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Advancing Research on Gene-Environment Interactions in Breast Cancer: Expert Panel Recommendations

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Highlights

- Breast cancer environmental research can be facilitated by long term animal studies
- Rodent models show mammary tumors to 130 weeks, similar to 80 years in women
- We recommend Whole Mount Mammary Methods to improve prediction and insights in GxE studies

Advancing Research on Endocrine Disrupting Chemicals in Breast Cancer: Expert Panel Recommendations

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Abstract

Breast cancer incidence continues to increase in the US and Europe, a reflection of the growing influence of environment factors that interact with personal genetics. The US Environmental Protection Agency estimates that over 85,000 endocrine disrupting chemicals are among the common daily exposures that could affect the risk of disease. The daunting tasks of identifying, characterizing, and elucidating the mechanisms of endocrine disrupting chemicals in breast cancer need to be addressed to produce a comprehensive model that will facilitate preventive strategies and public policy. An expert panel met to describe and bring attention to needs linking common environmental exposures, critical windows of exposure, and optimal times of assessment in investigating breast cancer risk. The group included investigators with extensive experience in the use of rodent models and in leading population studies and produced a set of recommendations for effective approaches to gaining insights into the environmental origins of breast cancer across the lifespan.

Keywords

Endocrine disrupting chemicals

Breast cancer

Mammary gland biology

Abbreviations

BPA	Bisphenol A
BMD	Benchmark dose
BMI	Body mass index
DEHP	Di(2-ethylhexyl) phthalate
DHEA-S	Dehydroepiandrosterone sulfate
DES	Diethylstilbestrol
DTT	1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane
EDC	Endocrine disrupting chemicals
EDSTAC	Endocrine Disruptor Screening and Testing Advisory Committee
EPA	Environmental Protection Agency (US)
ERα	Estrogen receptor alpha
IBCERCC	Interagency Breast Cancer and Environmental Research Coordinating Committee
MEP	mono(2-ethyl-5-oxohexyl) phthalate
NOAEL	No-observed-adverse-effect-level
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
SD	Sprague Dawley rat
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin

1.0 Introduction

Evidence is accumulating that the risk of breast cancer in the US and Europe is influenced by common exposure to endocrine disrupting chemicals (EDC). A wealth of data on animal models suggests that significant developmental changes occur in the breast and ovaries following early or chronic exposure to common chemicals. The data have guided studies of populations of young girls that, although few in number, are consistent in finding that common exposure to select EDC (as found in everyday materials, e.g. cosmetics, plastics, and food) are associated with established risk factors for breast cancer. The US Federal Interagency Breast Cancer and Environmental Research Coordinating Committee (IBCERCC) emphasized in 2013 the need to elucidate the influence of chemical exposures, particularly EDC, on breast cancer [1] and recent reports are suggestive that exposure to EDC alters timing of entry into puberty, defined as the appearance of secondary sexual characteristics including pubic hair, thelarche (appearance of postnatal breast development), and menarche (onset of first menstrual cycle) [2-4]. However, documentation of chemically - induced early developmental changes leading to breast cancer remains elusive.

In response to this gap in our knowledge, an expert panel was convened to identify needs for improved research linking common environmental exposures, critical windows of exposure, and optimal times of assessment in understanding breast cancer risk. We briefly review some of the compelling evidence that EDC, even at low doses, contributes significantly to developmental effects influencing breast cancer and provide a set of recommendations to investigate such effects through continuous monitoring of animal models. The literature cited is not intended to be comprehensive and interested readers are referred to recent, excellent short reviews on EDC and windows of susceptibility [2, 5, 6] and the IBCERRC Report [1]. However, the field is in need of a more comprehensive review addressing the limits on the windows of susceptibility for

breast cancer, evidence supporting the relative risks for specific exposures in each window, and the potential for reversibility of changes that might occur. Such reports would be valuable for highlighting insights on environmental exposure influencing breast cancer risk and as a guide for research leading to preventive and treatment strategies.

