Final Degree Thesis

Pharmacy Degree

# Bisphenols, PCBs, Parabens, and Dioxins: The Silent Threat of Endocrine Disruptors in Breast Cancer

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## ABBREVIATIONS

| | |
|---|---|
| AhR | Aromatic hydrocarbon receptor |
| AP-1 | Activator protein 1 |
| AREG | Amphiregulin |
| BC | Breast cancer |
| BCL2 | B-cell lymphoma 2 |
| BMI | Body Mass Index |
| BPA | Bisphenol A |
| BRCA1 | Breast cancer type 1 |
| BRCA2 | Breast cancer type 2 |
| CAT | Catalase |
| CAAs | Cancer-associated adipocytes |
| CCL28 | C-C motif chemokine ligand 28 |
| CCL5 | Chemokine ligand 5 |
| CXCL1 | Chemokine ligand 1 |
| CXCL12 | C-X-C motif chemokine ligand 12 |
| CXCL2 | Chemokine ligand 2 |
| CYP1A1 | Cytochrome P450 1A1 |
| DNMT1 | DNA methyltransferase 1 |
| E1 | Estrone |
| E2 | 17β-estradiol |
| E3 | Estriol |
| E4 | Estetrol |
| EDC | Endocrine disrupting chemical |
| ER | Estrogen receptor |
| ER- | Estrogen receptor-negative |
| ER+ | Estrogen receptor-positive |
| EREs | Estrogen response elements |
| ESR1 | Estrogen receptor 1 |
| FOXM1 | Forkhead box M1 |
| GLUT1 | Glucose transporter 1 |
| GPR30 | G protein-coupled receptor 30 |
| GREB1 | Growth regulation by estrogen in breast cancer |
| HBECs | Primary benign breast epithelial cells |
| HDI | Human Development Index |
| HER2 | Human epidermal growth factor 2 |
| IGF-1 | Insulin like growth factor 1 |
| IL1A | Interleukin 1 alpha |

| | |
|---|---|
| IL1B | Interleukin 1 beta |
| MAPK/ERK | Mitogen-activated protein kinases and extracellular signal-regulated kinases pathway |
| MEHP | Mono-2-ethylhexyl phthalate |
| MMP7 | Matrix metallopeptidase 7 |
| MYC | V-myc avian myelocytomatosis viral oncogene homolog |
| NF-κB | Nuclear factor kappa-light-chain-enhancer of activated B cells |
| p53 | Tumour suppressor protein p53 |
| PCBs | Polychlorinated biphenyls |
| PDEs | Patient-derived explants |
| PFAS | Polyfluorinated Alkyl substances |
| PI3K | Phosphatidylinositol-3-kinase |
| PI3K/AKT | Phosphatidylinositol-3-kinase and Akt protein pathway |
| PKA | Protein kinase A |
| PKC | Protein kinase C |
| PR | Progesterone receptor |
| PTEN | Phosphatase and tensin homolog |
| pRb | Retinoblastoma protein |
| ROS | Reactive oxygen species |
| SNPs | Single nucleotide polymorphisms |
| SOD | Superoxide dismutase |
| Sp1 | Specificity protein 1 |
| TCDD | 2,3,7,8-Tetrachlorodibenzo-p-dioxin |
| TEF | Toxic equivalency factor |
| TNBC | Triple negative breast cancer |
| Tregs | Regulatory T cells |

## ABSTRACT

**Bisphenols, PCBs, Parabens, and Dioxins: The Silent Threat of Endocrine Disruptors in Breast Cancer**

This study investigates the potential role of endocrine disrupting chemicals (EDCs) in breast cancer development and progression. The primary objective is to analyse how bisphenols, polychlorinated biphenyls (PCBs), parabens, and dioxins contribute to oncogenic processes by disrupting hormonal balance, altering gene expression, and modifying the tumour microenvironment. Specifically, this review aims to identify key exposure pathways, elucidate molecular mechanisms of action, and evaluate their public health implications.

To achieve this, a comprehensive literature review was conducted using peer-reviewed scientific databases, focusing on studies published from 2019 onward. The findings reveal that BPA promotes cell proliferation, migration, epigenetic alterations, and immune suppression, accelerating tumour progression. PCBs influence gene expression and cellular metabolism, but their correlation with breast cancer remains inconclusive. Parabens exhibit estrogenic activity, increasing oxidative stress and tumour growth, while dioxins contribute to metastasis, inflammation, and therapy resistance. Epidemiological studies suggest that high exposure levels-mainly through diet, environmental contamination, and personal care products-are associated with increased breast cancer risk.

In conclusion, EDCs play a significant role in breast cancer, although further research is needed to address existing knowledge gaps. Moreover, stronger regulatory preventive measures should be reinforced to minimize exposure and mitigate associated health risks. Moreover, raising public awareness and promoting the development of safer alternatives are crucial steps in reducing the impact of these chemicals on human health.



**Key words:** Endocrine disrupting chemicals, breast cancer, bisphenols, polychlorinated biphenyls, parabens, dioxins.

## RESUM

**Bisfenols, PCBs, Parabens i Dioxines: L'amenaça silenciosa dels disruptors endocrins en el càncer de mama**

Aquest estudi investiga el paper dels disruptors endocrins (DEs) en el desenvolupament i la progressió del càncer de mama. L'objectiu principal és analitzar com els bisfenols, els bifenils policlorats, els parabens i les dioxines contribueixen als mecanismes oncogènics, ja sigui alterant l'equilibri hormonal, l'expressió gènica, i el micro-ambient tumoral. Entre els objectius específics del treball s'inclouen la identificació de les principals vies d'exposició als DEs, la comprensió dels mecanismes moleculars implicats i l'avaluació del seu impacte en la salut pública.

