Xenoestrogens Alter Mammary Gland Differentiation and Cell Proliferation in the Rat

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We investigated mammary gland differentiation and cell proliferation in rats after acute exposure to xenoestrogens. Pubertal female Sprague-Dawley rats (six/group) were treated for 1 week with diethylstilbestrol (DES), genistein, *o,p'*-DDT, Aroclor 1221, Aroclor 1254, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), or the vehicle, sesame oil. Animals were killed 18 hr after the last treatment. Analysis of mammary whole-mounts revealed that exposure to DES, genistein, and *o,p'*-DDT resulted in enhanced gland differentiation and increased epithelial cell proliferation as measured by proliferating cell nuclear antigen immunohistochemistry. TCDD treatment inhibited cell proliferation and gland development. Aroclor 1221 and Aroclor 1254 treatments had slight but not statistically significant effects on cell proliferation and mammary gland development. We conclude that DES, genistein, and *o,p'*-DDT given to pubertal rats act as morphogens; i.e., they increase cell proliferation, which promotes maturation of the undifferentiated terminal end buds to more differentiated lobular terminal ductal structures. *Key words*: Aroclor, cell proliferation, diethylstilbestrol, dioxin, genistein, mammary gland, *o,p'*-DDT, xenoestrogens. *Environ Health Perspect* 103:708–713 (1995)

Breast cancer is the most common cancer among females in the United States and is the second leading cause of death among women in this country. In the last decade, the incidence of breast cancer has increased at a rate of approximately 2% per year (*1*). Speculation that environmental chemicals, especially estrogenically active chemicals, may be responsible for breast cancer is supported by epidemiological reports. Elevated breast cancer rates have been reported for women exposed to polychlorinated biphenyls in Japan (*2*). A recent analysis of chemical plant workers found a more than twofold increase in breast cancer in female workers exposed to dioxin (*3*), although other studies report negative associations of polychlorinated biphenyls and dioxin with breast cancer (*4,5*). In one report DDT was associated with a higher risk of developing breast cancer (*6*), although another study failed to observe a higher risk after DDT exposure (*7*). DDT, dioxins, polychlorinated biphenyls, and a number of other organochlorine pesticides have been found in breast milk and human adipose tissue (*8*). Some hormonally active chemicals are synthetic; others occur naturally. We selected six such chemicals to investigate their actions on mammary gland differentiation and cell proliferation. Diethylstilbestrol (DES), genistein, *o,p'*-DDT, and Aroclor 1221 are documented to be estrogenically active xenobiotics (*9–11*); 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) has been reported to be antiestrogenic (*12*). Aroclor 1254 is not estrogenic, but it is more toxic in mammalian systems than Aroclor 1221 (*11*).

Alterations to mammary gland development and to cellular proliferation from chemical exposure could alter cancer susceptibility. From birth through the first week postpartum in the rat, the mammary gland is composed of a single primary or main lactiferous duct that branches into three to five secondary ducts (*13–15*). During the second week, further sprouting of ducts occurs up to the sixth generation. This sprouting of ducts causes an increase in density of terminal end buds in the growing periphery of the mammary gland. Some of the terminal end bud differentiate in response to each estrous cycle, giving rise to alveolar buds which can be found in type I lobules. Type I lobules can mature to type II lobules. These lobules respond to hormones of pregnancy by differentiating further into type III lobules, which form the functional units of the lactating gland.

The differentiation of terminal end buds to lobules appears to be a basic and protective mechanism against chemical carcinogenesis. Terminal end buds and terminal ducts are less differentiated structures that are more susceptible to chemical carcinogenesis than the more differentiated alveolar buds and lobules (*13–19*). This may be due to the increased mitotic activity of terminal end bud and terminal duct cells as opposed to cells in alveolar buds and lobules.

Methods

Weanling female Sprague-Dawley CD rats were purchased from Charles River Breeding Laboratories (Raleigh, North Carolina). Upon receipt, animals were placed on AIN-76A diet (Harlan Texlad, Madison, Wisconsin). Animals were maintained in a climate-controlled room at 21°C ±1°C on a 12-hr light/dark cycle. Diet and tap water were available *ad libitum*. Animals in experiment I were injected subcutaneously with 50 µg DES, 50 µg genistein, or 50 µg *o,p'*-DDT per gram body weight, or the vehicle, sesame oil (200 µL/rat), on days 23, 25, 27, and 29 postpartum. Rats in experiment II were treated by gavage with 25 µg Aroclor 1221, 25 µg Aroclor 1254, or 2.5 ng TCDD per gram body weight, or sesame oil (200 µL/rat), on days 25, 27, 29, and 31 postpartum. The doses were derived from the literature which showed these chemicals caused alterations to the endocrine system, reproductive tract, or the liver, or from our unpublished data. We killed the rats 18 hr after the last treatment and removed both abdominal glands—one for preparation of a whole mount and the other for processing as a tissue block for sectioning.

The whole mount was spread on a slide, fixed in 10% neutral buffered formalin (8–24 hr), defatted in acetone (8–24 hr), rehydrated in 70% ethanol (30 min), rinsed in water (15 min), and stained in alum carmine (2 g/L) overnight. The stained gland was progressively dehydrated in ethanol from 35% to 95% in four steps (30 min/step), then left in 100% ethanol overnight. The glands were subsequently transferred to xylene for clearing for 24 hr, compressed with a second glass slide held with paper clips for 24 hr, and then released and allowed to expand for 6–24 hr before coverslipping using Permount (Fisher Scientific, Atlanta, Georgia).