2.0 Windows of Susceptibility for Mammary Development and Breast Cancer

While a current topic of interest in environmental health, details of the windows of susceptibility, which proposes that individuals are more vulnerable to exposures during particular life periods, are incomplete. In concert with the concept, there is compelling evidence indicating that exposure – responses vary with mammalian developmental periods [2, 7]. A powerful illustration is the experience of female survivors of the atomic bomb blasts in Hiroshima and Nagasaki. Girls and women exposed to radiation before age 20 are at much higher risk of breast cancer than women who were older at the time of exposure [8]. The windows of susceptibility concept complements Barker's suggestion of the "thrifty phenotype" advancing the idea of *in utero* conditions or early exposure laying the foundation for adult disease [9]. Regarding mammary biology and breast cancer, the generally accepted windows, including gestation, puberty, and pregnancy appear to be periods of intense morphologic changes and cell proliferation all of which indicate periods of potential increased risk for breast cancer [2].

With a long developmental period, the mammary gland would appear to be at particular vulnerability to exposure. The structural basis for the mammary glands is in place before birth in women, forming the epithelium *in utero* by invading and branching into the mammary fat pad [10]. The mammary gland is not terminally differentiated for much of a woman's life, becoming fully formed and functional only during pregnancy, and partially de-differentiating at the end of lactation in a process called involution [11]. This pattern is different from many organs in which

terminal differentiation results in a loss of most of the stem cell populations and reflects the need in the mammary gland to maintain a colony of highly proliferative cells to sustain multiple pregnancies. The stem and progenitor cell populations constitute approximately 1% of normal breast and represent the opportunity for chronic and varied exposure to founder populations that could lead to the evolution of cancer stem cells [10, 12]. Adult stem cells are slowly dividing, long lived cells that may be exposed to EDC and damaging agents for decades, potentially accumulating greater numbers of mutations. Diet, radiation, and chemical exposures experienced by the mother may make their way to the fetus and could affect its long term health [9, 13]. In animal models, endocrine disruptors such as phthalates, bisphenol A (BPA), and 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane (DTT), have *in utero* effects on the architecture of the mature breast, altering number or structure of mammary gland lobules [5, 14, 15]. Early treatment with EDC may effect changes to the architecture of the mammary gland that are observed at the time of exposure or, as in the case of genistein, and varying with the dose, alterations may be delayed until times when ducts and terminal end buds are expected to become more extensive [16–18].

The pre-pubertal period is likely a sensitive window of susceptibility and a recent study suggests that the process of hormonal regulation setting the stage for entry into puberty begins earlier than previously thought. A recent longitudinal study of 252 US girls found that dehydroepiandrosterone sulfate (DHEA-S) concentrations rose 24 months and androstenedione and estrone rose 12 to 18 months before breast development. Estradiol and testosterone rose while sex hormone - binding globulin fell during the relatively shorter period of between 6 to 12 months before breast development [19]. EDC could influence the hormonal balance during this period, affecting the onset of puberty, an established risk factor for breast cancer [2].

Pregnancy is obviously a period of intense developmental change for mothers and, though parity reduces the lifetime risk of breast cancer compared with nulliparous women, there is a brief postpartum period of increased risk with each pregnancy [20].

Menopause is a well-described period of significant female hormonal change that corresponds to an increase in the risk for breast cancer and most cases are diagnosed in the US and Europe in women older than age 60 [21]. The level of sex hormones is positively associated with risk for breast cancer in post-menopausal women [22]. The potential for hormone mimetics, such as EDC, to influence risk is further demonstrated by the increased incidence of breast and endometrial cancer brought on by hormone replacement therapy [23].

Thus, from before birth through the child-bearing years, women experience multiple potential windows of susceptibility of variable length in which EDC could potentially affect the balance of hormones and play a central role in development of breast cancer. The exposures could induce tumorigenesis immediately or create an initiating event to potentially exert its effects many decades later.