Per assolir aquests objectius, s'ha dut a terme una revisió bibliogràfica a partir de bases de dades científiques, incloent-hi únicament estudis publicats a partir de 2019 per garantir l'actualitat de les dades. Els resultats mostren que, el bisfenol A promou la proliferació i migració cel·lular, així com les alteracions epigenètiques i la supressió del sistema immunitari. Els bisfenils policlorats influeixen en l'expressió gènica i el metabolisme, tot i que la seva correlació amb el desenvolupament del càncer de mama encara no està prou establerta. Els parabens presenten activitat estrogènica, augmentant l'estrès oxidatiu i el creixement tumoral, mentre que les dioxines contribueixen a la metàstasi, la inflamació i la resistència als tractaments. Per altra banda, els estudis epidemiològics suggereixen que l'exposició elevada a aquests compostos- principalment a través de la dieta, l'ambient i els productes d'ús personal- pot incrementar el risc de càncer de mama.

En conclusió, els disruptors endocrins tenen un paper rellevant en el càncer de mama, tot i que continua sent necessària la recerca en àmbits on encara hi ha buits de coneixement. Així mateix, s'haurien de reforçar les mesures reguladores per minimitzar l'exposició i mitigar els riscos per a la salut, així com sensibilitzar la població i promoure el desenvolupament d'alternatives més segures.



**Paraules clau**: disruptors endocrins, càncer de mama, bisfenols, bisfenils policlorats, parabens, dioxines.

## INTEGRATION OF FIELDS

The present project examines the effects of endocrine disrupting chemicals (EDCs) - to which we are continuously exposed in daily life- on the development and progression of breast cancer. Given the complexity of this issue, the study integrates different fields of knowledge that are essential to understand the subject.

The main field of this study is **Toxicology** - as EDCs are classified as xenoestrogens- chemical compounds that mimic or interfere with endogenous hormones. Human exposure to these substances occurs through ingestion, inhalation, or dermal absorption, allowing them to enter the body and exert their toxic effects. Toxicology plays a key role in identifying the mechanisms through which these chemicals interact with endocrine pathways and in determining their long-term health effects.

In addition to Toxicology, the fields of **Physiology and Pathophysiology** play a complementary yet fundamental role in identifying the biological targets affected by EDCs when they exert their toxic effects. These disciplines also provide insights into how these disruptions create a favourable environment for cancer development.

Finally, **Public Health** is another key discipline addressed in this study. By studying the health effects of EDCs exposure, this field allows for a broader evaluation of its societal impact. Moreover, identifying risk factors and sources of exposure is essential for developing preventive measures, such as regulatory policies and awareness campaigns, aimed at reducing exposure and mitigating associated risks.

## SUSTAINABLE DEVELOPMENT GOALS

Studying the role of EDCs in cancer has become crucial due to their widespread presence in daily life and the alarming rise in cancer incidence rates. Understanding these compounds is essential for developing preventive measures and legal policies that will help improve humans' health.

The present study aims to gather information on how EDCs contribute to the progression and spread of breast cancer. By doing so, it aims to inspire new therapeutic targets and treatment strategies in the ongoing battle against breast cancer. Additionally, this project aligns with **Goal 3: Good health and well-being** of the Sustainable Development Goals (SDGs), specifically **Goal 3.9**, which seeks to reduce deaths and illnesses caused by hazardous chemicals in the air, water, and soil-key exposure routes for EDCs.

Indirectly, this work also addresses **Goal 11: Sustainable cities and communities**, as the level of exposure to EDCs can be influenced by living near industrial facilities that release these substances into the environment. Research has shown that environmental accidents have led to massive chemical spills containing EDCs, posing serious health risks to affected populations. This directly relates to **Goal 11.5** which aims to reduce disaster-related mortality and economic losses, particularly for vulnerable populations.

Finally, this study also contributes to **Goal 12: Ensure sustainable consumption and production**, as mitigating EDC exposure requires collective actions from industries, governments, and individuals. Preventive measures should focus on reducing the production and release of toxic substances, thereby minimizing human exposure. On an individual level, conscious consumer choices - such as opting for packaging-free food and non-toxic alternative products - can help mitigate risk of exposure. These principles align with **Goals 12.6 and 12.7**, which emphasize sustainable production and consumption practices.

## INDEX

# 1. INTRODUCTION

## 1.1 Breast cancer

### 1.1.1. Incidence of breast cancer

Female breast cancer is the most diagnosed cancer among women, with 2,308,897 new cases recorded in 2022, representing 11.6% of all new cancer cases that year. It is also the leading cause of cancer-related deaths in women worldwide, accounting for 665,684 deaths, comprising for 6.9% of all cancer-related mortality globally in the same year. More alarmingly, it is expected that by 2040, new breast cancer cases will raise up to a 40% resulting in 3 million new cases, and mortality will grow over 50%, leading to 1 million deaths from the disease (1–4).

Incidence and mortality vary remarkably across geographical regions and according to the Human Development Index (HDI)—a composite index derived from scores in three dimensions: health, education, and standard of living—**.** Developed countries have higher incidence rates (54.1 per 100,000) and lower mortality rates (11.3 per 100,000), while less developed countries show the opposite trend, with lower incidence (30,8 per 100,000) but lower survival rates (15.3 per 100,000). These disparities are believed to emerge from the different exposure to risk factors and the availability of diagnosis and treatment resources in these regions (2).

Although breast cancer is curable in approximately 70 - 80% of patients diagnosed at an early, non-metastatic stage −which means that the malignancy is confined to the breast or has spread only to auxiliary lymph nodes−advanced breast cancer with distant organ metastasis is still considered incurable. In such cases, only palliative measures to extend survival and alleviate symptoms are available (2).

### 1.1.2. Breast cancer carcinogenesis and molecular subtypes

Cancer is a highly heterogeneous disease characterized by a wide range of molecular changes that can lead to its development, such as genetic mutations, gene expression dysregulation and alterations in the tumour microenvironment, among others. While the exact mechanism by which breast cancer initiates remains unknown, extensive research has provided valuable insights regarding the main factors contributing to its development (2).

