

Endocrine-Disrupting Compounds and Mammary Gland Development: Early Exposure and Later Life Consequences

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Breast cancer is the most common non-skin cancer among women in this country. Breast cancer risk is significantly influenced by genetics, but over 70% of the women that are diagnosed have noninherited or sporadic cancer. The risk of breast cancer is thought to be modified by lifestyle and environment. Exposures to certain chemicals and hormone-mimicking or endocrine-disrupting compounds (EDCs) are suspected of contributing to increased breast cancer incidence as well as precocious puberty in the United States. Studies of EDC effects in rodents indicate that multiple toxicants can alter mammary gland development, with or without changing other markers of puberty. EDCs can cause transient and persistent effects on mammary gland development depending on dose, exposure parameters, and whether exposure was during critical periods of gland growth or differentiation. Adverse effects from these abnormal developmental patterns include

the presence of carcinogen-sensitive structures in greater numbers or for longer periods in the gland and inhibited functional differentiation leading to malnutrition or increased mortality of their offspring. Developmental toxicants of the mammary gland could lead to an increase in the incidence of mammary tumors if they alter circulating or tissue-localized hormone levels, gland receptor expression patterns, hormone transport, or metabolism that results in altered response to endogenous hormones or growth factors. Environmental disruptors of rodent mammary gland development must be identified for informed decisions in epidemiological studies aimed at identification of environmental factors contributing to breast cancer risk, altered breast development during puberty, or inability to produce sufficient breast milk. (*Endocrinology* 147: S18–S24, 2006)

THE RODENT MAMMARY gland has been used for decades as a model of the human breast. Animal models have been used to study both developmental events, including the function of genes in development via the use of knockout mouse technology, and the mechanisms, treatment, and potential prevention of breast cancer. These models have many fundamental benefits (beyond cost and ethical considerations), such as the ability to delete, suppress, or overexpress a gene in the tissue of interest, use of a large tissue pool to provide the statistical power necessary to substantiate a finding, the fact that rats and humans produce similar types of tumors in their mammary tissue (1, 2), and that the rodent and human undergo mammary gland development at a similar biological pace (albeit the absolute time is quite different) with a few exceptions (reviewed in Ref. 3). Rats and mice have been used to test endogenous components (those that are produced naturally by the body) or events for their ability to confer sensitivity to or protection from mammary tumor development (*e.g.* protective properties of pregnancy and adverse effects of 17β -estradiol). In recent years, animal models have been used to test exoge-

nous agents (accidental or unperceived exposures from the environment) for their mammary carcinogenicity or conferred sensitivity to other chemicals, in addition to their developmental effects on the mammary tissue. This review summarizes the developmental effects of exogenous components, such as endocrine-disrupting compounds (EDCs), on the mammary gland and highlights epidemiological examples that relate to findings in the rodent. Discussion of critical periods of exposure that may make the mammary gland more or less susceptible to additional carcinogenic/chemical exposures is also included.

Critical Periods of Mammary Gland Development

The epithelial bud and ductal outgrowth of the mouse and rat mammary gland begins to form during late gestation, approximately 6–7 d before birth (4). However, the little information available on fetal breast epithelial development suggests that the human tissue begins ductal development early in gestation, about embryonic wk (EW) 12–14 (5, 6). Both the rodent and human mammary epithelia grow at an isometric rate (at the same rate as the body) until just before puberty, although there is a short burst just before birth (5–7). Peripubertal growth of the mammary tissue, controlled by the rapidly changing hormonal milieu, is exponential, and the fat pad rapidly fills with epithelium to manifest the adult form of the gland. The gland will stay in this form, with minor changes depending on the stage of the estrous (rodent) or menstrual (human) cycle, or will undergo dramatic differentiation during pregnancy (7). The comparison of human

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Abbreviations: BPA, Bisphenol A; DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene; EDC, endocrine-disrupting compound; EW, embryonic week; GD, gestational day; PCB, polychlorinated biphenyl; PND, postnatal day; TEB, terminal end bud.

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and rodent mammary gland developmental periods is reviewed in Table 1 (modified from Ref. 3).

There are three phases of mammary gland growth that are suggested to be critical because they are the times during which paramount developmental events occur. Summarized in Fig. 1, the prenatal development of the mammary epithelial sprout (when the primary ducts form at the bud site coincident with the nipple) serves as a critical event. The mammary epithelial bud receives signals from the surrounding fat pad to form primary ducts and begin extension into the fat before birth (4). Interference of this occurrence could lead to altered timing of mammary development or formation of the glandular structures (altered number of primary ducts or blind ducts or unusual presence of nipple/areola), leaving lasting effects on the gland.