The limits of the windows of susceptibility and the role of exposures and lifestyle across those periods on breast cancer are unclear. The lack of clarity in the details of the windows of susceptibility limits construction of a comprehensive model to guide research and preventive strategies. More precise determination of the beginning and end of vulnerable periods needs to be performed. Studies such as that mentioned, above [19], will be valuable in providing a basis for improved definitions of developmental periods such as puberty. However, improved characterization of the “opening and closing” of a window still need to be determined, as the degree of vulnerability may vary throughout any particular window. For example, in the case of the atomic bomb survivors, the risk of breast cancer linearly decreased with age of exposure

from birth to age 20 [8]. The variability in sensitivity raises the question as to whether there are multiple sub-windows within the pubertal one. Furthermore, many studies link exposures during a putative window of susceptibility with early outcomes, rather than directly to breast cancer that, as the concept suggests, may occur in women decades after a single or set of exposures. One might also ask whether there are, as yet, undescribed windows of susceptibility. These could be peak periods of sensitivity within known windows or new windows altogether. As suggested earlier, hormonal changes years prior to secondary sexual characteristics may eventually be interpreter as several windows, rather than a single pre-pubertal one.

Examination of typical study protocols suggests a strong possibility that investigators may frequently miss critical periods for assessment. Data collection in laboratory studies is generally performed during a select number of days within a narrow life period [see, for example, 16, 24]. Further efforts are often limited by cost, available labor, predisposition or available time in the busy life of subjects, etc. However, as suggested above, windows of susceptibility may be brief [19, 20] and significant effects of EDC may be overlooked if serial sample collection or continuous observation is not performed.

Finally, a key question is whether EDC effects on mammary gland development and breast cancer risk are reversible. Epigenetic changes, for example, can occur either permanently or reversibly, as illustrated by studies of prostate gland in the rat [25]. The report indicates that BPA treatments may exert one of three potential methylation states. Methylation of the nucleosome binding protein-1 promoter in prostate appears to be a permanent epigenetic mark of early, neonatal exposure to BPA and, once altered in early life, remains unchanged. In contrast, BPA – induced methylation of the phosphodiesterase promoter appears to be a relatively silent effect, with observed effects on expression observed only at the time of sexual maturation. Finally, methylation of the hippocalcin-like 1 promoter changes with developmental

age or other exposure challenges throughout the lifespan. Exposures that result in structural changes to morphology may less likely to be reversible. However, the mammary gland appears to be very plastic, as the pattern from pre-pregnancy to lactation to involution indicates. Studies of EDC effects on epigenetics, gene expression, and breast architecture have begun [11, 14-16, 24, 26], but need to be extended in-depth on animal models focusing on exposures in common use by human populations.

3.0 Endocrine Disrupting Chemicals and Breast Cancer Risk

A multitude of likely or confirmed EDC have been introduced into common use, many of which are likely to be associated with breast cancer. In 2012, the US Environmental Protection Agency Endocrine Disruptor Screening Program reconfirmed its 1998 estimate of "the initial universe of chemicals that needs to be considered for prioritization for endocrine disruptor screening and testing … at approximately 87,000" [27]. The Tox21 project, a collaboration of US Federal partners including the National Toxicology Program, that is examining the toxicity of thousands of compounds, recently determined that a significant proportion of common chemicals qualify as EDC, as defined as estrogen receptor alpha (ERα) binding agents. The assays indicate that among ~10,500 screened compounds are 588 – 1092 (5.6 -10.4%) active ERα agonists and 430 – 493 (4.1 - 4.7%) active ERα antagonists, depending on the assays applied [28]. The figures reflect only those compounds that detectably bind the human estrogen receptor and, thus, might under estimate the number of compounds that could have an effect along a relevant pathway. While not intended to be a comprehensive review, a few select, materials under mainstream investigation as EDC are mentioned here that highlight the risks of everyday exposure.