We examined coded whole mounts under light microscopy at 40× and 100× magnification and scored them for the numbers of terminal end buds, terminal ducts, alveolar buds, and lobules. In our evaluations, terminal ductal structures with diameters ≥100 µm were called terminal end buds, while those of <100 µm in diameter were called terminal ducts. Terminal ductal structures composed of 5–10 alveoli were called type I lobules. The criteria for identification of the structures were based on the work by Russo and Russo (*13*). We evaluated the outer portion of the entire gland (periphery to 1.78 mm inward).

We measured cell proliferation in the contralateral gland using proliferating cell nuclear antigen (PCNA) as a marker of mitotic activity (*20*). Formalin-fixed glands were processed for paraffin embedding within 24 hr of their removal from the animal. We cut 5-µm sections and mounted them on Superfrost Plus Electrostatic Slides (Fisher Scientific, Atlanta, Georgia). The sections were deparaffinized and subjected to 3% hydrogen peroxide to quench

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endogenous peroxide activity. Tissues were blocked with 5% normal horse serum to suppress nonspecific binding of IgG. Next the tissues were incubated with 1) PCNA antibody (PC10 clone; Signet Laboratories, Dedham, Massachusetts), 2) biotinylated horse anti-mouse secondary antibody, and 3) avidin–biotin-bound peroxidase. Secondary antibody and avidin–biotin complex were purchased from Signet Laboratories. Color was developed by 3,3′-diaminobenzidine tetrahydrochloride and cells were counterstained with Gills no. 2 hematoxylin (Sigma, St. Louis, Missouri).

We analyzed the cell cycle using the immunocytochemical staining patterns of PCNA as described by Foley et al. (*20*). Cells in S-phase were characterized by uniform dark-brown to black nuclear staining. We calculated the labeling index by dividing the number of epithelial nuclei in S-phase by the total number of epithelial nuclei counted and expressed the results as a percentage. Because mammary cells have a high nuclear to cytoplasmic ratio, we were not able to distinguish between cells in G_1 and G_2, but we did report our data as the percentage of cells in the active cell cycle. Proliferative index was defined as the percentage of epithelial cells in the active cell cycle, i.e., $(G_1 + S + G_2 + M)$/total number of epithelial cells. PCNA analysis was carried out with an image analysis system (Image-1 Universal Imaging Corporation, West Chester, Pennsylvania) using an Olympus AH3 microscope, a Dage CCD 72 video camera, and a 486 computer.

Mammary gland size was determined from whole mounts using an image analysis system connected to a Vidicon Black & White video camera designed for gross specimens and a 486 computer. The glands were magnified 8.55 times, projected to the video screen, and video prints were made on a Seikosha video printer. Video prints of the carmine stained whole mounts of the right abdominal gland were measured with a sonic digitizer system (Graf Bar, Science Accessories Corporation, Southport, Connecticut) using computer programs developed in-house. The system was calibrated with a micrometer photographed with the glands. Uterine–ovarian weights represent fluid-filled wet weights of both uterine horns and ovaries. Statistical comparisons between treatment groups were performed using Student's *t*-test (two-tailed).

Results

Pubertal female rats treated with DES for 1 week had reduced body weights as compared to the vehicle-treated females (Table 1). The other treatments did not significantly affect body weights. Uterine–ovarian weights were increased after treatment with DES and genistein, decreased by TCDD

Table 1. Effects of xenoestrogens on body weights, uterine–ovarian weights, and mammary gland size in pubertal female rats[a]

Treatment (*n*)	Body weight (g)	Uterine–ovarian weight (mg)	Mammary gland size (mm^2)
Experiment 1			
Sesame oil, 200 µl SC (6)	82±2	185±30	122±10
DES, 50 ng/g SC (5)	74±2*	298±17*	104±8
Genistein, 50 µg/g SC (5)	81±1	265±18*	149±7*
o,p′-DDT, 50 µg/g SC (6)	82±2	220±9	131±7
Experiment 2			
Sesame oil, 200 µL IG(6)	93±3	230±33	132±6
Aroclor 1221, 25 µg/g IG (6)	94±3	255±27	133±7
Aroclor 1254, 25 µ/g IG (6)	97±2	250±27	122±8
TCDD, 2.5 ng/g IG (6)	88±2	110±12*	81±9**

Abbreviations: SC, subcutaneous; DES, diethylstilbestrol; IG, intragastric (gavage); TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.
[a]Rats in experiment 1 were treated on days 23, 25, 27, and 29 postpartum. Rats in experiment 2 were treated on days 25, 27, 29, and 31 postpartum. Eighteen hours after the last treatment, rats were killed and mammary whole mounts of the left abdominal glands were prepared. Numbers in parentheses indicate the numbers of rats in each group. Data are expressed as means ± SEM.
*$p<0.05$, **$p<0.01$ compared to respective sesame oil-treated animals.