Another critical interval of mammary gland development is the peripubertal period, when mammary growth is exponential in nature. This span of time, several weeks in rodents or years in girls, features unique, highly proliferative, terminal end buds (TEBs) present throughout the gland (5–8). The ends of these teardrop-shaped structures are multiple cell layers thick and are the sites of further ductal branching (7). These structures eventually disappear from the mature gland as differentiation proceeds. Several studies have determined that TEB structures are sensitive to chemical carcinogens in rodent models, and in fact, TEB presence at the time of carcinogen exposure is positively associated with tumor multiplicity (the number of tumors per tumor-bearing animal (1, 8)). Potentially, any compound that prolonged the period of TEB presence in the developing gland, or slowed differentiation, could affect the sensitivity of the gland to chemical carcinogen action. Alternatively, environmental exposures could cause precocious TEB differentiation of the breast or confer protection from breast cancer risk.

Finally, the gland undergoes a third critical period of development during pregnancy. During this time, the gland prepares itself for functional lactation. Interruption of this process can lead to mortality or malnutrition of the offspring. This is particularly important in wildlife and domestic species that rely solely on maternal nourishment for reproduction. Pregnancy has been shown to be protective of breast tissue to later-life disease (cancer) (reviewed in Ref. 9). Whether this is because of some permanent change in receptor status, cell morphology, or signaling mechanisms necessary for cancer initiation or progression or is simply because of the final pregnancy-induced differentiation of breast TEBs is under investigation.

EDCs and Mammary Gland Development

There are relatively few studies that have reported on the effects of EDCs (or lack thereof) on mammary gland development. There are several possible reasons: 1) the primary focus of traditional 2-yr bioassay studies is to look for potential carcinogens in animals dosed in adulthood, after the most sensitive windows, for effects in the mammary gland; 2) the mammary gland is not a tissue suggested to be evaluated in tiered testing strategies for EDC effects on reproductive or endocrine tissues after peripubertal exposures (not looking for the effects at all); 3) only a small portion of government-based funding has prioritized the area of EDCs to cover these type of studies, even though expertise and ideas are available (*e.g.* EPA STAR grants support EDC research); 4) lack of information on the importance of this area of study; or some combination thereof. This review is meant to inform on the importance of this area of study but is not a breast cancer review. The relationship between critical-period exposure and later-life disease susceptibility is an important area of discussion.

Of the several EDCs that have been reported to affect mammary gland development in a rodent model, there is probably more information on the potent aryl-hydrocarbon receptor ligand 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (dioxin) than any other. This persistent EDC, recognized as a component of Agent Orange (10), is of note because its effects in humans have been (or are being) evaluated because of occupational or accidental environmental exposures. One report (11), looking specifically at pubertal development in a cohort of 200 Belgian adolescents (aged 15.8–19.6 yr old) exposed to polychlorinated aromatic hydrocarbons (including dioxin) via pollution demonstrated a significant delay in breast development in girls that was associated with a doubling of serum dioxin concentrations. The young women did not exhibit delayed menarche, but both girls and boys exhibited delayed adrenarche associated with serum contaminant levels. Another study evaluating pubertal timing in girls exposed to polybrominated biphenyls during gestation and lactation (12) found no change in mammary developmental timing but suggested that exposure caused earlier menstruation (average of 1 yr earlier).

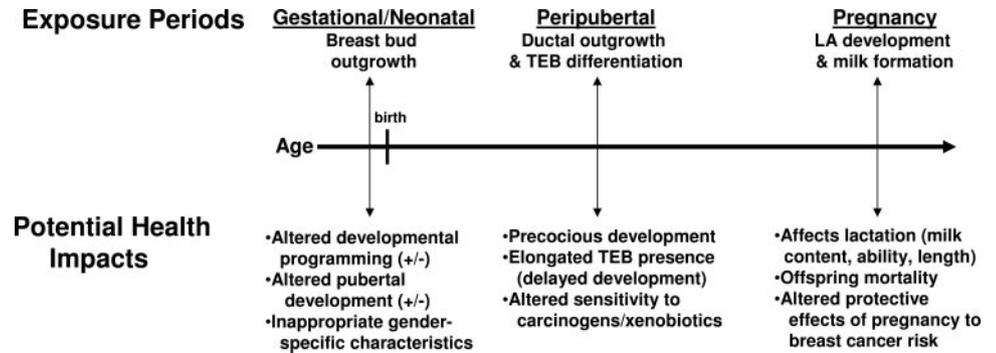
Early-life exposure to dioxin in animal models leads to delayed mammary gland development when exposed during critical periods of mammary development. Brown and co-workers (13) demonstrated, in Sprague-Dawley rats, that female offspring exposed to 1 $\mu\text{g}/\text{kg}$ dioxin on gestation d 15 (GD15) and cross-fostered to control dams after birth have