3.1 DES

Several common chemicals stand out among the suspected EDC that may alter female developmental processes. The classic example is DES that was widely used in the United States

and Europe in the mid-Twentieth Century with the goal of reducing menopausal discomfort and preventing miscarriage [5, 16]. The form of cancer (vaginal clear cell carcinoma) associated with DES was rare and drew attention to its carcinogenic effects, likely shortening the time to alerting providers and policy makers of the inherent risk. The effects of DES included an increase in relative risk of 1.40 for women who directly received the drug [16, 29]. Although one study conducted on a Dutch cohort found no increase of breast cancer risk for DES - daughters [30], studies of cohorts in most areas, including the US indicate an increased risk to about twice that of the general population [31]. The impact appears to be more moderate on grand-daughters of the exposed adult population with that cohort having a normal risk, although the incidence of vaginal and cervical clear cell carcinoma remains increased. Never banned in the US, the Food and Drug Administration began steps to reduce prescribed use of DES in 1971 [32]. The youngest DES daughters in Europe are expected to reach the age of menopause around 2040 and related breast cancer cases will likely continue to climb until then [5, 16].

3.2 Dioxin

Another intensely examined EDC is dioxin, which has been linked to multiple forms of cancer, although for breast, the findings are conflicting [33, 34]. Longitudinal studies of a population exposed to extreme levels of dioxin following an explosion at a chemical plant near Seveso, Italy initially suggested a strong association (2.1:95% CI, 1.0 - 4.6) of breast cancer incidence, showing the hazard ratio for breast cancer associated with each 10-fold increase in serum TCDD levels significantly increased to 2.1 [35]. However, a recent follow-up study found that, while confirming the trend, breast cancer cases associated with dioxin did not achieve significance [36].

Animal studies of dioxin, on the other hand, consistently demonstrate more frequent mammary tumorigenesis and at a frequency greater in females. However, the conditions of exposure seem

to be critical. Reports suggest that the effects of dioxin through the aryl hydrocarbon receptor may have differential effects depending on the level of competing hormones [37, 38] Dixoin appears to have anti-estrogenic effects on mouse reproductive organs in the presence of endogenous estrogen but estrogenic effects in its absence [38]. Another report demonstrates that maternal exposure to dioxin doubles mammary tumor incidence in the offspring with considerable changes to mammary gland branching and morphology, but only in mice fed a high fat diet [39].

3.3 Perfluoroalkyl Acids

Perfluoroalkyl acids are found in numerous cooking and clothing materials and persist indefinitely in the environment. Recent reports suggest effects of elevated levels of PFOA on pubertal outcomes in young girls. A study of girls in the UK shows birthweights reduced by 140 g among girls born to mothers with prenatal concentrations of perfluorooctane sulfonate (PFOS) in the upper versus the lower tertile [40]. Though controversial, birth weight is used as an indirect measure of estrogen exposure in utero and been linked to breast cancer risk through associations with adolescent height and lower age of menarche [2, 41, 42].

Mouse models indicate perfluorooctanoic acid (PFOA) induces developmental defects of the mammary gland, lactation deficits, restricted growth potential, and decreased postnatal survival [43]. Even low doses have been reported to influence the pubertal window of susceptibility, with modification of epigenetics, gene expression, delayed vaginal opening, and defective estrous cycling. The effects, though, are species specific. Balb/c mice exposed to 5 mg/kg body weight PFOA exhibit inhibition of mammary gland and uterine function and C57BL/6 mice show stimulatory effects at that dose but inhibition at 10 mg/kg [44]. Other data show that mice treated with physiologically relevant levels of PFOA during the prenatal period undergo changes to mammary tissue structure including enhanced stromal density, as well as altered steroid

hormone expression patterns [45, SE Fenton; personal communication]. Based on the available data, PFOA appears to have significant effects on female reproductive health, but the results are difficult to generalize from the laboratory.