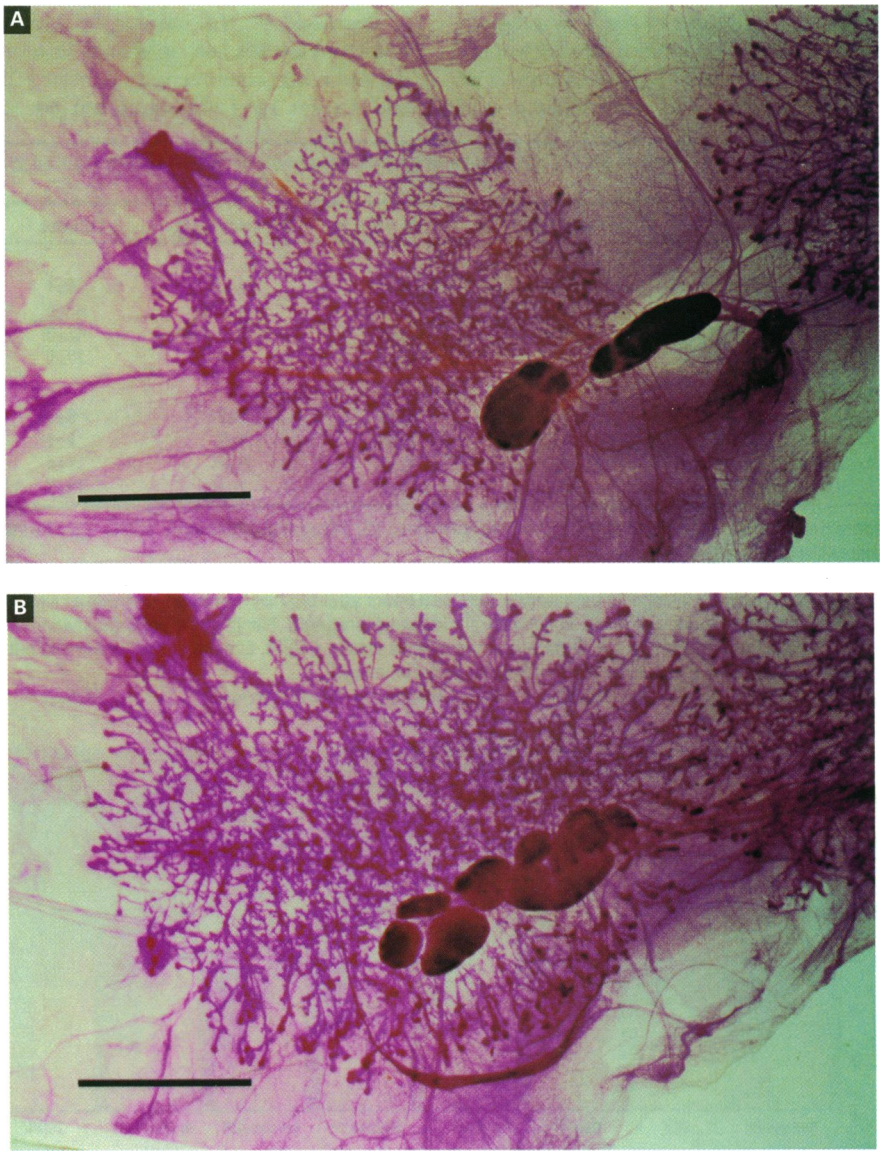

Figure 1. Whole mount of carmine-stained abdominal mammary glands from 30-day-old (A) sesame oil-treated and (B) genistein-treated rats. The gland grows from the nipple at the upper left toward the lymph nodes (dark red) at the lower right. The cranial inguinal gland can be seen (top right) growing toward the abdominal gland; 4.6×; bar = 3.9 mm.

treatment, and not significantly affected by the other chemical treatments. Mammary glands were significantly larger in genistein-treated animals and smaller in TCDD-treated animals as compared to vehicle-treated rats. The other treatments did not have a significant effect on gland sizes. Examples of glands from genistein-treated and vehicle-treated female rats are presented in Figure 1. Note the larger gland size in the genistein-treated female rat.

The periphery or actively growing part of the abdominal glands of 30- to 32-day old female rats treated with vehicle was composed of terminal end buds, terminal ducts, alveolar buds, and type I lobules (approximately 64%, 23%, 7%, and 6%, respectively; Table 2). DES treatment resulted in significantly fewer terminal end buds, terminal ducts, and alveolar buds with a concomitant increase in lobules. The only other treatment to result in a significantly increased number of lobules was the genistein treatment, but the numbers of terminal end buds and terminal ducts were only slightly decreased. TCDD treatment resulted in a significant decrease in terminal end buds, but no significant effects on terminal ducts, alveolar buds, or lobules. Treatment with *o,p′*-DDT, Aroclor 1221, and Aroclor 1254 caused slight but not statistically significant alterations in the number of terminal ductal structures.

Cell proliferation, as measured by total PCNA staining, was approximately 30% in cells of terminal end buds and terminal ducts and 10% in cells of type I lobules in vehicle-treated animals (Fig. 2). PCNA staining was not analyzed in the alveolar buds because there were too few of these terminal ductal structures. DES-, genistein- and *o,p′*-DDT-treated animals had higher percentages of PCNA-stained cells in all terminal ductal structures than did cells from sesame oil-treated animals (experiment 1). TCDD treatment resulted in decreased PCNA staining in terminal ducts and lobules and no change in terminal end buds (experiment 2). Aroclor 1221 and Aroclor 1254 had slight, but not statistically significant, effects on cell cycling. Figure 3 contains photomicrographs of PCNA-stained terminal end buds from animals treated with sesame oil, genistein, *o,p′*-DDT, and TCDD. The differences in proliferative indexes are obvious.

Analysis of the PCNA staining for S-phase revealed that DES and genistein treatments resulted in a greater percentage of cells from terminal end buds, terminal ducts, and lobules in S-phase (Fig. 4). *o,p′*-DDT caused an increase in S-phase cells in terminal end buds and terminal ducts but not in lobules. TCDD treatment resulted in a decreased percentage of cells in S-phase in terminal ducts only (Fig. 4). Aroclor 1221 and Aroclor 1254 had no significant effect on PCNA staining for S-phase.

When total cell proliferation in the periphery of an abdominal gland was considered (mean number of terminal ductal structures × mean number of PCNA-stained cells per terminal ductal structure), DES-treated females had more PCNA-labeled lobular cells and fewer PCNA-labeled terminal duct cells than the vehicle-treated animals (Fig. 5). Total S-phase cells were increased in terminal end buds and lobules and decreased in terminal ducts of DES-treated animals (Fig. 6).

Genistein treatment resulted in increased total PCNA-stained terminal end bud and lobular cells (Fig. 5) and in S-phase cells (Fig. 6). There were no proliferative changes in terminal ducts.