TABLE 1. Developmental events in human and rodent mammary tissue

Developmental event	Human	Rodent
Milk streak evident	EW4–6	GD10–11 (mice)
Mammary epithelial bud forms	EW10–13	GD12–14 (mice), GD14–16 (rat)
Female nipple and areola form	EW12–16	GD18 (mice), GD20 (rat)
Branching and canalization of epithelium	EW20–32	GD16 to birth (mice), GD18 to birth (rat)
Secretion is possible	EW32–40 (ability lost postnatally)	At birth, with hormonal stimuli
Isometric development of ducts	Birth to puberty	Birth to puberty
TEBs present (peripubertal)	8- to 13-yr-old girls	23–60 d old (rat)
Formation of lobular units	EW32–40, or within 1–2 yr of first menstrual cycle	Puberty and into adulthood

Human events are from Refs. 5 and 6; rodent events are from Refs. 4 and 7 and the author's personal observations.

FIG. 1. Timeline highlighting the critical periods of mammary gland development most likely to be altered by EDCs and the correlating potential health effects of these exposures. Three time periods are suggested from rodent model literature. LA, Lobuloalveolar.



delayed mammary differentiation at 50 d old. Lewis *et al.* (14) demonstrated stunted mammary development in 11-wk-old Holtzman rats after a single 1 $\mu\text{g}/\text{kg}$ dose of dioxin on GD15 and ovariectomy at 9 wk. Another study (15) demonstrated that Long-Evans rat offspring (a third strain) exposed to 1 $\mu\text{g}/\text{kg}$ dioxin on a single GD had delayed epithelial migration through the fat pad of the gland that was persistent into adulthood. The abnormal development was evident as early as postnatal d 4 (PND4), characterized by stunted longitudinal growth and fewer primary ducts and lateral branches. A critical period for the long-lasting developmental effect of dioxin on the mammary gland was determined to be around GD15, because single doses administered as late as GD20 (as well as several days of the postnatal period) had relatively little effect on the morphological development of the pup glands at weaning (15). This critical period corresponds to breast bud development taking place in the first trimester of women's pregnancies, EW10–13 (Table 1), a time when some women may not even know they are pregnant, and others are probably not aware of the sensitive developmental processes going on in their growing baby. Furthermore, when dioxin-exposed female rat offspring were bred and allowed to raise their litters, the mammary glands of female offspring in the second generation were also found to be significantly smaller than those of control animals. Although the abnormal development was apparent in the mammary epithelium of dioxin-exposed offspring, a transplantation study of affected epithelia into cleared fat pads of control rats (and vice versa) demonstrated a significant contribution from the stromal compartment of the gland for the abnormal epithelial development (15).

Another period of time during which dioxin-like compounds are reported to affect mammary gland development is during the peripubertal period (16). Animals exposed to multiple doses of 2.5 $\mu\text{g}/\text{kg}$ dioxin (PND25, 27, 29, and 31) had inhibited mammary epithelial outgrowth and fewer TEBs on PND32 as a result of their exposure. Dioxin doses as low as 1 $\mu\text{g}/\text{kg}$ on GD15 are known to delay puberty in rat offspring (17, 18) without altering serum estradiol levels. Yet, the effects of dioxin on the mammary gland, after identical exposure, persist into adulthood (15).

A third critical period of mammary gland development that is significantly affected by dioxin is development of the lactating gland. Studies in C57BL/6 mice, dosed with 5 $\mu\text{g}/\text{kg}$ dioxin on GD0, 7, and 14 demonstrated significant abnormal development of the pregnant gland as early as GD9, with the most dramatic effects seen on the day of

parturition (19). Dioxin exposure caused a decreased milk protein gene expression in the gland and early postnatal mortality in a group of dams allowed to deliver their offspring. Importantly, when mammary glands from age-matched nonpregnant mice given the same doses of dioxin were evaluated (with identical time between doses), there was no effect of dioxin. The impact of dioxin, or the related polychlorinated biphenyls (PCBs), on lactational performance in other species has been described (reviewed in Ref. 15). This is yet another example of how important it is to consider the timing of exposure and critical periods of development in the gland of interest. The impact of EDCs on breast milk production in women is not known, but many laboratories are currently investigating the environmental toxicants that are found in breast milk as a means of understanding exposures and potential risk to mother and child (3).