3.4 Phenols

A recent report from the US Centers for Disease Control and Prevention implicates an early effect of phenols, found in common household products such as insecticide and indoor disinfectants, on age of menarche [4]. Early onset of puberty is an established risk factor for lifetime breast cancer risk and the results indicate a dose-dependent, inverse association of 2,5-dichlorophenol with age of menarche. The report does not show significant associations of menarche with other exposures, including BPA, triclosan, and total phthalates.

3.5 Phthalates

Due to their ubiquitous presence in the developed world, exposure to phthalates could pose a significant breast cancer risk and recent reports are suggestive of their effects on developmental processes in young girls. In a Danish cohort, the concentration of 12 phthalate metabolites determined from first morning urine samples of 725 healthy girls (5 to 19 years old) is directly associated with older age at pubarche, but not breast development or precocious puberty [46]. Another report of a prospective study of 1239 girls in the US indicates an inverse association of high molecular weight phthalates with puberty, as determined by pubic hair development [3]. Delay of thelarche is observed in obese girls whose urine levels indicated higher di(2-ethylhexyl) phthalate (DEHP) but, somewhat surprisingly, the effect appears significantly greater among girls in the normal weight class. The authors suggest that obesity may have influenced the results, as detection of earlier puberty may have been masked by a strong influence of body mass index (BMI) at earlier as opposed to later ages of puberty.

An association of phthalates with breast cancer in women has been reported only in two studies, one of which just recently appeared. The first is an age-matched investigation of 233 breast cancer cases and 221 female adult subjects in northern Mexican women and demonstrates a strong association of breast cancer risk with DEHP, the parent compound of mono(2-ethyl-5-oxohexyl) phthalate (MEP) [47]. The highest MEP levels, as measured in urine samples, correlate with covariate adjusted odds ratios of 1.94 for increased risk of breast cancer. The association appears to be specific to MEP and is highly significant for pre-menopausal women, although the trend just escapes significance for post-menopausal women. A second study finds an association of MEP with breast cancer in an Alaskan Native American population [48]. The investigators assayed urine samples for a set of EDCs and compared confirmed cases (in which surgical procedure resulted in a diagnosis of invasive or *in situ* breast cancer) for women appearing at the Alaska Native Medical Center in Anchorage with those eventually diagnosed as having benign breast disease. The univariate odds ratio for confirmed cases was 2.16 (OR 2.16, 95% CI 1.16-4.05, p=0.02) associated with levels of MEP above the median of the cohort (n=75 cases; 95 controls). However, the study is limited by a small sample size and the authors could not control for confounding effects including BMI.

In animal studies, phthalates have documented effects on the female reproductive system treated with, for example butyl benzyl phthalate (BBP) but tumorigenesis is generally not reported. Prenatal BBP has been shown to delay vaginal opening and induce changes in the rat post-natal mammary gland as long as 35 days after treatment [15]. Modifications in mammary gland architecture and proliferative index are observed, largely in the terminal end buds. Multiple reports indicate phthalates induce altered gene expression patterns in metabolic and proliferative genes [15, 49] and epigenetic state [50] and stimulate proliferation in cell lines [51, 52]. However, we could find no reports of mammary cancer in whole animals in response to physiologically relevant levels of phthalate treatment.

4.0 Recommendations for Future Research on Windows of Susceptibility in Breast Cancer

On the whole, the available data represent compelling evidence that EDC have significant effects on female developmental processes, especially when exposures may have occurred during windows of susceptibility. Despite these advances, it is difficult to identify and confirm the effective EDC from the mix of common chemical exposures or the precise timing of exposure that impacts breast cancer risk.

To address these issues, a team of investigators experienced in observing long term animal models and in leading population research took part in a Meeting on Gene-Environment Interactions of Endocrine Disrupting Chemicals in Breast Cancer at the Ramazzini Institute in Bentivoglio, Bologna in 2014. The panel considered strategies and posed recommendations to advance insights into the impact of exposure to EDC across the life course influencing mammary development and breast cancer risk.

