Reproductive Malformation of the Male Offspring Following Maternal Exposure to Estrogenic Chemicals (44516)

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Abstract. **Recently, significant concerns have been placed on the widespread use of chemicals with persistent estrogenic activity for their long-term effects on human health. In this communication, we investigated whether fetal exposure to some of these chemicals at doses consumed by people, has any long-term effect on the reproductive functions of the male offspring. Thus, time-pregnant CD-1 mice were fed diethylstilbestrol (DES), bisphenol A (BPA), and aroclor (aroclor 1016) at an average concentration of 100 ng/kg/day, 50 μg/kg/day, and 50 μg/kg/day, respectively, during Days 16–18 of gestation. A high dose of DES (200 μg/kg/day) was also tested to compare the results of the current study with those of others using the high dose only. The offspring were examined at Day 3, Day 21, and Day 60 following birth. We demonstrated that BPA, aroclor, and the lower dose of DES enhanced anogenital distance, increased prostate size, and decreased epididymal weight. No effect was found on the testicular weight or size. The chemicals also permanently increased androgen receptor (AR) binding activity of the prostate at this dosage. This is the first demonstration that environmental chemicals program AR function permanently at the dosage consumed by the general population. The higher dosage of DES, on the other hand, produced an opposite effect, decreasing prostate weight, prostate AR binding, and anogenital distance, thus confirming the previous reports. To investigate whether the above mentioned effects of the chemicals represent direct or indirect effects, we also tested the effect of the chemicals on prostate development *in vitro*. Thus fetal urogenital sinus (UGS), isolated at the 17th day of gestation was cultured with the chemicals in the presence and absence of testosterone (10 ng/ml) for 6 days, and prostate growth was monitored by determining the size and branching of the specimen following histology. Results showed that these chemicals induced prostate growth in the presence and absence of testosterone. They also increased androgen-binding activity. Thus, the results of the *in vivo* studies were reproduced in the *in vitro* experiments, suggesting a direct effect of these chemicals on the development of fetal reproductive organs. This is the first demonstration that estrogenic chemicals induce reproductive malformation by direct interference with the fetal reproductive organs and not by interfering with the maternal or fetal endocrine system. The chemicals are able to induce malformation even in the absence of fetal testosterone; however, they are more effective in the presence of testosterone.** [P.S.E.B.M. 2000, Vol 224:61–68]

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Use of chemicals with mild but persistent estrogenic activity has increased significantly in our lives. Everyday household products, namely, pesticides, components of plastics, detergents, hand creams, and birth control pills are the source of such activity (1–4). There is some evidence that inappropriate exposure to estrogens in man as in animals can cause abnormal male reproductive functions, such as, lower sperm production, prostatic hyperplasia, and testicular cancer (5–7). However, little is known

about the effect of the estrogenic chemicals on the developing organism at the dosage that is consumed by people in their daily lives. Recently, it has been shown that an increase in estrogen level by only 0.1 pg/ml in the fetal mouse induces permanent enhancement in prostate size and androgen action (8). This prompted us to determine the effect of some of the commonly used estrogenic chemicals on the development of the fetal mouse reproductive system using the dosage that falls within the environmental level of these chemicals approved by the FDA. Bisphenol A (BPA, 2,2-bis [4-hydroxyphenyl] propane) is one such compound that is used to line food and drink cans (9, 10). Bisphenol A is also a component of plastic used in dental fillings, which are often used to protect teeth in children (11). A significant amount of BPA is released from these commonly used products (9), the level of which (20–40 μg/kg) was shown to have estrogen-like activity when tested on the MCF7 cells (12). Some of the polyclorinated biphenols (PCB) also showed estrogenic activity (13, 14). A high concentration of these chemicals is found in the environment of the big cities. As an example, a high concentration of PCB was found in the soil and air of the National Priorities landfill of Southern Illinois (13). Both soil (346 mg PCB/kg) and air (175 mg PCB/kg) extracts of this landfill caused a greater than 30% increase in uterine wet weight, suggesting presence of an estrogen-like effect in the extract (13). In this study, we tested the effect of three such environmental estrogenic products, namely, BPA, aroclor 1016 (a PCB product), and a very low dose of diethylstilbestrol (DES) for their effect on the development of male reproductive functions. Additionally, we examined whether these chemicals have a direct effect on the developing fetus by determining the effect of these chemicals on the fetal prostate grown *in vitro*. The results showed that environmental levels of all these chemicals had a profound effect on the developing reproductive functions. The effects were permanent and produced by direct interaction of the chemicals on the developing organs.