Mammary glands from female rats treated with *o,p′*-DDT as compared to sesame oil-treated females had increased total PCNA-stained cells in terminal ducts and lobules (Fig. 5) and in S-phase cells (Fig. 6). While the increase of total PCNA-stained cells in terminal end buds was not statistically significant, the number of cells in S-phase was increased.

TCDD treatment resulted in fewer total PCNA-stained cells in terminal end buds, terminal ducts, and lobules (Fig. 5). The mammary glands of the TCDD-treated females also had fewer terminal end bud and terminal duct cells in S-phase, whereas S-phase cell numbers were unchanged in the lobules (Fig. 6).

Table 2. Number of terminal ductal structures in mammary glands of pubertal rats treated with xenoestrogens[a]

Treatment (*n*)	Terminal end buds	Terminal ducts	Alveolar buds	Lobules I
Experiment 1				
Sesame oil, 200 µL SC (6)	92±8	37±10	13±3	10±1
DES, 50 ng/g SC (5)	55±5**	3±2†	4±3*	43±6†
Genistein, 50 µg/g SC (5)	79±4	18±4	10±1	43±8**
o,p′-DDT, 50 µg/g SC (6)	83±4	43±6	7±1	17±5
Experiment 2				
Sesame oil, 200 µL IG (6)	105±8	33±6	7±2	8±3
Aroclor 1221, 25 µg/g IG (6)	91±3	35±4	9±3	2±1
Aroclor 1254, 25 µg/g IG (6)	85±8	28±5	5±1	11±4
TCDD, 25 ng/g IG (6)	43±3+	26±2	2±1	10±3

Abbreviations: SC, subcutaneous; DES, diethylstilbestrol; IG, intragastric (gavage); TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

[a]Rats in experiment 1 were treated on days 23, 25, 27, and 29 postpartum. Rats in experiment 2 were treated on days 25, 27, 29, and 31 postpartum. Eighteen hours after the last treatment, rats were killed and mammary whole mounts of the left abdominal glands were prepared. Numbers in parentheses indicate the numbers of rats in each group. Data are expressed as means ± SEM. Evaluations were carried out in the outer portion of the entire gland (periphery to 1.78 mm inward).

*$p<0.05$, **$p<0.01$, †$p<0.001$ compared to respective sesame oil-treated animals.

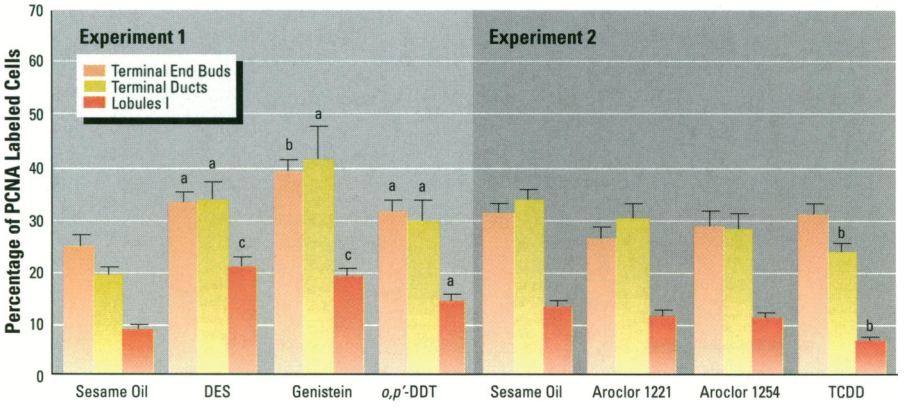

Figure 2. Cell proliferation as represented by the proliferating index for mammary terminal ductal structures of pubertal female rats exposed to xenoestrogens. The proliferating index represents the percentage of proliferating cell nuclear antigen-labeled cells. Values represent means ± SEM of six animals. Proliferation was determined in two structures from each gland; [a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$ compared to vehicle-treated animals.

Discussion

Body and Uterine–Ovarian Weights and Mammary Gland Sizes

We selected xenobiotic doses that have been reported to exert estrogenlike actions but yet would not cause overt toxicity. However, DES at 50 ng/g body weight, when injected on alternate days for 1 week to pubertal animals, had an adverse effect on body weight. As expected, DES also exerted a uterotropic effect. The lack of effect of DES on the mammary gland size