Breast cancer initiation results from the transforming events that occur in a single cell, driven by both genetic and epigenetic alterations. The subsequent tumour progression is induced by accumulative additional genetic changes as well as clonal expansion and selection. These processes are mainly explained by the clonal evolution model and the cancer stem cell model, in addition to molecular and morphological changes (5).

On the one hand, the clonal evolution model proposes that a differentiated somatic cell undergoes a series of mutational events that confer it the ability to acquire a malignant genotype. This cell proliferates and generates a new clonal population. As the tumour evolves, this population accumulates further mutations, resulting in a tumour composed of genetically diverse subclones. Some of these mutations can make cells resistant to treatment or apoptosis, alter the tumour microenvironment or promote other characteristics that contribute to tumour development and growth. Subclones within the tumour evolve continuously during its development, undergoing natural selection (**Figure 1**) (6).

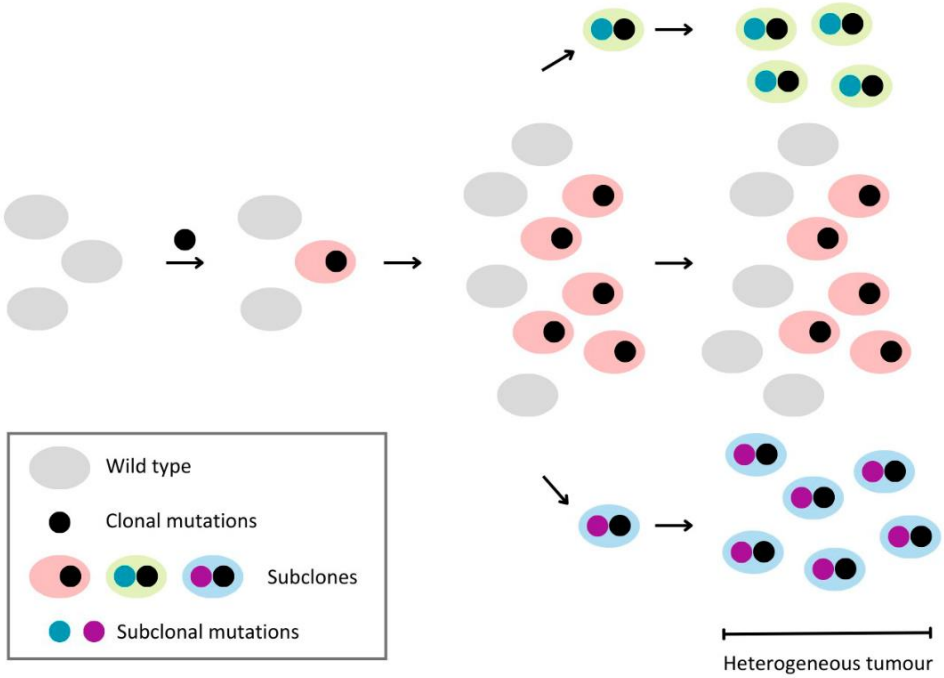


**Figure 1. Clonal evolution model diagram.** Adapted from (6). Created with Canva.

On the other hand, the cancer stem cell model suggests that in tumours, a small population of tumour stem cells drives their growth and progression, due to the accumulation of mutations and shifts into a more stimulatory microenvironment. This model also states that tumour heterogenicity can be explained by the remnants of the hierarchical organisation that cancer stem cells induce in normal tissues, being able to generate different cellular types. In fact, the cell origin may be the same for different types of tumour (**Figure 2**) (2,5).

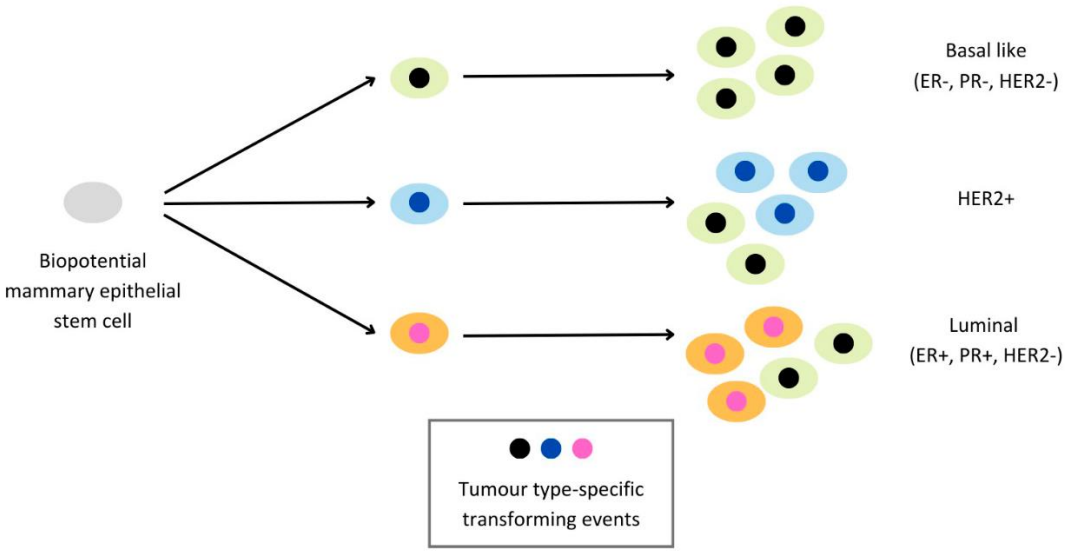


**Figure 2. Cancer stem cell model diagram**. Adapted from (5). Created with Canva.

At the morphological level, accumulative lesions and genetic modifications progressively transform normal glands into cancer. Hormone receptors play a key role in this process, as hormonal imbalances between oestrogen and progesterone during each menstrual cycle, for example, are believed to enhance cell proliferation and DNA damage. When this repetitive damage is defectively repaired, mutations can render cells pre-malignant, eventually leading to malignancy (2).

