences were observed in the number of antral follicles among the three treatment groups ($p > 0.05$).

Discussion

In the present study, administration of 50 mg/kg of OP during the early postnatal period altered development of the reproductive system in the form of accelerated vaginal opening, persistent estrus, the absence of a LH surge, no positive feedback action to estradiol, and anovulation. NP, 10-fold less potent than OP, failed to produce any significant effects on the development of the female reproductive system. These findings suggest that neonatal OP treatment defeminizes the neuroendocrine brain and therefore alters gonadotropin levels and, subsequently, the development of the acyclic reproductive function.

Development of the cyclic reproductive function is dependent upon the sexual differentiation of the neuroendocrine



D. Mean ± SEM number of various follicles and corpora lutea in each ovary.

	Control (n = 8)	Octylphenol (n = 13)	DES (n = 4)
Preantral follicles	5.3 ± 0.6	9.92 ± 1.8*	13.2 ± 1.2*
Antral follicles	5.9 ± 0.64	6.15 ± 1.13	5.3 ± 0.8
Atretic follicle	0.5 ± 0.19	16.3 ± 2.8*	21.0 ± 5.7*
Corpora lutea	8.6 ± 0.8	1.9 ± 0.4*	0.2 ± 0.2*

*$p < 0.01$, compared to control; **$p < 0.01$, compared to DES.

Fig. 7. Ovarian histological features. Animals given oil alone, OP, or DES were compared with regard to their ovarian histology. *Top*. Representative photographs of a ovary of vehicle (control), DES-, and OP-treated, 60-d old rats on the day of estrus. "–" equals 10 μm. *Bottom*. Summarizes the data on follicular maturation. Note that both OP-treated and DES-treated animals contained significantly ($p < 0.05$) more preantral follicles than the control animals. Antral follicles showed no significant difference among the three treatment groups ($p > 0.05$). Both OP ($p < 0.01$) and DES ($p < 0.01$) exhibited a greater number of atretic follicles when compared to the control group. OP ($p < 0.001$) and DES ($p < 0.001$) ovaries contained significantly fewer corpora lutea when compared to the control group.

brain. During the critical period of sexual differentiation, the female brain is protected from exposure to estrogen by systemic alpha-fetoprotein which has the ability to bind estrogen *(11)*. Owing to the fact that alpha-fetoprotein cannot cross the blood–brain barrier, circulating estrogen does not affect brain development in the female. Exposure to synthetic estrogens, which may not have the ability to bind to alpha-fetoprotein and therefore able to cross the blood–brain barrier, alter normal female brain development *(13)*. The biological effects of estrogenic compounds are mediated through binding to the estrogen receptor *(14)*. This has been observed to occur with DES, a non-steroidal estrogenic compound *(15)*.

In the present study, the age of vaginal opening of OP-treated animals was found to be accelerated as compared to the control animals. In addition, all OP- and DES-treated animals exhibited a persistent estrus as opposed to the normal 4–5-d cycle of the control group. These results are consistent with the effects in studies on neonatal administration of DES to rodents *(6)*. Previously, persistent estrous has been observed when OP was treated neonatally at doses of 100 mg/kg or above *(16)*, but these effects have not been seen until now at the dose of 50 mg/kg. We have also used a 5-mg/kg dose of OP, but this dose produced a moderate or no effect on puberty onset or on the development of the reproductive tract. Also, we did not see any significant effects of

NP on puberty onset or on the reproductive tract development. The failure of the NP doses we used to significantly affect reproductive development might be related to its low estrogenic activity *(8)*.

Serum LH levels of the OP-treated animals, when compared to the control animals, revealed slightly higher basal levels and the absence of a LH surge. This is consistent with data that identify the absence of cyclic release of pituitary gonadotropin in animals treated with steroids (such as testosterone propionate) neonatally *(17)*. It is hypothesized that the mechanism of this dysfunction is due to the fact that the increase in plasma estrogen in the defeminized female is not at a sufficient amount to induce the LH surge.