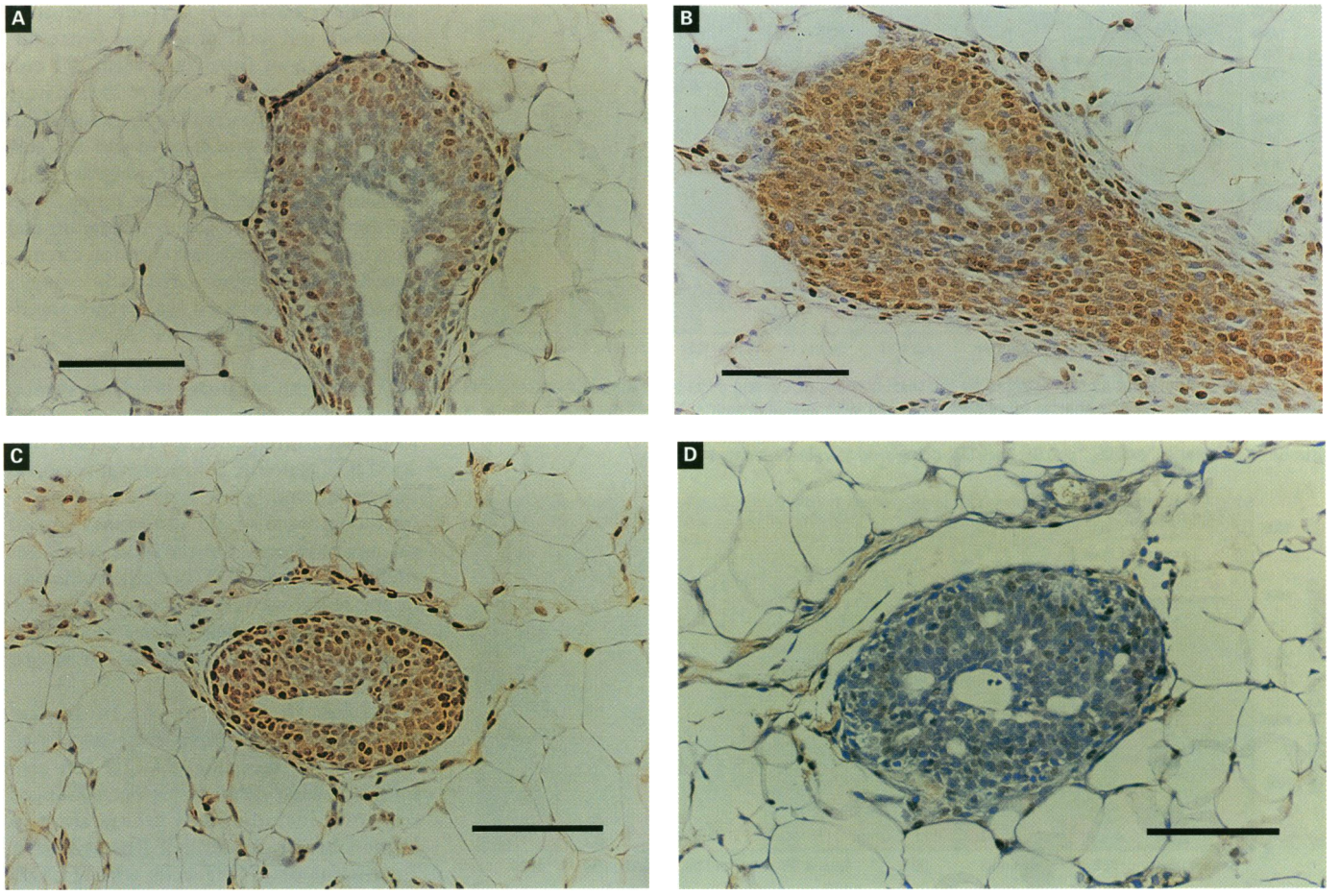

Figure 3. Cell proliferation in terminal end buds as quantitated by proliferating cell nuclear antigen staining. (A) Longitudinal section from a control rat treated with sesame oil; (B) longitudinal section from a genistein-treated rat; (C) cross-section from an *o,p'*-DDT-treated rat; (D) cross-section from a TCDD-treated rat. (A–D) 171×, bar = 64.3 µm.

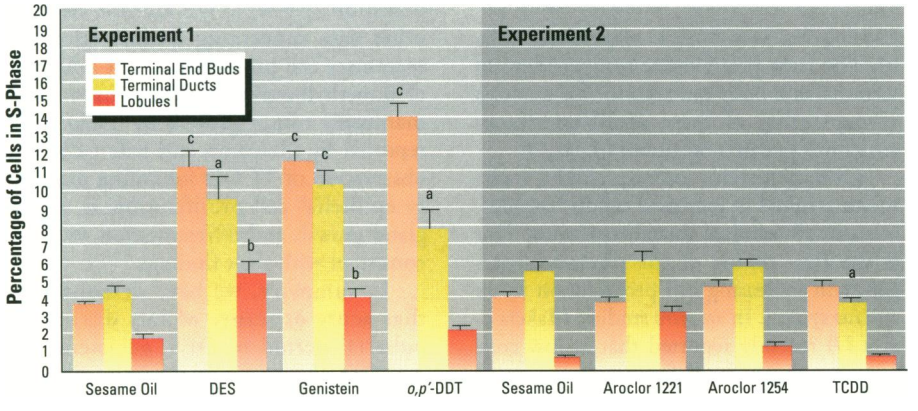

Figure 4. Cell proliferation as represented by the labeling index of cells in mammary terminal ductal structures of pubertal female rats exposed to xenoestrogens. The labeling index represents S-phase of proliferating cell nuclear antigen-stained cells. Values represent means ± SEM of six animals. Proliferation was determined in two structures from each gland; $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ compared to vehicle-treated animals.

may be due to a balance between its estrogenic properties and its suppressive effect on growth at these high concentrations. Genistein treatment resulted in significantly increased uterine–ovarian weights, confirming reports of its properties as a weak estrogen (*9,21,22*). There was also a significant increase in the size of mammary glands from genistein-treated animals. This is consistent with our previous report of early gland maturation in neonates treated with genistein (*23*).

TCDD treatment resulted in significantly reduced uterine–ovarian weights and mammary gland sizes. This could be a consequence of the antiestrogenic properties of TCDD (*12*) or extreme toxicity (*24*). Treatment with *o,p'*-DDT, Aroclor 1221, and Aroclor 1254 had no significant effects on body weights, uterine–ovarian weights, or mammary gland sizes. The slight but not statistically significant changes in these endpoints between experiments I and II may be due to the fact that animals in experiment 2 were slightly older (2 days).

Mammary Gland Development

Since terminal ductal structures of the mammary gland are sensitive to hormonal influence (*13,25–27*), we investigated the potential of these xenoestrogens to alter gland differentiation. DES treatment reduced the numbers of terminal end buds and terminal ducts and concomitantly increased lobules. This phenomenon appears to be due to accelerated maturation of terminal ductal structures (i.e., progression of terminal end buds and terminal ducts to lobules). Genistein and *o,p'*-DDT also appeared to act in a similar manner, even though the progressive changes in terminal ductal structures after treatment with these two compounds were not as profound.