Finally, several studies suggest that breast cancer progresses mainly through two different molecular pathways that differ in estrogen receptor (ER) expression, tumour grade and proliferation rates. The first pathway, also known as the low-grade-like pathway, includes a gain of 1q, loss of 16q, occasional rare amplification of 17q12 and changes in the expression of genes related to the ER phenotype. In contrast, the second pathway, referred to as high-grade-like pathway, is characterised by the loss of 13q, gain of the chromosomal region 11q13, amplification of 17q12 and the expression of genes that influence the cell cycle and cellular proliferation. This pathway is associated with HER2-positive tumours and triple-negative breast cancer (TNBC) (2).

Given the differential expression profiles and other specific characteristics that define breast cancers, numerous classifications have been proposed for the disease. However, the most used classification in clinical practice involves the surrogate intrinsic subtypes, which are based on histology and immunohistochemical expression of key proteins such as ER, progesterone receptor (PR), human epidermal growth factor 2 (HER2) and the proliferation marker Ki67. The characteristics of these subtypes are described in **Table 1**.

**Table 1.** Breast cancer's surrogate intrinsic subtypes

| | Triple Negative | HER2-enriched | Luminal B-like HER2+ | Luminal B-like HER2- | Luminal A-like |
|---|---|---|---|---|---|
| **ER expression** | - | - | + | + | ++ |
| **PR expression** | - | - | + | + | ++ |
| **Her2 expression** | - | + | + | - | - |
| **Ki67 index** | High | High | High | High | Low |
| **Histology** | NST histology, special type | NST histology | NST and pleiomorphic | NST, micropapillary and lobular pleiomorphic histology | NST, tubular cribriform and lobular histology |
| **Prognosis** | Poor | Intermediate | Intermediate | Intermediate | Good |

### 1.1.3. Risk factors for breast cancer

As mentioned in the previous section, breast cancer is a complex and heterogeneous disease which results from the accumulation and interplay of genetic alterations and environmental factors that can alter the normal function of cells. These risk factors are commonly divided in two groups: non-modifiable and modifiable factors.

Non-modifiable factors include genetic susceptibility, a family history of breast cancer, other breast diseases or high breast density, which collectively account for 5 to 10% of all breast cancer cases. Many patients inherit mutations in highly penetrant autosomal dominant genes, with *breast cancer 1* (BRCA1) and *breast cancer 2* (BRCA2) being the most studied ones. These genes are tumour suppressors, whose mutations significantly increase the risk of developing certain epithelial malignancies, particularly breast and ovarian cancer (7). In addition, other genetic variants, and single nucleotide polymorphisms (SNPs) are also regarded as predisposing factors for breast cancer. Aging is another significant risk factor, as the accumulation of genetic, epigenetic, and hormonal alterations becomes more pronounced with age, especially after the age of 50 (**Figure 3**) (8).

Modifiable risk factors include obesity (and other anthropometric traits), a sedentary lifestyle, reproductive factors such as early age of menarche, late menopause, low parity, late age at first childbirth, hormone replacement therapy, the use of oral contraceptive pills, exposure to radiation therapy and xenoestrogens. All these factors are thought to be the cause of 90% of all breast cancer cases (**Figure 3**) (8).

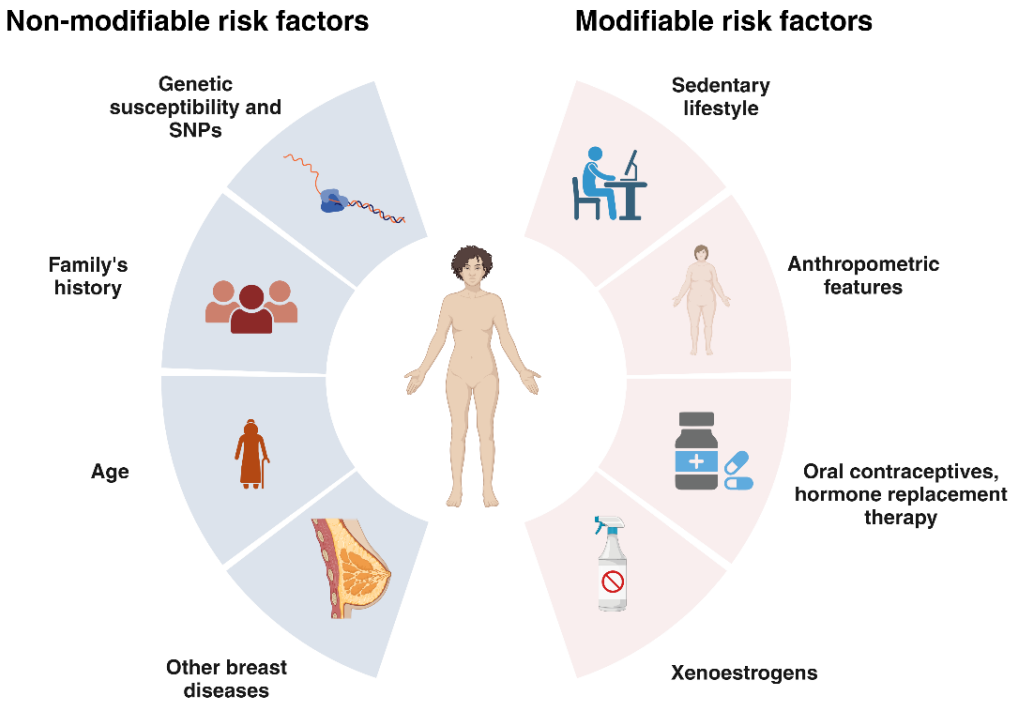


**Figure 3. Summary of risk factors for breast cancer.** On the left, most common non-modifiable risk factors. On the right, modifiable risk factors. SNPs: single nucleotide polymorphisms. Created with BioRender.

The complexity and variety of risk factors explains why the exact origin of any kind of breast cancer is almost untraceable, as one factor will hardly be the cause of the tumour, and many facets must be considered as contributing factors.