Materials and Methods

Animals. Time-pregnant CD-1 mice were purchased from the Charles River breeding laboratory (Wilmington, MA). The animals were received on the 12th day of gestation counting the day of breeding as Day 0 of gestation, and they were used in the experiments on the 16th day of gestation. The animals were maintained on Purina Chow-5 L9 diet in the Charles River Laboratory, and they were maintained on Purina Chow 5012 diet in our institute. Pregnant mice were fed different chemicals or vehicle in 25 μl of corn oil containing 10% ethanol at Days 16–18 (critical period of prostate development) of gestation as indicated in the text. Following delivery, each litter was adjusted to contain eight offspring (at least three of which were male) so that maternal nutritional effect remained constant in different litters. An equal number of offspring (1–3) was pooled from each litter in a group to compare the results from one group with the other. The offspring were weighed; their anogenital distance was measured; and the weight of different reproductive organs was assessed. To determine androgen function of the prostate, androgen binding was assessed as described in a later section.

For examination of prostate growth, whole tissue mount was prepared from the 15-day-old offspring. In brief, the entire prostate tissue was stripped off of the urethra with the aid of a dissecting microscope. The extraneous fibrous tissue and bladder were removed. The tissue was then carefully stretched onto filter paper, which was immediately immersed in 10% formalin in normal saline until ready for staining. The filter papers with the tissue were transferred to 70% ethanol overnight and then exposed to alcoholic toluidine blue (0.5 g in 20% ethanol) for 15–30 sec. The excess stain was rinsed off in water. The samples were taken in a stepwise fashion to absolute ethanol and then to xylene. After overnight exposure to xylene, the tissues were mounted in parmount.

Organ Culture of Prostate. Fetal UGS was microdissected from the junction of bladder and urethra at the 17th day of gestation (onset of prostate development). The organ culture assay of prostate development was carried out as described for rat prostate (15). In brief, stainless steel grids were placed inside the 60-mm organ culture Falcon Petri dishes (Miles Plastics, Oxnard, CA). A strip of 1% Difco Agar (3 mm thick, 45 mm long, and 10 mm wide) was placed on the grid. The UGS and its ventral prostatic region was placed on the surface of agar, and dye-free culture medium [Dulbecco's modified eagle medium (DMEM) and DMEM-F-12 1:1] was added in the culture dish. Serum was not used, but insulin and transferrin were added, each at 10 μg/ml, into the medium. The chemicals were added as described in the text, and the medium was changed every other day. To mimic the *in vivo* condition of testicular testosterone, the chemicals were tested in the presence of physiological levels of testosterone in some experiments. The culture dishes were then placed in a humidified 5% CO_2 incubator and incubated at 37°C with various agents. On Day 7, the cultures were terminated, and the organs were fixed in Bouin's solution for 4 hr, then transferred into 70% alcohol and finally transferred into 20% sucrose-PBS buffer and allowed to equilibrate for 20 hr each time. The fixed tissue was then used for histologic sectioning as described below.

Histology and Assessment of Prostate Differentiation *In Vitro*. The histology of the prostate from the organ culture experiments was performed as described earlier (15). In brief, the fixed tissue specimens were placed in Tissue-Tek OCT (Miles, Elkhart, IN) and frozen at –70°C. Ten-micrometer sections were cut transversely along the urethra using an AO histostat microtome (International Equipment Company, Needham Heights, MA) and stained with hematoxylin and eosin. All serial sections of the specimen were examined, and branching-appearance as well as the size of the specimen were monitored. On the average, each specimen produced 60–70 sections, and usually, one of

the 30th to 35th sections provided full details of the organ development. This section was used in determining the size and branching, shown in tables and figures. The size of the specimen was determined by calibrated micrometer disc attached to the microscope.

Preparation of Prostate Cells and Determination of Androgen Binding Using the Cell Preparation. Prostates were isolated at Day 3, Day 21, and Day 60 of age in RPMI 1640 medium and used in isolating cells as described elsewhere (16). Two to six prostates were pooled per isolation, depending on age of the offspring. The tissues were transferred to 1 ml of F-12 medium containing 10% fetal calf serum and minced into small pieces. Collagenase (type 1, Sigma, St. Louis, MO) was added at a concentration of 3 mg/ml, and the mixture was incubated at 37°C for 45 min with constant shaking. At the end, the cells were dislodged from the clumps by pipetting in and out. The undissociated cellular clumps were removed by allowing the mixture to settle for 5 sec and removing the cell suspension from the top. The collagenase treatment was repeated using the undissociated sediment, and the cell suspension from the two steps of isolation was combined. The cells were centrifuged at 1000x RPM, and the pellet was washed with 10 ml of the F-12 medium. The cell viability of the preparation ranged from 75%–85%. The mixture contained two types of small round cells (possibly muscle and stroma cells) and large multinucleated cells (epithelial cells).