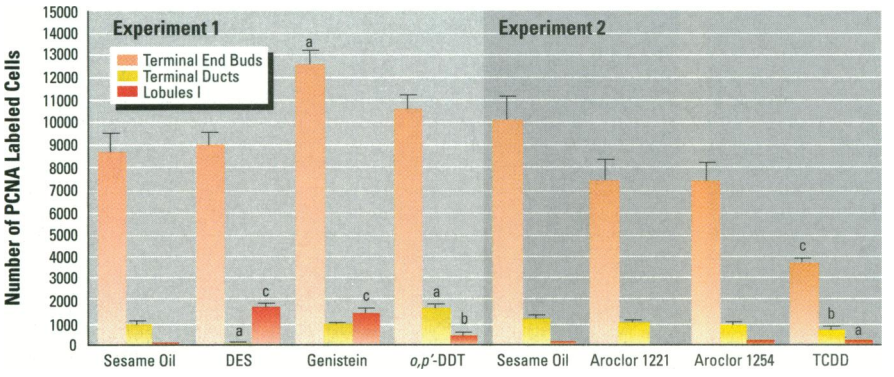

Figure 5. Cell proliferation as represented by the total number of cycling cells per gland in mammary terminal ductal structures of pubertal female rats treated with xenoestrogens. Values represent means ± SEM of cells in the cell cycle per terminal ductal structure multiplied by the numbers of terminal ductal structures per gland; [a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$ compared to vehicle-treated animals.

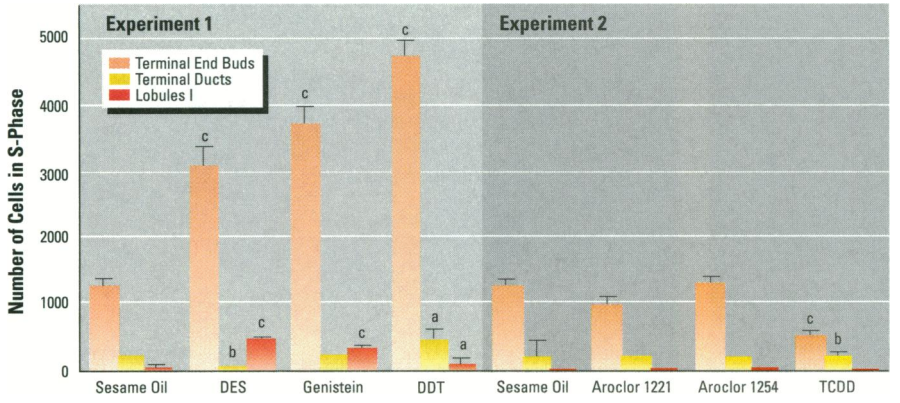

Figure 6. Cell proliferation as represented by the total number of cells per gland in S-phase in mammary terminal ductal structures of pubertal female rats treated with xenoestrogens. Values represent means ± SEM of cells in S-phase per terminal ductal structure per gland; [a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$ compared to vehicle-treated animals.

The overall decrease in numbers of terminal ductal structures and gland size from TCDD treatment may be due to its biological actions on estrogen metabolism (*28*) and the estrogen receptor (*29*), or as a consequence of overt toxicity (*24*). Aroclor 1221 and Aroclor 1254 caused slight but not statistically significant effects on the development of terminal ductal structures.

Cell Proliferation

Increased cell proliferation may be a critical factor in chemical carcinogenesis. Russo and Russo (*13,14*) and Nagasawa et al. (*30*) have shown that in the rat mammary gland there is a positive correlation between numbers of terminal end buds and terminal ducts with susceptibility to dimethylbenz[*a*]anthracene-induced adenocarcinomas. As shown in Figures 2 and 4, the highest proliferative rates occurred in the terminal end buds and terminal ducts as compared to the lobules. Furthermore, when one considers total epithelial cell proliferation in a gland (proliferation per structure × total number of structures per gland), the ratios of PCNA-stained cells in terminal end buds, terminal ducts, and lobules were 46:5:1, respectively (Fig. 5). The large proliferative compartment of terminal end buds can account for the high susceptibility of these undifferentiated structures to carcinogenesis (*13,14,23*).

DES, genistein, and *o,p'*-DDT treatments resulted in increased cellular proliferation in all terminal ductal structures. This is the first report of the phytoestrogen genistein increasing cell proliferation in an *in vivo* system. In *in vitro* studies, Makela et al. (*31*) recently reported that at low concentrations genistein functions as a transcriptional activator and cell proliferator through an estrogen receptor-mediated mechanism. On the other hand, Peterson and Barnes (*32*) demonstrated that at higher concentrations *in vitro* genistein inhibited the growth of estrogen-receptor positive and negative breast cancer cell lines. Until now, the *in vitro* inhibitory actions of genistein were consistent with the chemoprevention actions of genistein in the dimethylbenz[*a*]anthracene-rat mammary model (*23,33*). However, our data suggest that *in vivo* genistein acts as a morphogen on the mammary gland. When given before mammary gland maturation was complete and before carcinogen exposure, genistein promoted cell proliferation and hence differentiation of immature terminal end buds, causing them to evolve into mature differentiated structures (i.e., lobules). Lobules are less proliferative and less susceptible to the actions of carcinogens (*23*).