## 1.2. The physiological role of estrogenic hormones

It is well established that hormonal exposure is one of the main triggers of sporadic breast cancer. When estrogen binds to the ER, which is located in the nucleus and encoded by the *estrogen receptor 1* (ESR1) gene, the receptor is activated as it is ligand dependent. These hormonal changes are initiated during mammary gland development in adolescence, throughout each menstrual cycle, or during pregnancy, leading to hormonal imbalances, particularly between estrogen and progesterone. As a result, during cellular proliferation and repeated occurrence of these hormonal processes, DNA damage can accumulate. Improper DNA repair mechanisms may then result in mutations, ultimately giving rise to precancerous cells (2).

### 1.2.1. Estrogens

Estrogens are steroid hormones that play an important role in women's development during different life stages, as well as contributing to the health of bones, the heart and the brain in both men and women. They are classified based on their origin, whether they are endogenous, produced by glands or cells of living organisms, or exogenous, derived from synthetic estrogens, foods and drugs (9).

In humans, four types of estrogens have been identified: estrone (E1), 17-β-estradiol (E2), estriol (E3) and estetrol (E4). The general term "estrogen" usually refers to E2 (**Figure 4**), as it is the most widely distributed and biologically active estrogen across tissues and organs of the body. E2 has the highest affinity to ERα, ERβ and G protein-coupled estrogen receptor 1 (GPER1), and its principal activities include the development of secondary female characteristics, regulating the menstrual cycle and the growth of the endometrial lining from menarche to menopause. On the other hand, E1 has a lower affinity to ERs and is the predominant estrogen during menopause. E3 and E4 are the principal estrogens found during pregnancy and have lower affinities for ERs compared to E1 and E2 (1).

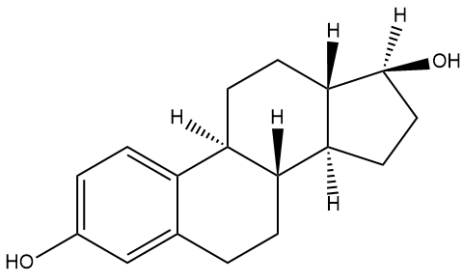


**Figure 4. 17-β-estradiol chemical structure**. Created with ChemDraw.

### 1.2.2. Canonical Estrogen Receptors

For decades it was believed that only one estrogenic receptor existed, later named as ERα. It was not until 1995 that a second type was discovered, ERβ, and since then many isoforms of the latter have been identified. Both receptors belong to the steroid/thyroid hormone superfamily of nuclear receptors and share a structure composed of three independents but interacting functional domains: NH2-terminal (A/B domain), the DNA-binding domain (C domain), and the ligand-binding domain (D/E/F domain) (**Figure 5**) (10).

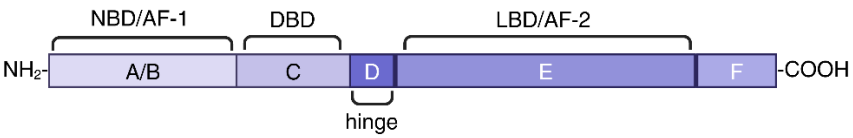


**Figure 5. General structure of estrogen receptors.** AF-1DBD: DNA-binding domain; LBD: Ligand-binding domain; NBD: NH2-terminal domain. Created with BioRender.

Regarding ER's mechanism of action, although the precise steps are not fully defined, it is known that when a ligand binds to the ligand-binding domain either on ERα or ERβ, it triggers receptor dimerization, allowing the complex to enter the cell nucleus (**Figure 6**). Once in the nucleus, the complex interacts with estrogen response elements (EREs) or activator protein-1 (AP-1) and specificity protein-1 (Sp1) on gene promoters, acting as transcription factors that regulate gene expression (11).

The AF-1 ligand-independent activation function, located in the A/B domain, participates in the receptor's protein interaction and target gene expression. It has been shown that in ERα this function is highly active and regulates a series of EREs, within the promoters of target genes in different cell lines, whereas in ERβ, this function is much less active. Specifically, ERα facilitates the expression of several factors like V-myc avian myelocytomatosis viral oncogene homolog (MYC), Cyclin D1, forkhead box M1 (FOXM1), growth regulation by estrogen in breast cancer (GREB1), B-cell lymphoma 2 (BCL2), insulin-like growth factor 1 (IGF-1) and C-X-C motif chemokine ligand 12 (CXCL12), which are potentially oncogenic (11).

Of note, although ERβ is homologous to ERα and interacts with known ERα ligands and transcriptional coregulators, it exhibits different affinities for them and often leads to distinct functional outcomes. ERβ's activity can vary significantly depending on its isoform, cellular context, and the co-expression of ERα. For instance, in a setting where ERα acts as a proliferative activator, ERβ may function as a proliferation inhibitor and apoptosis inducer. Another significant difference between the receptors is where they are mostly expressed. ERα is principally expressed in uterus, ovaries, bone, white adipose tissue, kidney, liver, and breast. However, ERβ, is typically found in male reproductive organs, central nervous system, cardiovascular system, lung, immune system, colon, and kidney (11).

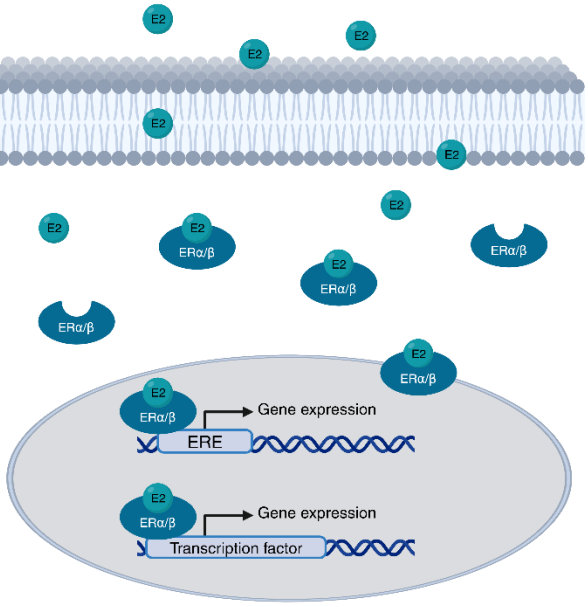

**Figure 6. Genomic effect of estrogen when it binds to ERα or ERβ**. E2: 17β-estradiol; Erαβ: Estrogen receptor alfa or beta; ERE: Estrogen response elements. Adapted from (11). Created with BioRender.