4.1 Refine the Definition and Improve the Characterization of Windows of Susceptibility

An overarching recommendation is to bring to bear a combination of laboratory, mechanistic, and epidemiological skills to better define and characterize windows of susceptibility over the lifetime so as to provide insights into the gene – environment effects that influence breast cancer risk. While epidemiologic studies are critical to identifying populations at risk and often provide initial clues to EDC of interest, population approaches may be restricted to surrogate outcomes or biomarkers, hampered by unknown or uncontrolled confounding, and may rely on participant recall rather than direct measures.

4.2. Extend Studies of Animal Models of Breast Cancer to Longer Periods

We recommend performance of investigations that are long-term, lifestage specific, and focus on susceptibility and cumulative exposure. Specific windows of susceptibility would optimally be examined in depth in sub-groups of larger long-term studies of animal models (e.g. rats) to help determine the limits of the windows of susceptibility to one or more exposures and improve risk assessment. Recognizing that breast cancer is a prolonged process, developmental exposure studies provide greater information than studies focused solely on adults at or near the time of disease diagnosis. Lifelong and early exposure models provide a greater opportunity to construct comprehensive mechanistic models reproducing the natural history of mammary cancers, their precursors, and metastases. Studies of shorter periods, each focused on a specific lifestage or outcome, are more likely to miss critical developmental effects. While not a blanket substitute for incremental advances, longer term investigations offer a more consolidated design and allow opportunities to observe multiple life stages in the same groups of animals and better define the limits of the windows of susceptibility. The results would provide a basis for other researchers to address the most likely developmental periods for EDC to affect processes concerning mammary or regulatory function.

4.3 Recommended Methods for Long-term Studies of Breast Cancer

Examination of early and developing mammary architecture is encouraged as a basic approach in conjunction with molecular determination, where appropriate, in understanding the role of EDC on primary targets in mammary gland biology. EDC can affect mammary glands directly as a carcinogen and resulting epigenetic patterns, gene expression, and cell proliferation can be quantified. However, changes in tissue composition and morphology that could be critical to tumorigenesis may be overlooked if measures are limited, for example, to cell proliferation. In fact, a thoughtful discussion that outlines the structural changes underlying disease pathogenesis has recently been published by Soto and Sonnenshein [6]. The authors suggest that exposure to some EDC triggers instigate defective interactions among cells and the

414 extracellular matrix, resulting in modification of regulatory control and an eventual rise in
415 tumors.
416
417 Procedures to visualize the whole mammary gland are suggested as a cornerstone to this level of
418 investigation. For example, a whole mount method allows for observation and prediction of
419 neoplasias and provides insights into changes in stromal density [53]. A procedure traditionally
420 used for quantifying neuronal dendritic patterns, the Sholl method can be applied to mammary
421 glands and allows quantifiable examination of branching density and characteristics. Using the
422 method, a recent report demonstrates detection of significant differences in branching density
423 in peripubertal female Sprague Dawley rats that have been exposed to vehicle or a potent
424 estrogen [54, 55 (this issue)].
425
426 The SD rat appears to be an optimal rodent model for primary examination of EDC, either
427 isolated exposures or mixtures. Rat mammary glands are large enough for accessible extraction
428 and for whole mount procedures and the process of mammary development is similar to that of
429 women. The three year lifecourse of rats can be considered in a human equivalent model; with
430 developmental parallel periods of *in utero*, pre-puberty, puberty, etc. [see Forman and Winn,
431 this issue]. Signs of puberty, inconsistent cycling patterns, and breast development occur in the
432 SD rat at about 16 weeks, corresponding to 10 years of age in girls [56]. With regard to cancer
433 studies, an average 80% of spontaneous tumors occur by 104 weeks in rats and by age 65 in
434 humans [56, 57]. Estimates from observations across the lifespan suggest that a human year
435 equals about two rat weeks across the entire life span [58]. Rats develop rapidly during infancy
436 and have a brief and accelerated pubertal window of susceptibility compared to girls. They
437 become sexually mature from 6 to 9 weeks of age while women enter puberty somewhat later in
438 their lifecourse, at about 10 - 12 years [59, 60]. The transition to adulthood in rats begins after

sexual maturity at about 8 weeks of age and from 7 to 10 weeks they are considered to be in the young adult period [61].