Androgen binding was measured using the prostate cell preparation as described previously (17). In brief, the cells (10^5 cells/tube) were mixed with mibolerone (17a-methyl-3H, 84 Ci/mmol, Dupont-NEN, Boston, MA) at a concentration of 0.1–1 n*M* for 1 hr in the presence and absence of 1 μ*M* unlabeled Mibolerone (Dupont-NEN, Boston, MA) in 1 ml of DMEM containing 0.1% BSA. The mixtures were incubated for 1 hr at 37°C in a shaking incubator. The reaction was stopped adding 500 μl of cold DMEM medium. The unbound steroid was removed by washing the cells with DMEM three times. The cells were transferred into scintillation vials to determine the bound radioactivity. The binding data were analyzed by a program, called EBDA (18). The files produced from this analysis were analyzed by a second program called LIGAND (18). Scatchard plots were generated, and the total number of binding sites and dissociation constant were calculated from the plots.

Experimental Plan and Data Analysis. The number of offspring was adjusted to eight per litter, each containing at least three males. Fifteen litters were assigned for each treatment. For determining organ weight and biochemical analysis, 15 offspring (1 from each of the 15 litters) were used for each time point. For body weight and anogenital distance, 45 animals (3 from each of the 15 litters) were examined at Day 3, 30 animals (2 from each of the 15 litters) were examined at Day 21, and 15 animals (1 from each of the 15 litters) were examined at Day 60. The data were tested by ANOVA to determine statistical significance.

Results

Effect of Prenatal Exposure to the Estrogenic Chemicals on the Development of External Genitalia. To investigate whether the estrogenic chemicals modulate androgen-dependent development, we first examined the anogenital distance of the male offspring that had been exposed to these chemicals prenatally. Thus, CD-1 pregnant mice (15 in each group) were fed different doses of BPA, aroclor, DES, or vehicle as described in the Materials and Methods section. The doses of these chemicals were chosen based on the environmental level of these chemicals (9–14) that is considered safe by the FDA. Thus, BPA and aroclor were fed to the CD-1 pregnant mice at 50 μg/kg. Since DES was shown to have 100-fold higher estrogen activity compared with BPA, DES was fed only at 100 ng/kg dosage. A higher dose of DES (200 μg/kg) was also examined to compare the current findings with those reported elsewhere (6). The offspring were examined for the development of anogenital distance using a micrometer. None of the chemicals induced fetal resorption or affected litter size (Gupta C, unpublished data). As shown in Table I, these chemicals, except a high dose of DES, produced no effect on the body weight. However, all of these chemicals, including a low dose of DES, enlarged anogenital distance of the male offspring, especially at the adult period (Table I). The anogenital distance results, shown in Table I, were corrected against the body weight; however, the uncorrected values also

Table I. Effect of Prenatal Exposure to Estrogenic Chemicals on Anogenital Distance (AG) of the Male Offspring

Drug	Dosage (ng/g)	Day 3		Day 21		Day 60	
		Body wt (g)	AG (mm/kg)	Body wt (g)	AG (mm/kg)	Body wt (g)	AG (mm/kg)
None	—	1.8 ± 0.1	1777 ± 55	15 ± 2.2	466 ± 26	39 ± 2.3	474 ± 13
DES	0.1	1.6 ± 0.1	2375 ± 66[a]	16 ± 2.5	512 ± 25[a]	34 ± 3.3	600 ± 32[a]
	200.00	1.4 ± 0.2[b]	1561 ± 114[b]	15 ± 1.7	413 ± 33[b]	29 ± 3.6[b]	406 ± 39[b]
BPA	50.00	1.7 ± 0.2	2176 ± 176[a]	16 ± 2.6	582 ± 43[a]	32 ± 1.5	631 ± 41[a]
Aroclor	50.00	1.8 ± 0.2	2222 ± 277[a]	17 ± 3.2	617 ± 64[a]	35 ± 2.6	634 ± 60[a]

Note. Data represent mean ± SD, n = 15 to 45; [a] $P < 0.05$ (larger) compared with the vehicle control; [b] $P < 0.05$ (smaller) compared with the vehicle control by ANOVA test. AG, anogenital distance. Forty-five offspring were examined in each group at Day 3; 30 offspring at Day 21, and 15 offspring at Day 60.

showed similar effects of different chemicals (Gupta C, unpublished data). DES at high dosage, on the other hand, decreased body weight and anogenital distance (Table I), thus confirming some of the previous findings with DES (6). These chemicals produced no effect on the anogenital distance of the female offspring (unpublished data), suggesting that the effects may be associated with modulation of androgen-induced effects.