### 1.2.3. Non-canonical Estrogen Receptors

GPER1 has the typical structure of a G protein-coupled receptor, consisting of seven transmembrane α-helices, four extracellular segments, and four cytosolic segments. This receptor is primarily found in skeletal muscle, neurons, vascular endothelium, immune cells, and target effector organs. Although active when interacting with estrogens, it has a lower affinity to them than canonical ERs. The pathway activated when estrogen binds to GPER1 causes a non-genomic effect, meaning that this interaction triggers nuclear transcription factors by changes on ion channel opening or activating enzymes that promote $Ca^{2+}$ mobilisation, phosphatidylinositol-3-kinase (PI3K) and mitogen activated protein kinase (**Figure 7**).

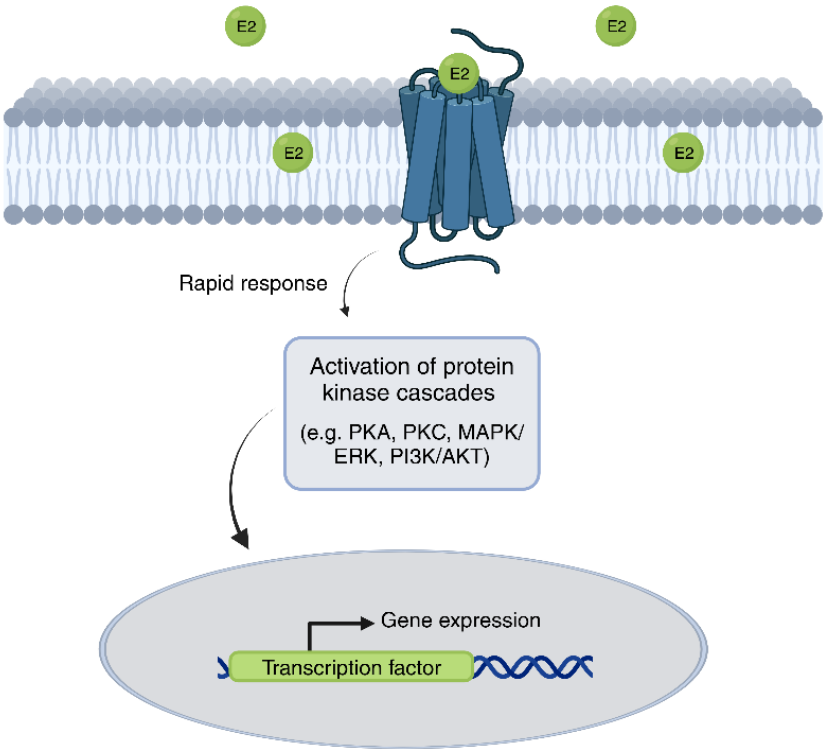


**Figure 7. Non-genomic effect of estrogen when it binds to ERs located in the cell's membrane, such as GPER1**. E2: 17-β-estradiol; PKA: Protein kinase A; PKC: Protein Kinase C; MAPK/ERK: mitogen-activated protein kinases and extracellular signal-regulated kinases pathway; PI3K/AKT: phosphatidylinositol 3-kinase and Akt protein pathway. Adapted from (11). Created with BioRender.

Bearing in mind the significance of hormonal pathways in physiological conditions for the human body, explains why it is important to study which EDCs and where they interfere or mimic our hormones, contributing to the development of breast cancer.

## 1.3. Endocrine Disrupting Chemicals

Endocrine-disrupting chemicals (EDCs) have been defined in many ways. However, the most accepted definition comes from *State of the science of endocrine disrupting chemicals 2012*, an official report from the World Health Organisation (WHO):

*"An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations"* (12).

The most common types of EDCs are listed below in **Figure 8**. However, given the vast amount of information available, this dissertation covers only a selected subset of them.

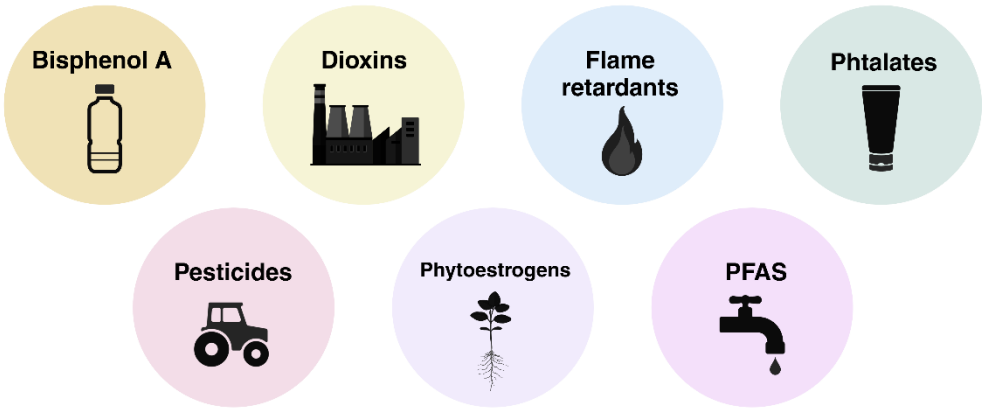


**Figure 8. Most common types of EDCs.** PFAS: Polyfluorinated Alkyl Substances. Created with BioRender.

### 1.3.1. Bisphenols

Bisphenols have been in the spotlight of research for many years. One of the most studied is Bisphenol-A (BPA), which is an organic synthetic compound belonging to the phenol's family, characterized by a hydroxyl group directly attached to the aromatic ring (**Figure 9**). BPA is used as a monomer in polycarbonates and epoxy resins, which are key materials in products such as thermal paper, baby bottles, medical equipment, electronic devices and even food and beverages. The most important source of exposure for humans is believed to be diet. BPA can migrate into ecosystems, food, and beverages when polycarbonate plastics and epoxy resins are subjected to high temperatures and

acidic or basic solutions. In addition to dietary exposure, dermal contact, and inhalation are other important ways of exposure (13).