During sacrifice, an increase in uterine weights was noted in OP-treated animals. Previous studies of OP treatment have found cell proliferative activity of the endometrium as well as an increase in uterine weights *(16,18)*. The present study did not observe a significant difference in somatic growth between the control and the OP-treated groups at a dose of 50 mg/kg. However, a significant reduction of body weight was previously demonstrated in animals treated with a higher dose of 100 mg/kg of OP *(16)*.

Ovarian histology of OP-treated rats revealed a significantly increased number of preantral and atretic follicles as well as a decrease in the number of corpora lutea, indicating anovulation. It has been reported that the persistent estrus stage in rats is accompanied by anovulation *(19)* that is consistent with the data obtained in this study. These effects are most likely due to the fact that although there is both natural and synthetic estrogenic material to cause follicular development, the estrogen does not increase at the rate necessary to induce a LH surge and is therefore unable to elicit ovulation. Anovulation is indicated by the absence of corpora lutea and the increase in number of atretic follicles. In humans, polycystic ovary syndrome—showing similar effects on ovarian structure—is a predisposing factor for many diseases including breast, uterine, and ovarian cancer *(20)* and is therefore a cause for concern in women's health. Alkylphenolic compounds have been found to have the ability to bioaccumulate in mammalian tissues and the additive effect of environmental estrogens has also been reported *(21)*. A source of concern is small doses exposed to a fetus for a prolonged periods of time during development and producing toxic effects on reproductive development.

In conclusion, neonatal subcutaneous exposure to OP at a dose of 50 mg/kg altered puberty onset, ovulation, estrus cyclicity, LH levels, estradiol's positive feedback action on LH, and ovarian histology. In addition, uterine weights were increased, while somatic growth did not show significant differences. These results suggest that the developmental hormone secretion is altered due to a hypothalamic–pituitary–ovarian disorder that works to defeminize the female neuroendocrine brain, and changes in estrus cyclicity and ovarian histology arise from this dysfunction.

Materials and Methods

Animals and Housing

All animals were maintained and cared for under controlled environmental conditions on a 12-h light, 12-h dark cycle, fed with laboratory food, and given tap water *ad libitum*. Animal surgery and care were in accordance with institutional guidelines and complied with the NIH policy. The animal protocol was approved by the University Animal Care and Facilities Committee.

Treatment Regime

Pregnant Sprague–Dawley female rats, obtained from Simonsen Laboratories (Gilroy, CA), were allowed to give birth and were used as donors for neonates. Newborn female rats were injected subcutaneously for a period of 9 d (d 1–10 after birth) with corn oil (negative control); OP (4-*tert*-octylphenol; 97% purity; cat. no. 29,082-3; Aldrich Chemical Co., Inc., Milwaukee, WI; 5 and 50 mg/kg); NP (4-nonylphenol; cat. no. 44-2873; Supalco, Bellefonte, PA; 5 and 50 mg/kg); and DES (diethylstilbestrol; cat. no. D4628, Sigma, St. Louis, MO; 0.5 mg/kg; positive control). Drugs were dissolved in corn oil, and all animals were given the conserved volume of 10 μL/g. Pups were switched in order to maintain genetic variation. This was done by anesthetizing the mother with halothane, a gas anesthetic, in order to stifle her sense of smell so that she would be unable to differentiate the pups. Pups with the same dam were given the same compound to avoid cross contamination. Litter sizes were kept at eight, with male pups present until weaning at 25 d. Weekly weights were taken to determine the effects on somatic growth.

Puberty

The day of vaginal opening was recorded and used as an indicator of puberty onset. Some of these pubescent animals were sacrificed, and their ovarian and uterine weights were recorded. The ovaries were examined visually for the presence of corpora lutea to determine if ovulation had taken place.