Effect of the Estrogenic Chemicals on the Development of Male Internal Genital Organs. The results described in the previous section demonstrated that the estrogenic chemicals modulate development of external genitalia. This prompted us to examine the effect of these chemicals on the development of internal genital organs in the male offspring. As shown in Table II, all of these chemicals enhanced prostate weight but decreased epididymal weight. No effect was observed on testicular and vas deferens weight (Gupta C, unpublished data). Enhancement of prostate weight by these chemicals was found even at age 3, but the effect was intensified when the offspring reached the adult age (Table II). Again, the organ weights, shown in Table II, have been corrected for body weight; however, the uncorrected values of the prostate and epididymal weight demonstrated similar effects of the chemicals (Gupta C, unpublished data). DES at a very low dosage increased prostate weight (Table II) but at high dosage, it actually decreased the prostate weight (Table II).

A group of animals (four in each group) from each treatment group was also examined at 15 days of age to determine the prostate growth. As shown in Figure 1, the size of this gland was significantly increased in the offspring, which were exposed to these chemicals prenatally.

Effect of the Estrogenic Chemicals on Androgen Binding. The mechanism by which the estrogenic chemicals modulate the androgen-dependent development is not known. One possibility is that they alter androgen function by stimulating androgen receptor binding activity. Previous studies with a low dose of estradiol demonstrated enhancement of AR binding activity (8). We examined whether these estrogenic chemicals induced similar effects in the male offspring. Thus, AR binding activity was determined in the cellular preparation of the prostate, isolated at different ages of development from the offspring exposed to the estrogenic chemicals prenatally. As shown in Figure 2, all of these chemicals induced prostate AR binding activity. The effect of aroclor was found as early as 3 days of age. However, the effect was more pronounced when the offspring reached puberty or adult age (Fig. 2). No significant effect was noted in the dissociation constant of the AR binding activity (Gupta C, unpublished data). DES at high dosage significantly reduced AR binding activity (Gupta C, unpublished data), thus confirming the findings of other reports.

***In Vitro* Effect of Estrogenic Chemicals on Prostate Growth.** In the above experiments, estrogenic chemicals were administered during the prenatal period. This approach suffered from uncertainties about the differential metabolism of these compounds in circulation and binding to serum proteins in mothers, their transport through the fetoplacental barrier, and the amounts of active substance reaching fetal target tissues. Additionally, it was not known whether the effect of these chemicals was mediated through a maternal effect or whether they directly affected prostate development. To answer these questions, we set up an organ culture assay using fetal prostate gland isolated at the 17th day of gestation (onset of prostate development) and determined the effect of estrogenic chemicals. To investigate the effect under a physiological setup of testicular testosterone, we also determined the effect of these chemicals in the presence of physiological levels of testosterone, and the results are shown in Table III. As expected, testosterone enlarged prostate size and increased glandular development and its branching (Table III and Fig. 3). A low dose of DES also enlarged the prostate size in the absence of testosterone by increasing stromal growth but produced no effect on branching (Table III, Fig. 3). The lumen size was also increased by DES exposure. DES enhanced testosterone-induced prostate growth and branching, causing reduction in the urethral lumen size (Table III, Fig. 3). The other two chemicals, BPA and aroclor, also induced prostate growth and branching in the absence and presence of testosterone (Table III and Fig. 4). DES also induced epithelial hyperplasia as seen in the insets (Fig. 3), but no such effect was observed using BPA and aroclor (Gupta C, unpublished data). Thus, estrogenic chemicals modulate prostate growth by acting directly on the developing prostate.

***In Vitro* Effect of Estrogenic Chemicals on AR Binding.** To further examine whether results from the *in vitro* experiments represented those from *in vivo* experiments, we measured AR binding activity of the cultured

Table II. Effect of Estrogenic Chemicals on the Weight of Reproductive Organs of the Male Offspring

Drug	Dosage (μg/kg)	Prostate weight (mg/kg body weight)			Epididymis weight at day 60 (mg/kg)
		Day 3	Day 21	Day 60	
None	—	225 ± 66	620 ± 45	1020 ± 210	1468 ± 420
DES	0.1	310 ± 45[a]	910 ± 105[a]	1780 ± 110[a]	1222 ± 345
	200.00	145 ± 60[b]	410 ± 62[b]	640 ± 220[b]	645 ± 210[a]
BPA	50.0	350 ± 51[a]	860 ± 72[a]	2050 ± 310[a]	952 ± 333[a]
Aroclor	50.0	295 ± 55[a]	730 ± 66[a]	1766 ± 154[a]	710 ± 420[a]

Note. N = Average ± SD, n = 15 in each group. The data were analyzed by ANOVA test. The other symbols are defined under Table I.