Once inside the body, BPA can accumulate in adipose tissue, such as breast adipose tissue, due to its lipophilic properties. BPA concentrations in this type of tissue could be a good marker for chronic exposure, while urinary BPA levels are preferable to evaluate recent exposure (10).

BPA is considered an EDC due to its structural similarity to the synthetic estrogen diethylstilbesterol, allowing it to bind to both canonical and non-canonical estrogen receptors.

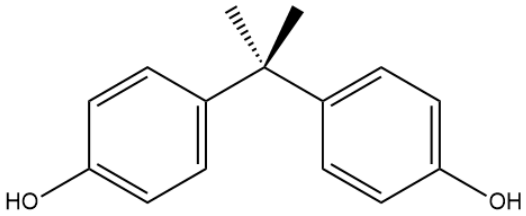


**Figure 9. Bisphenol A chemical structure.** Created with ChemDraw.

### 1.3.2 Polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) are a group of organochlorinated compounds that share a structure of two benzenic rings and from one to ten chlorine atoms, resulting in a series of 209 congeners (**Figure 10**). Their use was highly widespread throughout the 20th century due to their elevated thermic stability and resistance to flammability. Later, when their potential carcinogenic activity and toxicity were discovered, growing concerns led to their prohibition in 1986. Despite the ban, PCBs can still be found in machinery and products produced before their restriction (14).

PCBs are persistent organic pollutants, meaning that are stable in different environmental matrices, such as biota, air, water, and soil, for decades. This persistence still contributes to different routes of exposure for humans in the present time, such as ingestion of contaminated fatty foods, inhalation, dermal transmission through direct contact and even from mother to children via placenta or milk. Once inside the body, PCBs can accumulate like BPA (15).

Notably, among PCBs, PCB-153 is the most abundantly detected in the environment and the primary contributor to PCB body burden estimates.

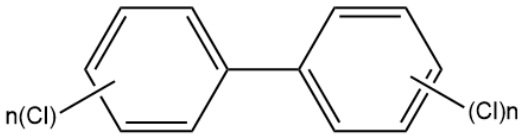


**Figure 10**. PCBs' general chemical structure. Created with ChemDraw.

### 1.3.3 Parabens

Parabens are esters of P-hydroxybenzoic acid with various alkyl chain lengths (**Figure 11**) (16). These molecules are highly used as preservatives in personal care products, such as lotions and deodorants, as well as pharmaceutical products, clothes, and food, to prevent the growth of unwanted microorganisms. Given their extensive use in daily use products, humans are highly likely to get exposed to these compounds, entering the body through dermal absorption or gastrointestinal uptake, and subsequently distribute systemically throughout the body. As a result, parabens have been detected in human tissues, blood, breast milk, placenta, and urine (17).

The most detected parabens in biological fluids are methylparaben (MP), ethylparaben (EP), propylparaben (PP), and butylparaben (BP) (14).

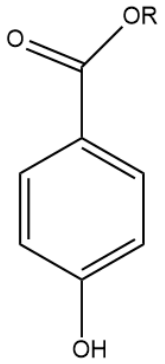


**Figure 11. Paraben's general chemical structure.** Created with ChemDraw.

### 1.3.4. Dioxins

The term dioxin encompasses different groups of persistent organic pollutants that are usually generated during industrial combustion processes, such as waste incineration, and production of herbicides, among others. This family's common structure is characterised by the presence of two benzene rings connected by a pair of oxygen atoms. Additionally, chlorine atoms can be paired with the carbon atoms that are not bonded to oxygen, increasing their toxicity (**Figure 12**). Similarly to other EDCs, dioxins are highly lipophilic allowing them to accumulate in adipose tissue and are also extremely environmentally persistent.

In humans, exposure comes from contaminated food, air, and dermal absorption. Remarkably, certain populations may receive especially high doses of dioxins if they work in an environment where high concentrations are produced, if they are victims of accidental releases, or live near municipal waste incinerators (18).

TCDD, or 2,3,7,8-tetrachlorodibenzo-p-dioxin, is the most biologically active dioxin congener and has also been classified as a group 1 carcinogen by the International Agency for Research on Cancer (IARC), a WHO agency that researches cancer causes and classifies carcinogens.

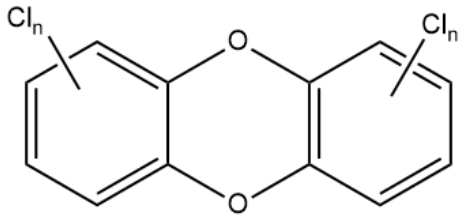


**Figure 12. Dioxin's general chemical structure**. Created with ChemDraw.

## 2. OBJECTIVES AND HYPOTHESIS

The hypothesis of this project is that EDCs significantly contribute to various aspects of breast cancer initiation, development, and progression.

To evaluate this hypothesis, the primary objective of this study is to analyse the impact of EDCs on human health, with a specific focus on their role in breast cancer development, by examining exposure pathways, hormonal disruption mechanisms, alterations on pro-oncogenic pathways and tumour microenvironment alterations. The study will specifically investigate the effects of BPA, PCBs, parabens, and dioxins.

The specific aims of this project include:

- Explain what EDCs are and the extent of daily human exposure.
- Investigate how selected EDCs contribute to breast cancer development in women, including hormonal disruption, epigenetic modifications, and microenvironment alterations.
- Analyse the impact of EDCs on tumour progression, focusing on their influence on cell proliferation, migration, and invasion.
- Discuss the public health implications of EDC exposure and highlight the importance of coming up with new preventive measures and regulations.