There are other examples of EDCs that have been shown to affect the development of the mammary gland. Atrazine, a high-use chlorotriazine herbicide brought to the attention of risk assessors because of its effects on mammary tumor formation in Sprague-Dawley rats (20), has been reported to cause abnormal mammary epithelial development in rats (21, 22). This nonlipophilic chemical is very different from dioxin (half-life in humans, 6–11 yr; rodent, 10–30 d; from <http://ntp.niehs.nih.gov/ntp/roc/toc11.html>) (23), having a far shorter half-life in people, rats, and the environment (day or days in mammals to several months in soil) (24). The reports on atrazine revealed that a 3-d exposure during a critical fetal period of mammary development can give rise to the most severe and long-lasting effects in Long-Evans rats, equivalent to that seen after a 7-d exposure (22). Cross-fostering studies revealed that both *in utero* and lactational exposures are needed to develop the persistent effects of the compound on the mammary gland of the offspring, suggesting that atrazine may be stored in the mammary gland of the dam during exposure and transferred to the offspring during lactation (21). Furthermore, if animals demonstrating abnormal mammary gland development after gestational atrazine exposure are bred, and allowed to rear offspring, the weight gain of their pups is significantly inhibited (12–25%), as is the mammary gland development of the pups (22). This is also a second example of an environmental toxicant that alters mammary gland development in multiple generations. The lowest doses of dioxin and atrazine able to cause these developmental abnormalities are yet to be determined, but it should be mentioned that the doses used in these animal

studies are significantly higher than reported human serum or urine levels, respectively.

Low-dose rodent studies using bisphenol A (BPA), a hormonally active compound found in plastics and dental sealants, have been performed (25, 26). This compound has a very short half-life, proposed to be about 90 min in rats (27). These studies suggest that low-dose (25 ng/kg·d) gestational or perinatal BPA administration to CD-1 mice caused a stimulation of mammary growth, with increased TEB early and more lateral branches per gland in the adult, compared with animals exposed to 0 or 250 ng/kg·d BPA. The higher dose of BPA had an opposite effect; reduced lateral and longitudinal growth compared with control (25, 26). In other studies using higher BPA doses (0.5 and 10 mg/kg·d; four daily sc injections starting on GD15), transient increases in mammary differentiation were noted (28). However, when the same lab repeated this experiment (four daily sc injections, 10 mg/kg) in 15-d-old CD-1 mice, they found no effect of treatment (29). These same studies revealed that zearalenone can also stimulate long-lasting proliferative effects on the mammary epithelium when exposure is transplacental (28). This information suggests, again, that the latter part of gestation is a critical period of development for the fetal mammary gland in rodents, correlating with first-trimester development in women (Table 1).

Only a handful of other environmental toxicants (nutritional, pharmaceutical, metals) have been evaluated for their effects on mammary gland development. Some heavy metals are being evaluated for reproductive tissue effects (arsenic, uranium), but cadmium is the only one (to date) with published effects on mammary proliferation (30). These metals are hypothesized to have estrogen-like attributes and are of interest because of their natural contamination of ground water.

Several dietary components known for their estrogen agonist or antagonist properties have been evaluated for their effects on mammary gland development, with a variety of outcomes reported depending on the delivery route (injection *vs.* oral), species evaluated, developmental time of delivery, exposure dose (many have U-shaped response curves), and diet of the animals on study (reviewed in Ref. 31). Among these compounds is genistein, a heavily studied component of soy. Genistein has been reported to have no effect on morphology of the mouse gland after gestational/lactational gavage exposure (32), to cause hyperplastic lesions in the adult rat gland after gestational/lactational dietary exposure (33) or *in utero* exposure (eight daily injections midpregnancy) (34), to dose-dependently increase the incidence of dimethylbenz[*a*]anthracene (DMBA)-induced mammary tumors after *in utero* exposure (35), or to confer protection from DMBA-induced mammary carcinogenesis after peripubertal exposure of female rats either by injection or via the diet (36, 37). This is an area of study on mammary gland development where few solid conclusions have been reached because of the variety of discrepancies in exposure conditions. The importance of dose for xenoestrogens is raised again in a recent study (38) that demonstrates differing effects of high- and low-dose diethylstilbestrol and tamoxifen on mammary gland proliferation and differentiation after neonatal exposure. Furthermore, the importance of re-

porting the dietary makeup of rodent study chow is also increasing, because whey (*vs.* casein) protein diets can alter rat mammary gland development (39). The same holds true for the use of soy-based diets.

Adverse Developmental Effects and Disease

A striking effect of the developmental perturbations in the mammary gland after environmental exposures is altered TEB differentiation (either hastened or delayed). The TEBs are well documented as the structure of the rodent mammary gland most sensitive to the effects of carcinogens (1) and when affected can give rise to adenomas and ductal carcinomas similar to those seen in women. Humans and rodents alike possess TEBs during the peripubertal period. Studies on brief gestational exposure to dioxin or atrazine (15, 21, 22) suggest that those EDCs can delay differentiation 2- to 3-fold compared with controls. This would leave the mammary gland vulnerable to the effects of carcinogens for a significantly longer developmental period. Girls that undergo precocious puberty may also be vulnerable to carcinogens for extended periods if they demonstrate longer time to final Tanner-stage breast development (start early but end at mean age for population). With this said, early or rapid breast development may confer protection from later-life disease.