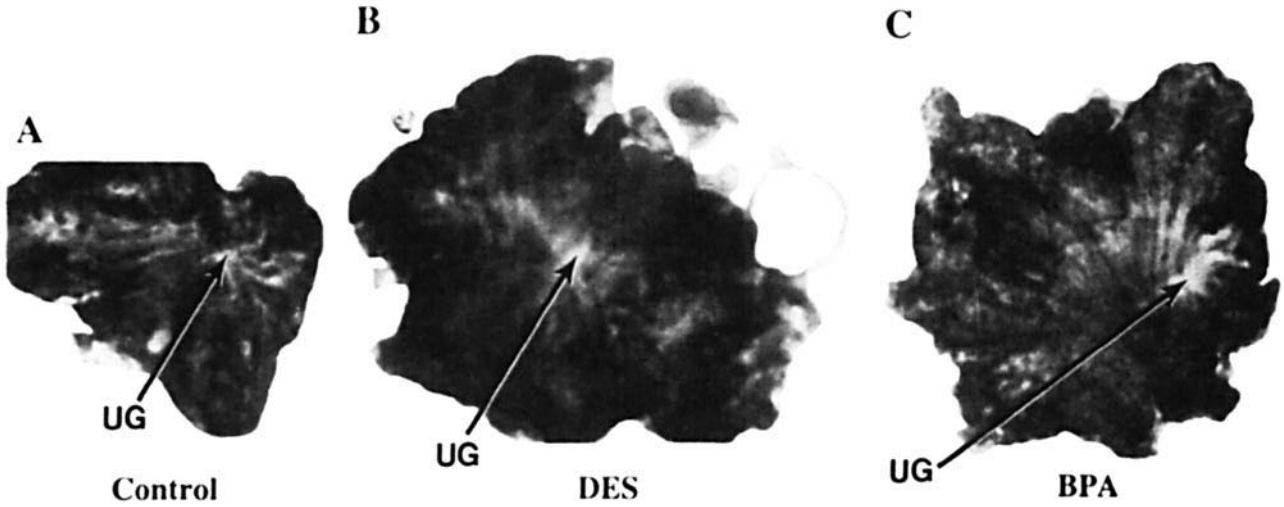

Figure 1. Effect of prenatal exposure to DES (100 ng/kg) and BPA (50 μg/kg) on prostate growth at 15 days of age. Four specimens from each group were analyzed, and a representative specimen from each group is shown in Figure 1. The arrow indicates the approximate region of urethral junction (UG) in different specimens.

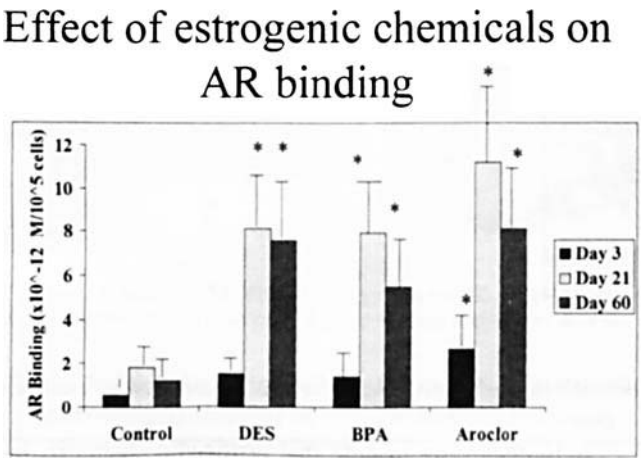

Figure 2. Effect of prenatal exposure to estrogenic chemicals on AR binding of the prostate gland at different ages. The data represent mean ± SD, $n = 5$; $^*P < 0.05$ compared with the control by ANOVA. 10^-12 and 10^5 are the same as 10^{-12} and 10^5, respectively.

prostates. Thus, fetal prostates at the 17th day of gestation were cultured with and without the presence of different estrogenic chemicals. After 6 days of culture, cells were prepared from the cultured prostates and used in the AR binding assay. One point assay using only 1 n*M* of 3-H-mibilerone was used in this instance, as sample size was too small to use for the entire Scatchard analysis. As shown in Table IV, all of these chemicals increased AR binding as compared with vehicle-treated control cells. The finding was similar to the *in vivo* treatment, although the values were slightly different.

