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

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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.

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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

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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

and the milk composition [45,46]. Factors that accelerate MG development also make the gland less sensitive to ovarian and pituitary hormones. Because effective lactation depends on these hormonal signals to stimulate the gland, a less responsive gland is less likely to be capable of normal milk production [43].

Gynecomastia, or benign breast enlargement due to proliferation of the ductal epithelium [12], occurs in up to two-thirds of pubertal boys [47]. It is hypothesized that the altered MG development that causes this condition arises due to an imbalance in estrogen and androgen [48,49].

4. Exposure to chemicals during windows of susceptibility can alter mammary gland structure and function, increasing cancer risk

4.1. The role of hormones and EDs

Many of the established risk factors for breast cancer are proxy measures for changes in the hormonal environment [50–52]. For example, high birth weight, birth length, and placental weight are associated with increased endogenous intrauterine estrogen exposure [53,54], while pre-eclampsia is associated with increased androgen levels [55]. Furthermore, DES, a synthetic estrogen prescribed to prevent miscarriage from the 1940s through 1970s, has been associated with breast cancer in women who were directly exposed, as well as their daughters [29,56].

Levels of circulating estrogens and androgens are associated with breast cancer risk in both premenopausal and postmenopausal women [57–59]. Breast cancer risk is elevated by extended exposure to high levels of endogenous hormones, which can occur with obesity [60] or as a result of early age at menarche or late age of first pregnancy and menopause [61,62]. However, the relationship between hormones and breast cancer is complicated by the fact that breast cancer is a heterogeneous disease with at least five molecular subtypes. The presence or absence of biological markers, such as estrogen receptors (ER+/ER–), progesterone receptors (PR+/PR–), and human epidermal growth factor receptor 2 (HER2+/HER2–), serve as approximations of the molecular subtypes [63]. The majority of information pertaining to hormonal risk factors is specifically associated with the ER+/luminal A subtype. The influence of hormones on ER– and triple negative (ER–, PR–, and HER2–) breast cancers are not as well characterized [57,64].

The endocrine system governs hormone production, and as such, plays a critical role in development and ongoing function of multiple organ systems. Disruption of the endocrine system is linked to multiple adverse outcomes, including metabolic disturbances (e.g., obesity) [65], and alterations to lactation, breast density [66], immune function [67,68], and the timing of puberty [69] and menopause [70]. An ED was originally defined as “an exogenous agent that interferes with the production, release, transport, metabolism, binding, action or elimination of natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes” [71]. This definition has been simplified by Zoeller et al. [72] to define an ED as “an exogenous chemical, or mixture of chemicals, that interferes with any aspect of hormone action”. Compounds that have estrogen-like activity have been studied the most, yet chemicals with anti-estrogenic, androgenic, anti-androgenic, progesterone-like, and thyroid-like activity are also EDs [73].

4.2. EDs as carcinogens

Developmental exposure to EDs may contribute indirectly to a variety of adverse outcomes by altering MG development in ways that raise the risk of later disease. For example, chemical

alterations to breast development in early life can increase the tissue's susceptibility to a subsequent carcinogenic exposure, a so-called ‘second hit’ [43]. Carcinogen challenge models in experimental animals have demonstrated these effects by using a known chemical carcinogen following an early life chemical exposure to show the increased rate of tumor formation in response to the second exposure, relative to tumor incidence from the known carcinogen alone [10]. This effect has been observed in rodents with prenatal exposure to both estrogens and androgens [74–76]. Additionally, increased tumor number and accelerated tumor development after carcinogen challenge have been observed following early life exposure to known EDs such as alcohol [77], dioxin [78–80], bisphenol A (BPA) [81], and the phytoestrogen genistein [82,83].

Research suggests that, in addition to increasing susceptibility to a second hit by a chemical carcinogen, EDs may have direct carcinogenic effects. For example, DES causes cervical, vaginal, uterine, endometrial, and breast cancer via a combination of genotoxic, estrogen-receptor-mediated, and epigenetic effects, independent of subsequent carcinogen exposure [84]. A recent study demonstrated that BPA caused a non-significant increase in atypical hyperplasia and adenocarcinoma of the MG at doses ranging from 0.25 to 250 micrograms/kg-day without later exposure to another chemical carcinogen [85]. Taken together, these findings suggest that in some cases EDs may be complete carcinogens, not requiring a second hit to cause cancer.