Consistent with genistein acting as an estrogen to promote gland maturation and thereby protecting against chemical carcinogenesis (*23*) are reports that early estrogen treatment also protected against carcinogen-induced mammary tumors (*26,27,34,35*). Furthermore, we have observed that neonatal DES treatment protects against spontaneously developing rat mammary tumors (*36*). Accordingly, we would expect that *o,p'*-DDT exposure before mammary gland maturation and before exposure to a genotoxic carcinogen would also protect against mammary tumors. On the other hand, our finding that *o,p'*-DDT is a cell proliferator in the mammary gland is consistent with the action of this chemical as a cancer promoter if given after a genotoxic carcinogen (*37,38*).

Although many of the biological actions of TCDD have been associated with the Ah receptor, TCDD can also inhibit a wide range of estrogen-induced responses including cell proliferation in human breast cancer cell lines (*28,39*). Treatment of MCF7 cells with TCDD also caused a decrease in nuclear estrogen receptor levels (*29*). TCDD has been shown to suppress estrogen-induced gene transcription, which may lead to cell proliferation (*39–41*). We are not aware of other data concerning TCDD effects on mammary cell proliferation, but inhibition of dimethylbenz[*a*]anthracene-induced rat mammary tumor growth by TCDD was reported by Holcomb and Safe (*42*). Our observation of TCDD inhibiting mammary epithelial cell proliferation as well as gland growth and differentiation *in vivo* is consistent with their findings.

In summary, we have demonstrated that acute exposure of rats during the pubertal period to DES, genistein, and *o,p'*-DDT increases mammary cell proliferation and enhanced gland differentiation. TCDD inhibited cell proliferation and gland development. Aroclor 1221 and Aroclor 1254 had a slight but not statistically significant effect on mammary gland development. We speculate that genistein and *o,p'*-DDT, like DES, act via estrogen receptor-mediated mechanisms to promote mammary epithelial cell proliferation and enhance mammary gland maturation. Exposure of the immature and undifferentiated terminal end buds to estrogenically active chemicals results in more differentiated and less susceptible terminal ductal structures (lobules). TCDD inhibition of

cell proliferation and gland growth may be a consequence of its extreme toxicity (*24*) or antiestrogenic properties (*12,29*). A lack of effect on the mammary gland from Aroclor 1221 and Aroclor 1254 exposure may be due to inadequate dose or weak estrogenic properties. In interpreting these results, one must keep in mind the window of exposure and the developmental maturation of the mammary gland—these chemicals administered perinatally or in adulthood could yield significantly different results in this same tissue.