Discussion

Many synthetic chemicals can bind to the estrogen receptor and thereby affect specific gene expression and cellular function (19). These compounds are now referred to as xenoestrogens (9, 20, 21). The persistence of these compounds and their metabolites in the environment poses risks for both the human and animal population (22), and studies are necessary to estimate this risk. Although some reports have described endocrine-disruptive effects of these chemicals, there is a great deal of controversy on these findings (23). Additionally, the endocrine disruptive activity of DES and other estrogens has been described less conclusively in the male than in the female. Furthermore, studies are needed to determine whether environmental estrogenic chemicals induce developmental malformation at the dosage that is comparable to the human consumption level (9–13, 21). In this study we chose to determine the effect of three such environmental chemicals, namely, BPA, aroclor, and DES. All of these chemicals have estrogen-like activity (12, 21, 22) and are consumed by the human population (9–13). Using a dosage that is comparable to the human consumption level (11, 13), we evaluated the prenatal effect of these chemicals on male sexual differentiation in the mouse. We report that these chemicals permanently affected male sex-

Table III. Effect of Estrogenic Chemicals on Fetal Prostate Growth *In Vitro*

Estrogens	Dosage (pg/ml)	Prostate size (mm^2)	
		In the absence of T	In the presence of T
Vehicle	—	.05 ± .01 (5)	.08 ± .02 (9)[b]
DES	0.1	.08 ± .03 (4)[a]	.15 ± .03 (5)[c]
	0.5	.12 ± .04 (6)[a]	.32 ± .07 (4)[c]
BPA	5.0	.04 ± .02 (4)	.07 ± .01 (5)
	50.0	.12 ± .06 (5)[a]	.20 ± .12 (5)[c]
Aroclor	5.0	.03 ± .01 (4)	.06 ± .01 (4)
	50.0	.14 ± .04 (6)[a]	.22 ± .12 (5)[c]

Note. Data represent mean ± SD; [a] $P < 0.05$ compared with estrogen-untreated control. [b] $P < 0.05$ compared with testosterone-untreated control by ANOVA test. [c] $P < 0.05$ compared with testosterone-control by ANOVA test. The number in the parenthesis indicates the number of specimens examined.

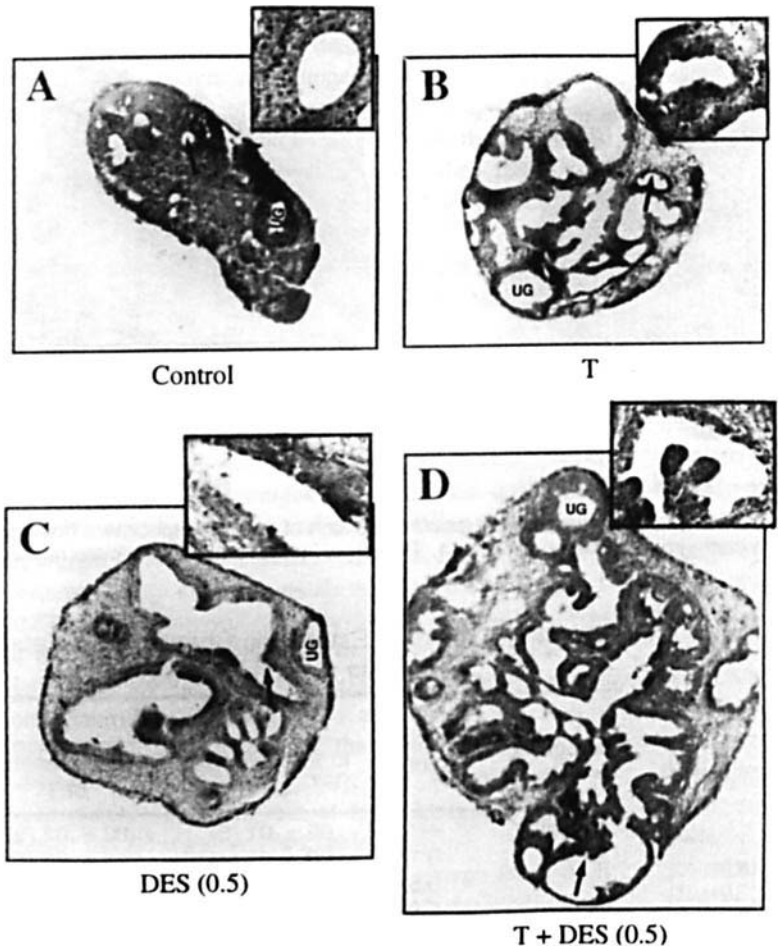

Figure 3. Effect of testosterone and DES on prostate development *in vitro*. T, testosterone (10 ng/ml); DES (0.5), DES 0.5 pg/ml. One of the four to six specimens examined in each treatment group is presented here. Note enhancement of size and branching by testosterone but only size by DES (panel C). DES also enhanced the testosterone effect (panel D). The insets demonstrate the development of the epithelium in the region indicated by the arrow.