Environmental exposures that promote or delay differentiation of the mammary epithelia may cause uncommon numbers of TEBs to be present at inopportune times. For example, studies evaluating the effects of dioxin on chemically induced tumor development in rodent models have found that toxicant exposure on GD15 (13) or PND18 (40) increased the number of mammary tumors in the adult rodent. Brown *et al.* (13) reported more TEBs present in dioxin-exposed animals on PND50 (the day that chemical carcinogen was delivered) compared with controls; some may translate that to mean that dioxin increased the number of TEBs in the gland, but careful examination of this event over time in Long-Evans rats (15) determined that dioxin causes an impairment of TEB development and delays both their formation and differentiation. Once they are present in the gland, they are there for a longer period of time. This finding might explain the increased mammary tumors after carcinogen exposure; more TEBs mean increased targets for carcinogen action. Interestingly, the stromal portion of the mammary gland is not only important for dioxin-induced developmental events (15), but mouse mammary fibroblasts lacking dioxin receptor demonstrate impaired tumorigenicity (nearly four times less than control) in a mouse xenograft model (41). Those findings suggested that the dioxin receptor signaling pathway may be involved in an angiogenic or extracellular matrix mediated response needed for tumor formation.

Few studies of the effects of atrazine on chemically induced mammary tumors have been conducted. Sprague-Dawley rats bearing chemically induced tumors or not were ovariectomized and were treated with atrazine (0, 5, 50, or 500 ppm in diet) to evaluate the chemical's effect on tumor cell proliferation (42). Only in the group of animals that had no tumors after ovariectomy, they found a significant increase in the percent incidence of mammary tumors at the

highest dose of dietary atrazine (500 ppm) and in the number of tumors per rat in the two highest atrazine dose groups (average per rat was less than one). Another study used Ha-ras transgenic rats, chemically induced on PND50 to form mammary tumors (43), that were also treated with 0, 5, 50, and 500 ppm atrazine in their diet. The lowest doses produced higher incidences of tumors than control, but a dose response was lacking. Other studies in Sprague-Dawley rats fed atrazine for nearly 2 yr demonstrated a dose response in increased incidence and earlier appearance of spontaneously forming adenomas and carcinomas, but this effect was due to precocious reproductive senescence (mode of action) caused by the herbicide (20, 44). This finding deems evaluation of the estrous cycle in aging rodents very important, especially in Sprague-Dawley rat tumor studies, because the atrazine mode of action is not thought to be applicable to women (44). The mode or mechanism of action for other mammary developmental toxicants is under investigation.

Several other xenoestrogens and organochlorines, specifically PCBs and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE), have been suspected of involvement in mammary tumor development. In two separate studies, Desaulniers and co-workers (40, 45) found no significant effect of organochlorine mixtures, delivered on 5 d between birth and weaning, on either mammary gland development or carcinogen-induced tumor development. Another study also demonstrated no effect of prenatal exposure to biologically relevant levels of organochlorine mixture on mammary gland development but that this mixture may interact with dietary genistein to promote ductal hyperplasia (34). Organochlorines have also been the focus of several large case-control studies on breast cancer risk (cohorts of middle-aged women). Results of multiple rodent and epidemiological studies have failed to demonstrate a consistent significant correlation between PCB/DDE (organochlorine) exposure and development of breast cancer (reviewed in Refs. 31 and 46). In fact, a review of five case-control studies (total of over 1400 cases and 1600 controls), which included the Nurses Study, demonstrated no positive relationship between serum PCB or DDE levels and risk of breast cancer (47). However, this developmental exposure review may address the reason for the lack of relationship of body burden and disease in the case-control studies. The studies in rodents described here focus on critical periods of mammary development (*in utero*, puberty, and pregnancy) and demonstrate effects of environmental compounds during these times. Dioxin, for example, has little if any noticeable effect on the mammary gland of the adult animal yet serious and long-lasting effects if exposure is during a critical period of development (15, 19). Case-control studies lack the ability to evaluate exposures to these women during their own critical breast developmental windows. Longitudinal cohorts are the best way to determine the early life factors or exposures necessary for increased breast cancer risk, even though study results may be painfully slow and costly.