4.3. Rodent evidence

Rodent studies suggest that many mammary carcinogens also alter MG development, often by accelerating or delaying development and/or alter branching and epithelial structure (specific chemicals reviewed in [10]). The effects of ED exposure are highly dependent on the level and timing of exposure, and can even have protective effects when exposure occurs at the right level and at a particular developmental stage. EDs can stimulate growth, causing the MG to mature with an increased ratio of fully differentiated structures compared to immature or undifferentiated structures. They can also reduce the ratio of proliferation to apoptosis in the epithelium [43], reducing the risk for tumor formation. For example, prenatal [78] and neonatal [79] dioxin exposure alters rodent MG differentiation, increasing MG tumor development following treatment with dimethylbenz[*a*]anthracene (DMBA). In contrast, dioxin exposure in long-term feeding studies decreases spontaneous tumor development [86], and dioxin exposure during pregnancy followed by treatment with DMBA four weeks later delays tumor formation compared to controls [87]. Likewise, genistein exposure during the peripubertal period decreases tumors after a carcinogen challenge, while perinatal and lifelong exposure appears to increase tumors in rats [88].

4.4. Human evidence

As with rodent studies, human studies demonstrate that outcomes depend on the timing of exposure. Cohn et al. [89] demonstrated that breast cancer is associated with DDT exposure, but only when exposure occurred before 14 years of age. This study used blood samples obtained before DDT use was banned to find that women who had high levels of serum DDT and were under 14 years old at time of exposure had a 5-fold increased risk of breast cancer, while women who were not exposed prior to 14 years of age demonstrated no association. Because puberty is a period of rapid development when the MG is particularly vulnerable to the effects of chemical exposure, lengthening puberty extends this window of susceptibility, thus increasing the possibility of ED exposure and adverse outcomes. This is particularly concerning in light of the

current shift toward earlier onset breast development in American and European girls, with girls now beginning breast development (thelarche) earlier and taking longer to reach full breast development than in previous generations [90]. Endogenous hormones play a central role in the onset and regulation of puberty; estrogen, the primary hormone in early breast development, promotes growth of the ducts, while progesterone promotes lobuloalveolar development [73,91]. The physical changes seen in premature thelarche may reflect the onset of pubertal maturation, or they may reflect exposure to endogenous or exogenous hormones independent of the maturity stage of reproductive or adrenal systems (pseudo-puberty) [92]. In general, precocious puberty is associated with various adverse outcomes, including metabolic syndrome and polycystic ovarian syndrome, behavioral disorders, testicular and prostate cancer, and breast cancer in later life [92]. While it is unknown if early thelarche is specifically associated with later breast disease, prolonging this sensitive developmental period increases the potential that susceptible tissues will be exposed to EDs or carcinogens [43].

4.5. The question of dose

In addition to timing, effects of ED exposure depend on dose: EDs can have complex dose response relationships – including showing opposite effects at lower doses relative to higher doses – which traditional toxicity testing methods are unable to detect [93]. Traditional toxicological studies typically use high doses to determine the maximum tolerated dose, lowest observed adverse effect level, and no observed adverse effect level, and extrapolate to estimate effects at lower doses. Developing tissues in particular can be exquisitely sensitive to very low doses of EDs. BPA, atrazine [94], PFOA [95], and dioxin [80] have each been shown to affect the MG at doses below those typically used in toxicological studies conducted for regulatory purposes. Exposure to concentrations of BPA expected to produce circulating free BPA levels above nanomolar concentrations has been shown to alter MG development, gene and protein expression, histogenesis, or to induce mammary hyperplasia in over a dozen studies (reviewed in [93]). Mammary epithelial cells exposed to nanomolar concentrations of BPA in culture demonstrate consistent effects [96].

Nonmonotonic dose-response (NMDR) curves are characterized by a nonlinear relationship between dose and effect, with the slope of the curve changing sign at some point in the range of doses examined. The effects of 17β -estradiol [97,98] and DES [99] on the MG have displayed NMDR curves in mice. Observations of NMDR curves in animal studies are particularly concerning because they raise questions about whether effects observed at higher doses can be extrapolated to lower doses more typical of human exposures, and raise concerns about missing important effects if testing is only performed at high doses.

4.6. Non-cancer effects

Finally, EDs that alter MG development may also affect the structure or function of the gland, causing changes such as gynecomastia in men or impaired lactation in women. In rodents, high doses of atrazine [100,101] and PFOA [95,102] have been shown to severely inhibit mammary development, which then affects lactation and impairs the growth of developing offspring. While these high doses are rarely seen in humans, it is possible that ecological systems may be exposed to levels that alter animals' development, impair lactation, and reduce offspring survival [43]. Other animal studies demonstrate that gestational exposure to dioxin [45,103], BPA [46,104–107], genistein [108], and Ziracin [109] not only alter mammary epithelial development, but impair lactation. It is possible that exposure to EDs contributes to

difficulty breastfeeding in women, but this outcome has not been investigated.