Pubertal LH Levels

Before the onset of puberty, the jugular vein was surgically cannulated by the following procedure in order to provide access to the blood supply for hourly blood collection. Animals were put under general anesthesia (sodium pentobarbitol, 40 mg/kg, ip), and a 2-cm incision was made over the left external jugular vein extending from the clavicle to the ear. The vein was isolated by gently removing overlying fat and connective tissue with forceps. Two ligatures were slipped under the jugular vein and were used to hold the catheter in place. The superior ligature was tied off, and microscissors were used to puncture the wall of the jugular vein. Silastic tubing was filled with a solution of 40% polyvinylpyrrolidone (PVP) in heparinized saline (40

U/mL), inserted through the hole and advanced. The two ligatures were then secured. The closed tip of a hemostat was inserted between the skin and muscle layer and was used to create a subcutaneous pocket to feed the catheter through to the dorsal side of the animal. This was secured by a third ligature in the muscle layer of the back. The incision was then sutured closed, and the cannula was tied shut. Cannulated animals were allowed a 48 h recovery time prior to blood collection and were housed separately during this time. Catheters were flushed daily with the 40% PVP in heparinized saline (50 U/mL) solution.

Blood collection occurred the day before the onset of puberty, generally the day before vaginal opening. We confirmed this by the presence of proestrous-type cells in vaginal smears from control animals. Each control animal's vagina was artificially opened with a glass pipet *(22)*. Polyethylene tubing was connected through a hollow metal tube to the silastic tubing of the cannula, hung outside the animal's cage and connected to a three-way valve and a 1-mL syringe filled with a 40% PVP heparinized saline solution. Beginning at 1300 h, blood was collected in these heparinized syringes every hour for 6 h from control and OP-treated animals. It was centrifuged, and erythrocytes were returned to the animals in equal volumes of 0.9% sterile saline. Serum samples were stored in an ultra-low freezer (−80°C) until use.

Estradiol-Induced LH Release

On the morning of d 27 after birth, the jugular vein was surgically cannulated as described previously. These cannulated rats were also implanted sc with an empty or estradiol-filled 0.5 mm silastic capsule (Dow Corning Corp., Midland, MI). These animals were allowed a minimum recovery time of 72 h prior to blood collection and were housed separately during this time. Blood collection from these rats occurred between 1400 and 1700 h on d 30 after birth. Blood was collected in heparinized syringes and centrifuged; erythrocytes were returned in equal volumes of 0.9% sterile saline. Plasma samples were stored in an ultra-low freezer (−80°C) until use.

Radioimmunoassay for LH

The LH levels of plasma samples were determined by radioimmunoassay (using the kit provided by the National Hormone and Pituitary Program, with values expressed in terms of NIDDK rLH-RP-3 equivalents). All samples were assayed in a single assay. Detection limits for the LH assay were <200 pg/mL, and the intra-assay coefficients of variation for this assay were 8%.

Estrus Cyclicity

Vaginal smears of rats were performed between 40 and 60 d of age in order to determine the effect of alkylphenols on the estrous cycle. This was done daily by flushing out the vaginal epithelium with 0.9% saline and microscopically examining the fluid. The cell types corresponding with the stage of the estrous cycle were used to determine reproductive problems. Animals were assessed for the presence of estrus cyclicity and categorized as exhibiting either persistent estrus or a typical 4–5 d cycle.

Ovarian Histology

Between 60 and 65 d of age, animals were sacrificed by rapid decapitation—this method is consistent with the recommendation of the panel on Euthanasia of the American Veterinary Medical Association. At the time of sacrifice, control animals ($n = 8$) were at the stage of estrus and the OP ($n = 13$)- and DES ($n = 4$)-treated animals showed vaginal smears containing nucleated and cornified epithelia. Ovaries were collected, fixed in 4% formalin, stored in 70% ethanol, and histologically processed in order to confirm whether ovulation had occurred. Histological processing of the ovaries included routine staining with hematoxylin and eosin, embedding in paraffin, and sectioning (4 μm sections) the resulting block, and routine staining of the serial sections with hematoxylin and eosin. Upon microscopic examination of the whole ovary, the presence and number of corpora lutea, preantral, antral and atretic follicles were determined in order to assess reproductive function.