There are many environmental compounds for which there is no information about human or rodent mammary tissue effects, yet levels of these compounds are on the rise in either the U.S. population or wildlife species or both. For example, many chemicals known to alter endocrine hormone

levels affect rodent pup weight gain or mortality (potential effect on the dam's milk production) or other reproductive tissues in the pup after gestational exposure remain to be evaluated for mammary gland developmental effects, not to mention tumor development. Some chemical classes that fall into this category include perchlorates, phthalates, perfluorinated alkyl acids, water disinfection by-products, alkylphenols, other heavy metals, and polybrominated flame retardants.

Recommendations

To translate rodent data on mammary gland effects to human risk assessment, more information is needed on the kinds, doses, and timing of toxicant exposures that affect this important tissue. Repetition of findings in the rodent model from more than one lab, as is the case with the dioxin effects on mammary gland development (13–15), will help epidemiologists determine which chemical or chemical classes to focus ever-limiting study funds on. Furthermore, determination of sources of these exposures (and potentially the mixture of exposures) will assist epidemiologists and risk assessors. Studies on rodent mammary gland development have shown that the majority of the effects are found after exposure during critical periods of development (bud outgrowth, puberty-induced exponential growth, and pregnancy). Studies that focus solely on exposure of mature animals or adult women and try to evaluate exposures once the disease or adverse health outcome has occurred may be missing important details on developmental effects of the exposure. Future studies on environmental exposures affecting mammary or breast development and their sensitivity to carcinogens must include a careful evaluation of dose-response studies delivered during these sensitive periods of gland development. Many animal studies are conducted with exposure doses that would result in a body burden higher than that found in humans; future work should strive to use doses relevant to humans or at least measure body burden in the rodent studies so that information can be used in risk assessment.

As we begin to better understand the sources of environmental exposures known to affect breast development, pregnant and lactating women and peripubertal adolescents should be informed of these potential sources of exposure by their health education teachers, pediatricians, obstetricians, lactation consultants, or midwives. A substantial learning curve lies ahead to accomplish this goal, but it is imperative that exposure during critical periods of growth of the mammary gland (as well as other reproductive tissues) be limited to decrease or prevent an increase in future adverse reproductive effects (*e.g.* cancer, fertility, inflammatory disease, and miscarriage).