ual differentiation by increasing anogenital distance, inducing prostate growth and its AR binding activity, and reducing epididymal weight. At high dosage, on the other hand, DES produced an opposite effect, inducing hypospadius and inhibiting prostate growth and AR binding activity. Some of the results obtained with BPA and DES are consistent with previous findings (8, 24), suggesting a teratogenic role of these chemicals on the developing organism at the dosage that is accepted by the FDA. This study, in addition, evaluated the effects of prenatal exposure to estrogenic chemicals in a more detailed fashion. Thus, it examined the prenatal effect of a number of chemicals with low estrogenic activity and studied their effects at all developmental phases. The results demonstrated that all of these chemicals acted very similarly and altered development of certain reproductive organs and functions at all phases of development.

The result of the *in vitro* studies is the other strength of this research. To our knowledge, this is the first demonstration that estrogenic chemicals induce reproductive malformation *in vitro*, suggesting that the chemicals have a direct effect on prostate development. Both prostatic enlargement and increased AR binding activity were demonstrated in the developing prostate, grown *in vitro* in the presence of all of the estrogenic chemicals. Thus, the *in vivo* and *in vitro* effects are comparable and therefore, the *in vitro* setup can be used in further elucidation of the mechanism of estrogen action.

It is interesting to note that estrogenic chemicals induced abnormal male reproductive functions in the presence of large concentrations of maternal circulating estrogen, estradiol, estrone, and estriol. One explanation for this observation could be that the fetal estrogen level is very low in spite of maternal high levels of estrogen (25). During the gestational phase, the fetus is protected from the maternal high-estrogen level by placental conversion of the maternal estrogen into biologically inactive estrogen. Estrogens are inactivated either by converting into conjugated estrogen, forming inactive estrogen metabolite, or binding with plasma protein (26). Thus, even a small change in the estrogen level during this fetal period may have a significant effect, and our findings, especially the *in vitro* findings, support this possibility.

The mechanism by which these chemicals induce male reproductive malformations is not known. The relatively low estrogenic potencies of suspected endocrine disrupters suggest that estrogenic activity of these chemicals alone is unlikely to produce adverse effects during fetal development. Some suspected endocrine disrupters have been shown to interact not only with the estrogen receptor (ER) but also with the androgen receptor (AR) or to interfere with