Statistical Analysis

The data shown in the figures and text are mean ± SEM. Comparisons between two groups were made using *t*-tests. Data comparisons between multiple groups were made using one-way or two-way analysis of variance (ANOVA) as appropriate. Student–Newmann–Keuls test was used as post-hoc test. A value of $p < 0.05$ was considered significant.

Acknowledgments

The authors thank Susan Chapman for technical assistance in animal maintenance and histology work, and the NIDDK for supplying the LH assay kit. This work is supported by NJAES-HATCH grant no. 06138 and NIH grant no. AA-00220 to D. K. S.

References

1. Colborn, T., vom Saal, F. S., and Soto, A. M. (1993). *En. Health Prospect.* **101,** 378–384.
2. Nimrod, A. C. and Benson, W. H. (1996). *Crit. Rev. Toxicol.* **26,** 335–364.
3. Clark, L. B., Rosen, R. T., Hartman, T. G., et al. (1992). *Int. J. Environ. An. Ch.* **47,** 167–180.
4. Dohler, K. D., Hancke, J. L., Srivastava, S. S., Hofmann, C., Shryne, J. E., and Gorski, R. A. (1984). *Prog. Brain Res.* **61,** 99–117.
5. White, R., Jobling, S., Hoove, S. A., Sumpter, J. P., and Parker, M. G. (1994). *Endocrinology* **135,** 175–182.
6. Bern, H. A., Edery, M., Mills, K. T., Hohrman, A. F., Mori, T., and Larson, L. (1987). *Cancer Res.* **47,** 4165–4172.
7. Bogh, I. B., Christensen, P., Dantzer, V., et al. (2001). *Theriogenology* **55,** 131–150.

8. Laws, S. C., Carey, S. A., Ferrell, J. M., Bodman, G. J., and Cooper, R. L. (2000). *Toxicol. Sci.* **54,** 154–167.
9. Harazono, A. and Ema, M. (2001). *Toxicol. Lett.* **119,** 79–84.
10. Herath, C. B., Watanabe, G., Katsuda, S., Yoshida, M., Suzuki, A. K., and Taya, K. (2001). *Biol. Reprod.* **64,** 1216–1224.
11. Gorski, R. A. (1976). *Annu. Rev. Physiol.* **38,** 425–450.
12. Urbanski, H. F. and Ojeda, S. R. (1987–1988). *Reprod. Toxicol.* **1,** 129–138.
13. Faber, K. A. and Hughes, C. L. Jr. (1991). *Biol. Reprod.* **45,** 649–656.
14. Korach, K. S. (1994). *Science* **266,** 1524–1527.
15. Iguchi, T. (1992). *Int. Rev. Cytol.* **139,** 1–57.
16. Yoshida, M., Katsuda, S., Ando, J., Kuroda, H., Takahashi, M., and Maekawa, A. (2000). *Toxicol. Lett.* **116,** 89–101.
17. Harlan, R. E. and Gorski, R. A. (1977). *Endocrinology* **101,** 741–749.
18. Katsuda, S., Yoshida, M., Watanabe, G., Taya, K., and Maekawa, A. (2000). *Toxicol. Appl. Pharmacol.* **165,** 217–226.
19. Jih, M. H., Lu, J. K., Wan, Y. J., and Wu, T. C. (1993). *Biol. Reprod.* **49,** 1208–1214.
20. Huber, J. C., Schneeberger, C., and Tempfer, C. B. (2002). *Maturitas* **41(Suppl. 1),** S55–S64.
21. Ramamoorthy, K., Wang, F., Chen, I. C., et al. (1997). *Science* **275,** 405.
22. Sarkar, D. K. and Fink, G. (1979). *J. Endocrinol.* **83,** 339–354.