The experience with the Ramazzini SD rat colony provides an illustration of the advantages of long - term studies on mammary cancer. A total of more than 200,000 male and female SD rats have been studied until spontaneous death or 130 weeks; corresponding to about 80 years in humans. Spontaneous mammary tumors of all types observed in humans are observed in untreated female animals in this colony. The incidence (10 - 12%) and age distribution of mammary carcinomas are very similar to those observed in women in industrialized countries with most of the tumors in the rat observed by 130 weeks; similar to the 85 years old reported for humans [62]. Such a dedicated multi-year study provides the opportunity to observe and assess the totality of spontaneous tumors and better characterize the windows of susceptibility for chemically – induced ones.

4.4 Study Lower Doses of EDC

Studies need to be performed for EDC at or below the no-observed-adverse-effect-levels (NOAEL), as appropriate for questions of human risk assessment in animal models and, where possible, in girls and women. The EDSP expressed concern on the controversy over the appropriate approaches towards resolving assessing EDC effects, especially at NOAEL. As an alternative, the benchmark dose method (BMD), addresses many limitations in the NOAEL method, being less dependent on dose selection and accounting for study sample size [63]. It is not uncommon for studies to indicate that low dose EDC induce changes to gene expression or epigenetic state without immediately observed changes to morphology or lactation. However, longer term studies indicate early, low dose EDC induce further changes on mammary gland architecture [43] or tumorigenesis [24, 64] at or beyond sexual maturation.

4.5 Consider Mixtures of EDC in Studies of Animal Models and Populations

Finally, studies need to be extended to identifying the mixtures of EDC in combinations and concentrations that reflect the daily exposure of populations to better determine the mechanisms of toxicity. While clearly daunting, select researchers are beginning to approach the issue [65]. Paul Price, an authority on models to assess exposure to multiple chemicals notes the primary challenges for considering mixtures: continuous changes to complex mixture permutations over time that require modifying the assessments and producing less certain conclusions; lack of available component data for component-based mixture assessment; and that biases exist in selecting test mixtures in toxicology studies [66]. Dr. Price and his colleagues are producing tools to enable researchers and policy makers to assess the need for performing cumulative exposure assessments [67]. These tools will be valuable to assess in construction of study protocols to determine whether they can are applicable to the effective mixtures experienced by a population of interest. Continuous monitoring of animal models run in parallel with population studies would allow comparison of the results with these probabilistic models and may provide support to that the models are appropriately precise for biomonitoring for breast cancer risk. The necessary expertise in advanced modeling is not traditionally found in toxicology or population work and training in probabilistic modeling will likely become a necessary component for future research on ECC mixtures in breast cancer.

5.0 Conclusions

In summary, we propose a set of recommendations for defining the effects of EDC in windows of susceptibility throughout the lifespan on breast cancer risk. The effects of EDC may induce significant effects at low doses, alone or in combination with other common chemicals, at known or, as yet to be determined, windows of susceptibility. The effects of early EDC exposure may not become clear until later in development or the lifespan. Whole mount mammary procedures are recommended, as are long term approaches using animal models, and a need for studies that

address EDC mixtures across the range of physiological doses, including low doses. Observations of tumorigenesis in women suggest the rat as an optimal animal model to be monitored for lifelong studies over an approximate time frame of 130 weeks of age. While many of the recommendations are aimed at investigation of animal models, we suggest that implementing them will be assist in planning of population studies and provide more rapid advances towards understanding the lifelong effects of EDC exposure and their mechanisms on mammary glands and breast cancer.

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