A limited number of epidemiological studies of prepubertal gynecomastia suggest an association with exposure to estrogenic or androgenic substances in boys [110,111]. Case reports have linked prepubertal gynecomastia to uses of tea tree oil and lavender oil, both of which have weak estrogenic and antiandrogenic activity [111]. While the mechanism is unclear, a variety of factors have been associated with gynecomastia in humans and domestic animals, including estrogen, androgen deficiency, and exposure to pyrethroid pesticides and some pharmaceuticals [48,112]. Since the growth of MG ductal epithelium is stimulated by estrogens and inhibited by androgens, EDs that alter the balance of estrogen or androgen stimulation may contribute to this condition [48]. Additionally, gynecomastia is frequently associated with other conditions that are related to hormonal imbalance. Vandenberg et al. [12] demonstrated that MG development was altered in male mice exposed to BPA during the perinatal period and studies of ethinyl estradiol and genistein show that the male MG is a highly sensitive marker of endocrine disruption [113], thus it appears that the male breast is also sensitive to EDs during susceptible periods of development.

5. Knowledge gaps

5.1. Understanding breast development

The three periods (i.e., fetal and neonatal growth, puberty, and pregnancy) of rodent MG development are known to be analogous to the periods of human breast development, however, research is needed to further describe the timing and characteristics of breast epithelial development and their relationship to adverse outcomes such as increased susceptibility to carcinogens or diminished lactation in both humans and rodents. Additionally, there are large gaps in our understanding of how MG development is regulated. It is important to understand what specific regulatory factors and genes are involved, as well as which receptor populations are present, and when, in order to understand the mechanisms through which these processes can be disrupted [55,73]. Elucidating the mechanisms involved in breast development would improve our understanding of breast cancer etiology, as well as non-cancer effects, such as lactation impairment and gynecomastia.

5.2. Understanding implications of altered development

Furthermore, while clear associations link various birth parameters to later life disease, the specific mechanisms through which perturbations to breast development cause disease or dysfunction years later are unknown. Understanding these mechanisms requires elucidation of the steps in mammary development and the processes that are susceptible to alteration. For example, epidemiologic evidence associates breast density with breast cancer risk. Density can be influenced by factors that are also associated with breast cancer risk (e.g., parity, number of births, and menopausal status) [114]. The stromal matrix of the breast tissue actively participates in the control of tumor growth [115–117] and may be a crucial target for carcinogens [118], suggesting that altered tissue architecture may be at the core of carcinogenesis. Research is needed to better understand the biological mechanisms responsible for these links and how perturbing them influences risk of cancer.

5.3. Understanding EDs' role in altered development and disease

Finally, animal studies demonstrate associations between ED exposure, altered MG development, and adverse outcomes.

However, the mechanisms by which EDs alter development and produce tumors are generally poorly understood. More information is needed on the types of chemicals, the doses, and the timing of exposure, as well as the ways that EDs act together, and the complex interactions among genetics, stress, diet, and endogenous and exogenous hormones.

Not only are there gaps in the knowledge concerning chemical causes of breast cancer, but the effects of ED on breast milk production and gynecomastia are largely unknown. Lactation impairment is usually identified by decreased pup weight or survival in multi-generational rodent studies. However, these surrogate markers do not adequately detect functional outcomes because they are not sensitive or specific for impaired lactation [13]. Additionally, multi-generational studies that can detect these effects are expensive and rare. An animal model has yet to be established to study gynecomastia, and there are very few studies investigating this condition.

6. Recommendations: improving test protocols to investigate how chemicals increase breast cancer risk and alter mammary gland structure and function

6.1. Include early developmental exposure in standard chemical testing protocols

While many knowledge gaps persist, recent research supports theories of development and later life outcomes in which environmental factors significantly alter phenotype [52]. Human epidemiologic studies are often unable to demonstrate associations between EDs and breast disease because exposures are not typically evaluated during critical periods of development. Longitudinal cohorts are of limited use in determining which early life exposures or factors increase breast cancer risk due to the difficulties of accurately measuring exposures and the length of time needed to see effects in humans. For example, it took 60 years for the effects of DES to appear [29]. As a result, the effects of EDs on the MG cannot be truly understood without animal studies. Such studies can be designed so that each animal model provides information concerning a particular aspect of human breast cancer and is able to represent a specific subset of the varieties of human breast disease [119].

