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# Evaluating chemical effects on mammary gland development: A critical need in disease prevention

Gwendolyn Osborne[a], Ruthann Rudel[b], Megan Schwarzman[a,*]

[a] Center for Occupational and Environmental Health, University of California at Berkeley, Berkeley, CA, USA
[b] Silent Spring Institute, Newton, MA, USA

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ABSTRACT

Although understanding the environmental factors that contribute to breast cancer could improve disease prevention, standard chemical testing protocols do not adequately evaluate chemicals' effects on breast development. Evidence suggests: (1) mammary gland (MG) development is a complex process that extends from gestation through fetal and neonatal growth, puberty, and pregnancy; (2) altered MG development can increase the risk of breast cancer and other adverse outcomes; and (3) chemical exposures during susceptible windows of development may alter the MG in ways that increase risk for later disease. Together, these highlight the need to better understand the complex relationship between exposure to endocrine disrupting compounds (EDCs) and the alterations in MG morphology and gene expression that ultimately increase disease risk. Changing guideline toxicity testing studies to incorporate perinatal exposures and MG whole mounts would generate critical knowledge about the effects of EDCs on the MG and could ultimately inform disease prevention.



## 1. Introduction

Breast cancer is the most common cancer in women worldwide and the second leading cause of cancer death in American women, after lung cancer [1,2]. The probability of a woman being diagnosed with breast cancer during her lifetime is one in eight [3]. While inherited risk factors explain up to a third of breast cancer cases [4,5], the risk factors for the remaining majority of cases are not well understood. In addition to a steady increase in female breast cancer, the incidence of male breast cancer has increased in the past few decades in the U.S. and internationally [6]. The correlation of male and female incidence rates over time suggests that there may be risk factors that are similar for both men and women [6,7]. Exposure to chemicals in the environment and consumer products is hypothesized to contribute to cancer risk [8–10], as well as to other breast health outcomes, such as impaired lactation [11] or male gynecomastia [12].

Despite emerging evidence that chemical exposure may contribute to breast cancer risk, most chemicals are not evaluated for their potential impact on breast tissue, particularly during vulnerable stages of development. Although understanding the environmental factors that contribute to breast cancer has the potential to dramatically improve prevention of the disease, standard chemical testing protocols do not adequately evaluate chemicals' effects on breast tissue. Most protocols lack an evaluation of the effects of exposures during critical periods of mammary gland (MG) development, as well as an assessment of functional outcomes such as lactation impairment [13].

As a result, we have only limited evidence of how chemicals such as endocrine disruptors (EDs) alter MG development, and how those effects contribute to adverse outcomes later in life. The evidence that exists suggests three key points: (1) MG development is a complex process that extends from gestation through multiple life stages; (2) altered MG development can increase the risk of breast cancer, as well as other adverse outcomes; and (3) chemical exposures during susceptible windows of development may alter the MG in ways that increase risk for later disease. Together, these points highlight the need to better understand the complex relationship between environmental exposures and the alterations in MG morphology and gene expression that ultimately increase the risk of disease.

## 2. Mammary gland development is a complex process extending from gestation through multiple life stages

Normal breast development in both humans and rodents consists of a series of well-orchestrated events that are finely regulated by a balance of hormones, growth factors, and

* Corresponding author. Tel.: +1 15106434685; fax: +1 510 642 5815.
*E-mail address:* mschwarzman@berkeley.edu (M. Schwarzman).

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stromal factors [14,15]. Growth depends on endocrine signaling from the hypothalamic–pituitary–gonadal axis, autocrine and paracrine hormones, and growth factors from outside tissues. Crosstalk between the epithelium and surrounding stroma also helps to balance proliferation and apoptosis during normal developmental remodeling of the MG [15,16].

The MG is distinct from other tissues in that it undergoes a significant portion of its development postnatally; in addition to the fetal/neonatal period, puberty and pregnancy are critical periods of MG development. In most mammals, MG development begins with the formation of the mammary, or milk, line. This separates into individual placodes, each of which develops into a ductal tree that embeds in a fat pad to form the mammary bud [15,17,18]. Factors that interfere with signaling from the surrounding fat pad to the mammary bud can potentially alter the timing of development or formation of glandular structures [19,20].

Subsequent to the neonatal period there is little epithelial growth until puberty. During puberty, mammary growth is exponential; this is a period of several weeks in rodents, or years in girls, during which the fat pad rapidly fills with epithelial cells to become the adult form of the gland. The epithelium develops bundles of ducts, which then form club-like structures, called terminal end buds (TEB) in humans. Each TEB cleaves into alveolar buds and sprouts into ductules. This structural unit, comprised of the terminal duct and the ductules, is called the terminal ductal lobular units (TDLU) [21]. Rodents also form TEBs, which are the structures most functionally equivalent to TDLUs in humans. These teardrop-shaped structures are the sites of future ductal branching and disappear as the gland differentiates [20].