References

1. ACS. Cancer facts and figures, 1994. Atlanta, GA:American Cancer Society, 1994.
2. Kuratsuna M, Imeda M, Nahamura Y, Hirohata T. A cohort study on mortality of Yusho patients: a preliminary report. Int Symp Princess Takamutsa Cancer Res Fund 18:61–66 (1987).
3. Manz A, Berger J, Dwyer JH, Flesch-Janys D, Nagel S, Waltsgott H. Cancer morality among workers in a chemical plant contaminated with dioxin. Lancet 338:959–964 (1991).
4. Nicholson WJ, Seidman H, Selikoff IJ. Mortality experience of workers exposed to polychlorinated biphenyls during manufacture of electrical capacitors. Report to the industrial disease standards panel. Ontario:Ontario Ministry of Labor (1987).
5. Bertazz PA, Zocchetti C, Pesatori AC, Guercilena S, Sanarico M, Radice L. Ten-year mortality study of the population involved in the Seveso incident of 1976. Am J Epidemiol 129:1187–1200 (1989).
6. Wolff MS, Toniolo PG, Lee EW, Rivera M, Dubin N. Blood levels of organochlorine residues and risk of breast cancer. J Natl Cancer Inst 85:648–652 (1993).
7. Krieger N, Wolff MS, Hiatt RA, Rivera M, Vogelman J, Orentreich N. Breast cancer and serum organochlorines: a prospective study among white, black, and Asian women. J Natl Cancer Institute 86:589–599 (1994).
8. Jensen AA, Slorach SA. Chemical contaminants in human milk. Boston, MA:CRC Press, 1991.
9. Folman Y, Pope GS. The interaction in the immature mouse of potent oestrogens with coumestrol, genistein, and other uterovaginotrophic compounds of low potency. J Endocrinol 34:215–225 (1966).
10. Welch RM, Levin W, Conney AH. Estrogenic action of DDT and its analogs. Toxicol Appl Pharmacol 14:358 (1969).
11. Nelson NM, Hammon PB, Nisbet ICT, Sarofim AF, Drury WH. Polychlorinated biphenyls-environmental impact. Environ Res 5:249–362 (1972).
12. Safe S, Astroff B, Harris M, Zacharewski T, Dickerson R, Romkes M, Biegel L. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and related compounds as antioestrogens: characterization and mechanism of action. Pharmacol Toxicol 69:400–409 (1991).
13. Russo IH, Russo J. Developmental stage of the rat mammary gland as determinant of its susceptibility to 7,12-dimethylbenzanthracene. J Natl Cancer Inst 61:1439–1449 (1978).
14. Russo J, Russo IH. DNA labeling index and structure of the rat mammary gland as determinants of its susceptibility to carcinogenesis. J Natl Cancer Inst 61:1451–1459 (1978).
15. Russo IH, Tewari M, Russo J. Morphology and development of the mammary glands in rat, including methods of study, collection and preparation of meterial. In: Integument and mammary gland, monograph series on the pathology of laboratory animals (Jones TC, Mohr U, Hunt RD, eds). Berlin:Springler-Verlag, 233–252; 1989).
16. Russo J, Russo I. Biology of disease. Biological and molecular bases of mammary carcinogenesis. Lab Invest 57:112–137 (1987).
17. Russo J, Russo I. Influence of differentiation and cell kinetics on the susceptibility of the rat mammary gland to carcinogenesis. Cancer Res 40:2766–2687 (1980).
18. Grubbs CJ, Peckham JC, Cato KD. Mammary carcinogenesis in rats in relation to age at time of N-nitro-N-methylurea administration. J Natl Cancer Inst 70:209–212 (1983).
19. Grubbs CJ, Juliana MM, Hill DL, Whitaker LM. Suppression by pregnancy of chemically-induced preneoplastic cells of the rat mammary gland. Anticancer Res 6:1395–1400 (1986).
20. Foley J, Ton T, Maronpot R, Butterworth B, Goldsworthy TL. Comparison of proliferating cell nuclear antigen to tritiated thymidine as a marker of proliferating hepatocytes in rats. Environ Health Perspect 101(suppl 5): 199–205 (1993).
21. Cheng E, Story CD, Yoder L, Hale WH, Burroughs W. Estrogenic activity of isoflavone derivatives extracted and prepared from soybean oil meal. Science 118:164–165 (1953).
22. Shutt DA, Cox RI. Steroid and phyto-estrogen binding to sheep uterine receptors *in vitro*. J Endocrinol 52:299–310 (1972).
23. Lamartiniere CA, Moore J, Holland M, Barnes S. Neonatal genistein chemoprevents mammary cancer. Proc Soc Exp Biol Med 208:120–123 (1995).
24. Vickers AE, Sloop TC, Lucier GW. Mechanism of action of toxic halogenated aromatics. Environ Health Perspect 59:121–128 (1985).
25. Grubbs CJ, Hill DL, McDonough KC, Peckham JC. N-nitro-N-methylurea-induced mammary carcinogenesis: effect of pregnancy on preneoplastic cells. J Natl Cancer Inst 71:625–628 (1983).
26. Grubbs CJ, Farnell DR, Hill DL, McDonough KC. Chemoprevention of N-nitroso-N-methylurea-induced mammary cancers by pretreatment with 17ß-estradiol and progesterone. J Natl Cancer Inst 74:927–931 (1985).
27. Welsch CW. Host factors affecting the growth of carcinogen-induced rat mammary carcinomas: a review and tribute to Charles Brenton Huggins. Cancer Res 45:3415–3443 (1985).
28. Gierthy JH, Lincoln DW, Kampcik SJ, Dickerman HW, Bradlow HL, Niwa T, Swaneck GE. Enhancement of 2 and 16α-estradiol hydroxylation in MCF-7 human breast cancer cells in 2,3,7,8-tetrachlorodibenzo-p-dioxin. Biochem Biophys Res Commun 157:515–520 (1988).
29. Harris M, Zacharewski T, Safe S. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin and related compounds on the occupied nuclear estrogen receptor in MCF-7 human breast cancer cell. Cancer Res 50:3579–3584 (1990).
30. Nagasawa H, Yanai R, Taniguchi H. Importance of mammary gland DNA synthesis on carcinogen induced mammary tumorigenesis in rats. Cancer Res 36:2223–2226 (1976).
31. Makela S, Davis VL, Tally WC, Korkman J, Salo L, Vihko R, Santti R, Korach KS. Dietary estrogens act through estrogen receptor-mediated processes and show no antiestrogenicity in cultured breast cancer cells. Environ Health Perspect 102:572–578 (1994).
32. Peterson G, Barnes S. Genistein inhibition of the growth of human breast cancer cells: Independence from estrogen receptors and the multi-drug resistance gene. Biochem Biophys Res Commun 179:661–667 (1991).
33. Barnes S, Grubbs C, Setchell KDR, Carlson J. Soybeans inhibit mammary tumors in models of breast cancer. In: Mutagens and carcinogens in the diet (Pariza M, ed). New York:Wiley-Liss, 239–253;1990.
34. Shellabarger CJ, Soo VA. Effects of neonatally administered sex steroids on 7,12-dimethylbenz(a)anthracene-induced mammary neoplasia in rats. Cancer Res 33:1567–1569 (1973).
35. Nagasawa H, Yanai R, Shodono M, Nakamura T, Tanabe Y. Effect of neonatally administered estrogen or prolactin on normal and neoplastic mammary growth and serum estradiol-17ß level in rats. Cancer Res 34:2643–2646 (1974).
36. Lamartiniere CA, Holland MB. Neonatal diethylstilbestrol prevents spontaneously developing mammary tumors. In: Hormonal Carcinogenesis (Li JJ, Nandi SA, Li SA, eds). Berlin:Springer Verlag, 1992;305–308.
37. IARC. Occupational exposures in insecticide application, and some pesticides. IARC monographs on the evaluation of carcinogenic risks to humans, vol 53. Lyon:International Agency or Research on Cancer, 1991;179–249.
38. Scribner JD, Mottet NK. DDT acceleration of mammary gland tumors induced in the male Sprague-Dawley rat by 2-acetamidophenanthrene. Carcinogenesis 2:1235–1239 (1981).
39. Biegel L, Safe S. Effects of 2,3,7,8-tetrachlorodibenzo-r-dioxin (TCDD) on cell growth and the secretion of the estrogen-induced 24-, 52- and 160-kDa proteins in human breast cancer cells. J Steroid Biochem Mol Biol 37:725–732 (1990).
40. Astroff B, Rowlands C, Dickerson R, Safe S. 2,3,7,8-tetrachlorodibenzo-r-dioxin inhibition of 17ß-estradiol-induced increases in rat uterine EGF receptor binding activity and gene expression. Mol Cell Endrocinol 72:247–252 (1990).
41. Zacharewski TR, Bondy KL, McDonell P, Wu ZF. Antiestrogenic effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on 17ß-estradiol-induced pS2 expression. Cancer Res 54: 2707–2713 (1994).
42. Holcomb M, Safe S. Inhibition of 7,12-dimethylbenzanthracene-induced rat mammary tumor growth by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Cancer Lett 82:43–47 (1994).