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References

- Russo IH, Russo J 1996 Experimentally induced mammary tumors in rats. *Br Cancer Res Treat* 39:7–20
- Cardiff RD, Anver MR, Gusterson BA, Hennighausen L, Jensen RA, Merino MJ, Rehm S, Russo J, Tavassoli FA, Wakefield LM, Ward JM, Green JE 2000 The mammary pathology of genetically engineered mice: the consensus report and recommendations from the Annapolis meeting. *Oncogene* 19:968–988
- Fenton SE, Condon M, Ettinger AS, LaKind JS, Mason A, McDiarmid M, Qian Z, Selevan SG 2005 Collection and use of exposure data from human milk biomonitoring in the United States. *J Toxicol Environ Health A* 68:1691–1712
- Sakakura T 1987 Mammary embryogenesis. In: Neville MC, Daniel CW, eds. The mammary gland: development, regulation, and function. New York: Plenum Press; 37–66
- Elston CW, Ellis IO 1998 Normal structure and developmental abnormalities. In: Elston CW, Ellis IO, eds. The breast. Edinburgh: Churchill Livingstone; 1–19
- Russo J, Russo IH 1987 Development of the human mammary gland. In: Neville MC, Daniel CW, eds. The mammary gland: development, regulation, and function. New York: Plenum Press; 67–93
- Daniel CW, Silberstein GB 1987 Postnatal development of the rodent mammary gland. In: Neville MC, Daniel CW, eds. The mammary gland: development, regulation, and function. New York: Plenum Press; 3–36
- Russo IH, Russo J 1978. Developmental stage of the rat mammary gland as determinant of its susceptibility to 7,12-dimethylbenzanthracene. *J Natl Cancer Inst* 61: 1439–1449
- Russo J, Russo IH 1997 Differentiation and breast cancer. *Medicina (B Aires)* 57(Suppl 2):81–91
- Mandal PK 2005 Dioxin: a review of its environmental effects and its aryl hydrocarbon receptor biology. *J Comp Physiol B* 175:221–230
- den Hond ED, Roels HA, Hoppenbrouwers K, Nawrot T, Thijs L, Vandermeulen C, Winneke G, Vanderschueren D, Staessen JA 2002 Sexual maturation in relation to polychlorinated aromatic hydrocarbons: Sharpe and Skakkebaek's hypothesis revisited. *Environ Health Perspect* 110:771–776
- Blanck HM, Marcus M, Tolbert PE, Rubin C, Henderson AK, Hertzberg VS, Zhang RH, Cameron L 2000 Age at menarche and Tanner stage in girls exposed *in utero* and postnatally to polybrominated biphenyl. *Epidemiology* 11:641–647
- Brown NM, Manzillo PA, Zhang JX, Wang J, Lamartiniere CA 1998 Prenatal TCDD and predisposition to mammary cancer in the rat. *Carcinogenesis* 19:1623–1629
- Lewis BC, Hudgins S, Lewis A, Schorr K, Sommer R, Peterson RE, Flaws JA, Furth PA 2001 *In utero* and lactational treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin impairs mammary gland differentiation but does not block the response to exogenous estrogen in the postpubertal female rat. *Toxicol Sci* 62:46–53
- Fenton SE, Hamm JT, Birnbaum LS, Youngblood GL 2002 Persistent abnormalities in the rat mammary gland following gestational and lactational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Toxicol Sci* 67:63–74
- Brown NM, Lamartiniere CA 1995 Xenoestrogens alter mammary gland differentiation and cell proliferation in the rat. *Environ Health Perspect* 103:708–713
- Flaws JA, Sommer RJ, Silbergeld EK, Peterson RE, Hirshfield AN 1997 *In utero* and lactational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) induces genital dysmorphogenesis in the female rat. *Toxicol Appl Pharmacol* 147:351–362
- Gray Jr LE, Wolf CJ, Mann P, Ostby JS 1997 *In utero* exposure to low doses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin alters reproductive development of female Long Evans hooded rat offspring. *Toxicol Appl Pharmacol* 146:237–244
- Vorderstrasse BA, Fenton SE, Bohn AA, Cundiff JA, Lawrence BP 2004 A novel effect of dioxin: exposure during pregnancy severely impairs mammary gland differentiation. *Toxicol Sci* 78:248–257
- Eldridge JC, McConnell CF, Wetzel LT, Tisdell LO 1998 Appearance of mammary tumors in atrazine-treated female rats: probable mode of action involving strain-related control of ovulation and estrous cycling. In: Ballantine LG, McFarland JE, Hackett DS, eds. Triazine herbicides: risk assessment. Washington, DC: American Chemical Society; 414–423
- Rayner JL, Wood C, Fenton SE 2004 Exposure parameters necessary for delayed puberty and mammary gland development in Long-Evans rats exposed *in utero* to atrazine. *Toxicol Appl Pharmacol* 195:23–34
- Rayner JL, Enoch RR, Fenton SE 2005 Adverse effects of prenatal exposure to atrazine during a critical period of mammary gland growth. *Toxicol Sci* 87: 255–266
- Report on carcinogens. 11th ed. U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program
- Wu J, Robinson R, Simoneaux B 1998 Metabolism of selected S-triazines in animals. In: Ballantine LG, McFarland JE, Hackett DS, eds. Triazine herbicides: risk assessment. Washington, DC: American Chemical Society; 95–103
- Markey CM, Luque EH, Munoz de Toro M, Sonneschein C, Soto AM 2001 *In utero* exposure to bisphenol A alters the development and tissue organization of the mouse mammary gland. *Biol Reprod* 65:1215–1223
- Munoz de Toro M, Markey CM, Wadia PR, Luque EH, Rubin BS, Sonneschein C, Soto AM 2005 Perinatal exposure to bisphenol A alters peripubertal mammary gland development in mice. *Endocrinology* 146:4138–4147