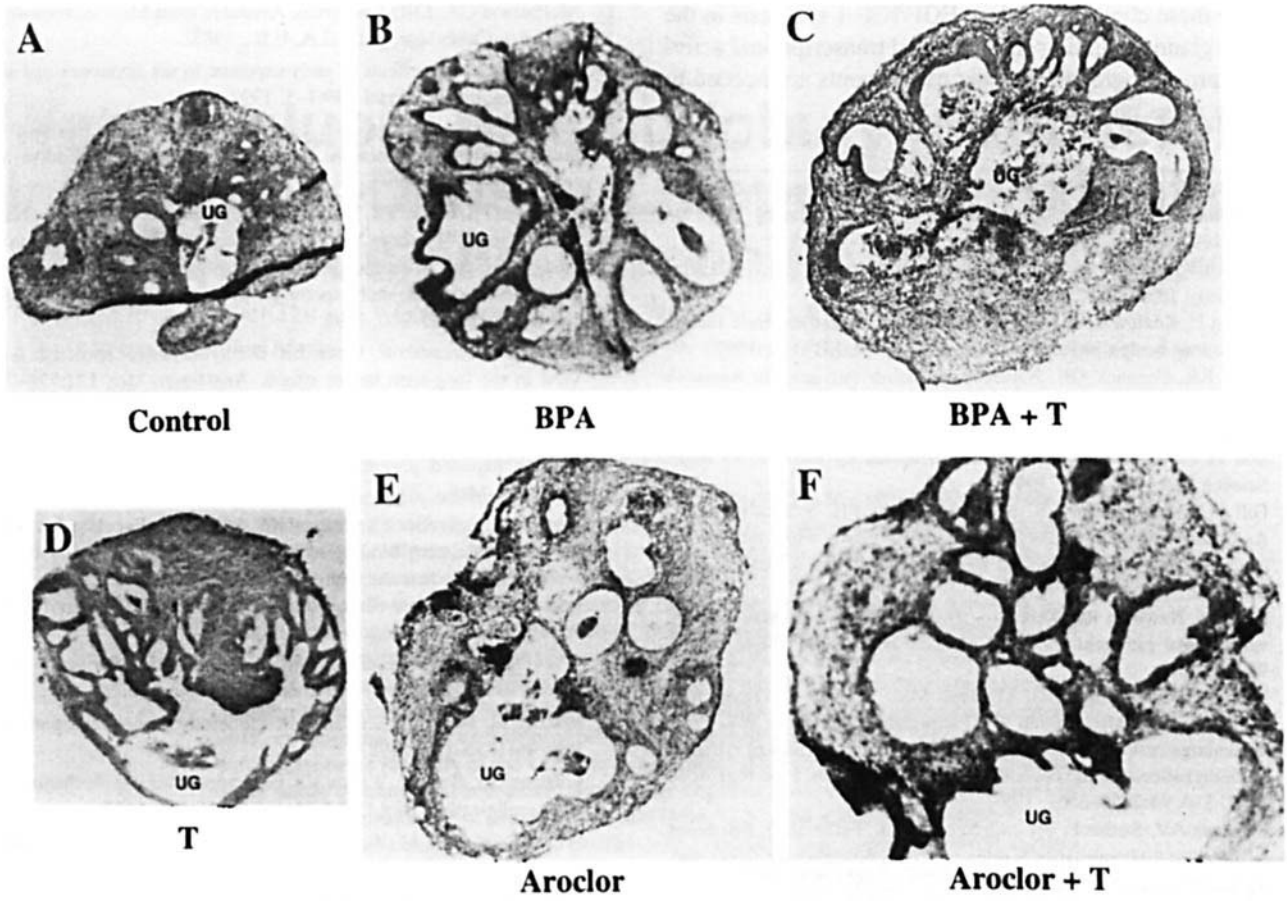

Figure 4. Effect of BPA (50 ng/ml) and Aroclor (50 ng/ml) on prostate development *in vitro*. C, control; T, testosterone. Note enhancement of size by BPA and aroclor both in the presence and absence of testosterone. One of the four to six specimens examined has been presented.

Table IV. *In Vitro* Effect of the Estrogenic Chemicals on Androgen Binding of the Developing Prostate

Estrogens	Dosage (pg/ml)	Number	Androgen binding ($\times 10^{-12}$ *M*)/10^5 cells
None	—	6	0.2 ± .12
DES	0.5	5	1.4 ± .32[a]
BPA	50.0	4	0.6 ± .22[a]
Aroclor	50.0	4	0.7 ± .26[a]

Note. Data represent mean ± SD. [a] $P < 0.05$ by ANOVA test.

steroid hormone synthesis or metabolism (27). At this time it is not known whether estrogenic activity is directly involved in the malformation induced by the chemicals. Additionally, there are two ER subtypes, Era and Erb, in the prostate (28). The xenoestrogens were shown to have higher binding affinity for the ER-β protein (29). The role of these two ER subtypes in relation to the action of these chemicals remains to be determined. The estrogenic potency of compounds is another issue. Although the chemicals are weak estrogens, the potency is dependent on a number of factors, such as differential effects on the transactivation functionalities of the receptor, the particular coactivator recruited and cell and target gene promoter context (30, 31). The apparently lower transcriptional activity of ER-β compared with ER-α has been reported in transient transfection experiments using different cell lines (32). In contrast, in human osteosarcoma or human endometrial cells, the transcriptional activity of ER-β was higher than that of ER-α (32). The reason for these differences in transcriptional activity of ER subtypes is unknown, but it might reflect differential expression of transcriptional coactivators or differential stability of the receptor proteins. At the present, it is not known whether any of the ER-mediated activities are involved directly or whether these chemicals alter recruitment of ER activators causing malformation of the prostate gland.

The results described here demonstrate that AR binding activity is permanently altered by these chemicals. There are several possibilities of how this effect is mediated. First, it is possible that the estrogenic chemicals enhance synthesis of the AR protein resulting in enhanced activity. Second, the estrogenic chemicals may enhance recruitment of AR coactivators, resulting in enhanced AR binding. Finally, since these xenoestrogen-exposed offspring have enlarged prostate growth, it is also possible that AR-mediated transcriptional activity has been enhanced in these animals. This possibility is supported by the observations that estrogenic chemicals enhance EGF and IGF-1 synthesis in the adult uterus (33) and that EGF and IGF-1 enhance AR-mediated transcriptional activity (34, 35). At this time it is not known

whether these chemicals induce EGF/IGF-1 synthesis in the prostate gland mediating AR-mediated transcriptional activity and prostate growth. Further experiments are needed to examine these possibilities.

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