In both humans and rodents, the key periods of development in MG maturation are regulated by the activation (in the fetal and neonatal periods) and later the reactivation (during puberty) of the hypothalamic–pituitary–gonadal (HPG) and hypothalamic–pituitary–adrenal (HPA) axes, which control the release of hormones [14,16,18].

The gland reaches a fairly static state from first menstruation until a pregnancy occurs. During pregnancy, the gland undergoes another period of rapid differentiation, involving branching and the development of lobulo-alveoli to prepare for lactation [19].

Male breast development also occurs in utero, but the androgen surge halts further development immediately prior to birth. Exposure to anti-androgens has been shown to lead to retained nipples in male rats [22–24].

## 3. Alterations to mammary gland development can increase the risk of breast cancer and other adverse outcomes

### 3.1. Human evidence

The fetal origins of adult disease hypothesis propose that disturbances to the fetal environment have physiological and structural consequences with the potential to alter an individual's disease risk as an adult [25]. Human epidemiological studies provide support for this hypothesis with respect to breast health outcomes, as alterations to the finely regulated process of MG development have been shown to produce changes that affect women later in life. For example, various birth parameters have been associated with breast cancer risk. Birth weight [26], longer birth length [27], older maternal and paternal age [28], and in utero exposure to synthetic estrogen and diethylstilbestrol (DES) [29] have all been associated with an increased risk of later breast cancer, while maternal preeclampsia is associated with a lower risk [28,30]. Each of these factors affects the earliest period of breast development that starts before birth.

The second period of breast development occurs during puberty. Ionizing radiation has the greatest effect on later breast cancer risk when exposure occurs before the age of 20 [31,32], suggesting that the period from childhood through adolescence is another significant period of vulnerability for the human breast.

Pregnancy is a third period of breast development during which external factors can alter disease susceptibility. Younger age at first birth, multiple gestation pregnancies (twins and greater), preeclampsia, pre-pregnancy obesity, and gestational hypertension may all lower maternal breast cancer risk (reviewed by [33,34]), while increased placental weight [35], higher infant birth weight [34], and DES exposure during pregnancy are associated with increased risk of maternal breast cancer. One factor driving these changes is total lifetime exposure to ovarian hormones. Lower cumulative exposure to estrogen – such as with pre-eclampsia – seems to protect against breast cancer [28]. Higher exposure to progesterone may increase risk of breast cancer, and affecting progesterone or progesterone receptor signaling pathways promotes breast cancer progression [36,37].

The relationship between lifetime estrogen and progesterone exposure and breast cancer risk provides a framework for understanding how chemicals that affect hormone homeostasis may alter breast development and ultimately cancer risk.

### 3.2. Rodent evidence

The rodent MG undergoes staged development analogous to that observed in humans during gestation, puberty, and pregnancy. Animal evidence further supports the hypothesis that there are periods of vulnerability during breast development that influence later life outcomes. A structure particularly important in rodent MG development and carcinogen susceptibility is the TEB [20,38]. The TEB has the greatest number of proliferating cells and the shortest cell cycle of the structures in the developing MG. Malignant tumors, such as adenocarcinomas induced by certain carcinogens, are most common in rodents following exposures that occur between days 40 and 46 of life (correlating with puberty in humans), the period of development when TEBs are most actively differentiating into alveolar buds. Benign tumors, such as adenomas, fibroadenomas, and mammary cysts, are thought to arise from the more differentiated alveolar buds [39].

Anything that changes the timing of mammary development will affect the timing of the presence of TEBs, and therefore the window of susceptibility to carcinogens. Earlier induction of MG development in rodents leads to a greater number of TEBs compared to terminal ducts and increased alveolar budding at the time of weaning, followed by the development of more lobules than in control animals [40,41]. On the other hand, late initiation of mammary development causes decreased longitudinal growth of the epithelium and fewer TEBs, and decreased alveolar budding at weaning [42]. As development progresses, these glands may have more TEBs at puberty, because the pace of development is slower [43]. It is hypothesized that factors that lengthen the period when TEBs are present lengthen the period during which the MG is susceptible to carcinogens.

### 3.3. Non-cancer effects

Altered MG development is also associated with non-cancer effects such as lactation impairment and gynecomastia in both rodents and humans. While it is recommended that infants are breastfed exclusively for at least the first six months of life [44], several million mothers are unable to breastfeed or have significant difficulty breastfeeding each year [45]. Research in rodents suggests that factors that interfere with MG growth and differentiation can negatively affect both the gland's ability to produce milk