- Long X, Steinmetz R, Ben-Jonathan N, Caperele-Grant A, Young PCM, Nephew KP, Bigsby RM 2000 Strain differences in vaginal responses to the xenoestrogen bisphenol A. *Environ Health Perspect* 108:243–247
- Nikaido Y, Yoshizawa K, Danbara N, Tsujita-Kyutoku M, Yuri T, Uehara N, Tsubura A 2004 Effects of maternal xenoestrogen exposure on development of the reproductive tract and mammary gland in female CD-1 mouse offspring. *Reprod Toxicol* 18:803–811
- Nikaido Y, Danbara N, Tsujita-Kyutoku M, Yuri T, Uehara N, Tsubura A 2005 Effects of prepubertal exposure to xenoestrogen on development of estrogen target organs in female CD-1 mice. *In Vivo* 19:487–494
- Johnson MD, Kenney N, Stoica A, Hilakivi-Clarke L, Stingh B, Chepko G, Clarke R, Sholler P F, Lirio AA, Foss C, Reiter R, Trock B, Paik S, Martin M B 2003 Cadmium mimics the *in vivo* effects of estrogen in the uterus and mammary gland. *Nature* 9:1081–1084
- Birnbaum LS, Fenton SE 2003 Cancer and developmental exposures to endocrine disrupting compounds (EDCs). *Environ Health Perspect* 111:389–394
- Fielden MR, Fong CJ, Haslam SZ, Zacharewski TR 2002 Normal mammary gland morphology in pubertal female mice following *in utero* and lactational exposure to genistein at levels comparable to human dietary exposure. *Toxicol Lett* 133:181–191
- Decloux KB,ucci TJ, Lomax LG, Latendresse JR, Warbritton A, Weis CC, Newbold RR 2001 Effects of dietary genistein exposure during development on male and female CD (Sprague-Dawley) rats. *Reprod Toxicol* 15:647–663
- Foster WG, Younglai EV, Boutross-Tadross O, Hughes CL, Wade MG 2004 Mammary gland morphology in Sprague-Dawley rats following treatment with an organochlorine mixture *in utero* and neonatal genistein. *Toxicol Sci* 77:91–100
- Hilakivi-Clarke L, Cho E, Onojafe I, Raygada M, Clarke R 1999 Maternal exposure to genistein during pregnancy increases carcinogen-induced mammary tumorigenesis in female rat offspring. *Oncol Rep* 6:1089–1095
- Murrill WB, Brown NM, Zhang JX, Manzillo PA, Barnes S, Lamartiniere CA 1996 Prepubertal genistein exposure suppresses mammary cancer and enhances gland differentiation in rats. *Carcinogenesis* 17:1451–1457
- Fritz WA, Coward L, Wang J, Lamartiniere CA 1998 Dietary genistein: perinatal mammary cancer prevention, bioavailability and toxicity testing in the rat. *Carcinogenesis* 19:2151–2158
- Hovey RC, Asai-Sato M, Warri A, Terry-Koroma B, Colyn N, Ginsburg E, Vonderhaar BK 2005 Effects of neonatal exposure to diethylstilbestrol, tamoxifen, and toremifene on BALB/C mouse mammary glands. *Biol Reprod* 72:423–435
- Eason RR, Velarde MC, Chatman Jr L, Till SR, Geng Y, Ferguson M, Badger TM, Simmen RC 2004 Dietary exposure to whey protein alters rat mammary gland proliferation, apoptosis and gene expression during postnatal development. *J Nutr* 134:3370–3377
- Desaulniers D, Leingartner K, Russo J, Perkins G, Chittim BG, Archer MC, Wade M, Yang J 2001 Modulatory effects of neonatal exposure to TCDD, or a mixture of PCBs, *p,p'*-DDT, and *p,p'*-DDE, on methylnitrosourea-induced mammary tumor development in the rat. *Environ Health Perspect* 109:739–747
- Mulero-Navarro S, Pozo-Guisado E, Perez-Mancera PA, Alvarez-Barrientos A, Catalina-Fernandez I, Hernandez-Nieto E, Saenz-Santamaria J, Martinez N, Rojas JM, Sanchez-Garcia I, Fernandez-Salguero PM 2005 Immortalized mouse mammary fibroblasts lacking a dioxin receptor have impaired tumorigenicity in a subcutaneous mouse xenograft model. *J Biol Chem* 280:28731–28741
- Ueda M, Imai T, Takizawa T, Onodera H, Mitsumori K, Matsui T, Hirose M 2005 Possible enhancing effects of atrazine on growth of 7,12-dimethylbenz(a)anthracene-induced mammary tumors in ovariectomized Sprague-Dawley rats. *Cancer Sci* 96:19–25
- Fukamachi K, Han BS, Kim CK, Takasuka N, Matsuoka Y, Matsuda E, Yamasaki T, Tsuda H 2004 Possible enhancing effects of atrazine and nonylphenol on 7,12-dimethylbenz(a)anthracene-induced mammary tumor development in human c-Ha-*ras* proto-oncogene transgenic rats. *Cancer Sci* 95: 404–410
- Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel, Atrazine: hazard and dose-response assessment and characterization.

- SAP Report No. 2000-05 of the FIFRA Scientific Advisory Panel Meeting, Arlington, VA, 2000
45. **Desaulniers D, Leingartner K, Musicki B, Cole J, Li M, Charbonneay M, Tsang BK** 2004 Lack of effects of postnatal exposure to a mixture of aryl hydrocarbon receptor agonists on the development of methylnitrosourea-induced mammary tumors in Sprague Dawley rats. *J Toxicol Environ Health* 67:1457–1475
46. **Safe S** 2004 Endocrine disruptors and human health: is there a problem? *Toxicology* 205:3–10
47. **Laden F, Collman G, Iwamoto K, Alberg AJ, Berkowitz GS, Freudenheim JL, Hankinson SE, Helzlsouer KJ, Holford TR, Huang HY, Moysich KB, Tessari JD, Wolff MS, Zheng T, Hunter DJ** 2001 1,1-Dichloro-2,2-bis(*p*-chlorophenyl)ethylene and polychlorinated biphenyls and breast cancer: combined analysis of five U.S. studies. *J Natl Cancer Inst* 93:768–776

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