In order to fully understand breast development and human breast disease, a variety of laboratory animal models are needed. Gaps in assessment of MG effects in the rodent toxicology guidelines produced by U.S. EPA, OECD, and NTP indicate that a paradigm shift is needed for these guideline studies to be able to detect critical alterations to MG development [13]. While the two-year carcinogenicity bioassays can assess MG histopathology, not all guidelines require exposure to the developing rodent. Developmental toxicity studies, which could provide information on developmental effects of EDs on the MG, typically do not evaluate MG tissue. Similarly, the standard one- or two-generation reproduction study could provide useful information on MG developmental effects but is not routinely used for this purpose.

The value of using the rat MG to elucidate potential modes of action of known mammary carcinogens has been shown by several investigators [119]. In most cases, these studies have been conducted in specialized study designs with treatment periods that replicate the developmental processes known to be important in the development of cancer. It is important to note that these inquiries and discoveries have not been conducted within the context of the standard 2-year carcinogenicity bioassay, as the usual protocols do not begin dosing until early adulthood. Reasons for this include (a) 2-year studies do not adequately assess changes that occur during especially sensitive life stages; (b) the studies are not designed to assess the consequences of early exposures that may

change tissue susceptibility to later exposures; and (c) the tests do not assess low-dose exposures to mixtures of chemicals that better approximate actual human exposure to chemicals found in the environment [120].

6.2. Evaluate MG development using whole mounts

Changing the two-year carcinogenesis study, developmental toxicity study, and multigenerational toxicity study guidelines would address these problems and improve our ability to understand ED effects on the MG. A protocol for evaluating whole mounts of MGs needs to be developed and validated that includes offspring exposed during perinatal and pubertal stages and that ensures outcomes are consistently reported among different laboratories. A whole mount is a tissue preparation technique that enables evaluation of the entire unsectioned organ [121]. This technique can identify morphological changes, as well as the temporal and spatial progression of epithelial development because it enables visualization of ductal branching [43]. Many laboratories use tissue sections, which alone are insufficient to detect developmental changes. Ideally, data from histological analyses could be used in combination with early life developmental end points seen in whole mounts to assess the later life impacts of early developmental changes [122]. Data generated from whole mounts could inform further investigations into the lactational impairment in offspring or tumor susceptibility using carcinogen-challenge protocols.

6.3. Other tools to improve testing

Another potential tool for evaluating chemical effects on the MG is the use of the male rat, which has demonstrated more sensitivity than other ED endpoints in male or females. A range of studies [113,123–125] indicate that the male rat MG is very sensitive to estrogen and may serve as a model for investigating the effects of EDs, although relevance for the female MG would have to be established [113,126].

Additional information can be gleaned from early biomarkers associated with MG cancer that can be measured using MG tissue blocks, serum samples, and other stored tissues. These include changes in levels of hormones or hormone receptors, receptor sensitivity, hyperplasia, gene markers, immunohistochemical markers, MG-specific gene markers, and effects on stromal–epithelial interactions [10]. Understanding these mechanisms would improve our knowledge of the relationship between altered MG development and later life outcomes.

Finally, toxicity information needs to be applicable to human-relevant endpoints. This can be achieved by including more time points during the investigation of MG developmental stages along with blood collection for dosimetry to understand the transition from normal mammary tissue to tumor development [43,73]. Careful characterization of internal dose is needed in all (not just guideline) studies [10,73]. Unlike laboratory animals, humans are exposed to many different environmental chemicals simultaneously, thus an understanding of biologically relevant mixtures that affect the mammary tissue is an important area of research.

7. Conclusion

Experimental evidence in rodent models and human epidemiological studies provide preliminary evidence that some EDs can affect normal MG development and function, and so may increase breast cancer risk. These clues indicate that traditional toxicology tests are likely missing many important effects on the MG. Chemical testing protocols should be standardized to require dosing during critical periods of development, assessment of MG development, structure, and function, and an assessment of the male MG.

A greater understanding of these mechanisms will help to clarify the risks of environmental exposures, provide evidence to reduce exposure, and ultimately reduce the burden of disease.

Conflict of interest statement

All authors confirm that they have no actual or potential competing interests regarding the submitted article.

Transparency document

The [Transparency document](#) associated with this article can be found in the online version.

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