## 3. MATERIALS AND METHODS

This study consists of a literature review exploring the effects of EDCs on breast cancer. The aim of this project was to identify and analyse recent scientific research on the mechanisms through which EDCs influence the development, progression, or metastases of breast cancer.

The literature search was primarily conducted by consulting publications from reputable scientific databases, including PubMed and Scopus, which are recognized for their extensive collections of peer-reviewed articles in biomedical and environmental sciences. Access to these databases was provided by CRAI UB (Centre de Recursos per l'Aprenentatge i la Investigació de la Universitat de Barcelona).

To ensure optimal search results, it was essential to have a clear definition of the information sought. Once the research focus was established, specific search terms related to the topic were selected. These terms were combined using Boolean operators such as "AND" and "OR" to refine the search results and ensure alignment with the study's objectives. Finally, to maintain scientific rigor and prioritize recent research, only articles from 2019 onwards were selected, making a few exceptions for remarkable articles that provided valuable insights.

# 4. RESULTS

This section presents key findings regarding the relationship between some of the most well-known EDCs and breast cancer. Research has been done in cellular and animal models, as well as epidemiological studies. Numerous studies have demonstrated that bisphenols, polychlorinated biphenyls (PCBs), parabens, and dioxins have shown influence in breast cancer by altering different physiological processes. The following subsections aim to provide a detailed overview of the effects of these compounds on breast cancer development and progression.

## 4.1. Bisphenol A

BPA is one of the most studied bisphenols due to its role as an EDC. Consequently, numerous preclinical studies have investigated its connection with the development of breast cancer, as well as other types of cancer (24).

Awada and colleagues studied the influence of BPA and its derivates on MCF-7 breast cancer cells, observing a time- and dose-dependent increase in metabolic activity mediated by ERs. Additionally, BPA exposure promoted cell cycle progression, increasing the proportion of cells in the S and G2/M phases of the cell cycle in a dose-dependent manner, also through ER interaction. Moreover, BPA exposure led to a significant increase in cellular migration in a time- and dose-dependent manner (**Figure 13**). These findings underscore the multifaceted effects of BPA on cellular behaviour, including proliferation and motility (19).

Building on these observations, researchers further examined the molecular changes induced by BPA and other analogues in MCF-7 cells. They detected an increased ER-dependent expression of DNA methyltransferase 1 (DNMT1), indicating BPA's role in genomic instability and epigenetic modifications. Some of these DNA methylation alterations affected cancer-related pathways, such as focal adhesion and cyclic guanosine monophosphate (cGMP)-protein kinase G (PKG) signalling, highlighting BPA's potential role in oncogenic transformation (**Figure 13**) (19).

Tumour suppressor genes like p53 and retinoblastoma protein (pRb), which normally prevent damaged DNA from progressing past the G1 checkpoint into the S phase, can become dysfunctional, promoting malignant transformation when inactivated (20). Acknowledging this, Victoria Lloyd and her team investigated BPA's impact on ERα and p53 in breast cancer cells. Their experiments revealed that BPA increased p53 expression and promoted cell proliferation, while simultaneously down-regulating ERα expression (**Figure 13**). They further hypothesized that BPA effects might be mediated

through c-Myc gene expression, a key regulator of cell growth, proliferation, metabolism, differentiation, and apoptosis in breast cancer (20).

The role of BPA in promoting cancer aggressiveness was further explored by Del Favero and colleagues, who conducted short-term exposure assays in MCF-7 and MDA-MB-231 cells. They observed that BPA significantly increased cell mobility in both cell types, suggesting a potential metastatic activity. Additionally, changes in actin fibre organization were detected, which contributed to enhanced mobility. Regarding gene expression changes, they detected a rise in the expression of integrin β1 and cathepsin D, which may contribute to greater invasive potential and metastasis formation (**Figure 13**). These findings suggest that BPA's effects extend beyond canonical and non-canonical ERs signalling pathways (21).

Interestingly, BPA's interactions with ERs are not exclusively linked to ERα. ERβ, which can act both as a tumour suppressor and a tumour promoter in breast cancer, exhibits variable effects depending on its isoforms and binding affinities. BPA exposure has been shown to disrupt ERβ's protective role, either by altering the expression of ERβ-regulated genes or by shifting the ERα/ERβ balance (**Figure 13**) (22).

The impact of BPA was also studied in vivo using BALB-neuT mice, a transgenic model predisposed to mammary carcinomas due to a mutated ErbB2/neu receptor gene. Mice exposed to BPA via drinking water developed tumours earlier (week 12) than the control group (week 16). BPA-treated mice exhibited lower survival rates, increased tumour burden, and enhanced tumour aggressiveness. Despite low ERα expression in tumours from both groups, GPR30 was highly expressed in pre-invasive and invasive stages, suggesting its role in mediating BPA's estrogenic effects in ER-negative breast cancer cells. Additionally, BPA exposure led to a significant upregulation of PR expression and increased levels of proliferation and angiogenesis markers (Ki67 and CD31). Moreover, BPA activated the Akt signalling pathway and induced PD-1/PD-L1 expression, promoting an immunosuppressive microenvironment marked by increased regulatory T cells (Tregs) and exhausted T lymphocytes (**Figure 13**) (23).

Kwon and colleagues further investigated BPA's role in modulating the tumour microenvironment. They concluded that BPA activates cell proliferation via ERs while also disrupting macrophage and lymphocyte functions, thereby promoting the immune system's evasion. Additionally, BPA was found to enhance collagen deposition that leads to mammary gland stiffness, which inhibits the expression of key tumour suppressors and activates Akt, driving cell proliferation and motility (**Figure 13**). Furthermore, BPA induced epigenetic modifications which increased adipocyte differentiation and inflammation, creating a pro-inflammatory microenvironment that favours cancer progression (24).

In a similar line of research, a recent cross-sectional study examined the potential role of BPA-stimulated adipocytes on the proliferation of breast cancer cells. The results first indicated that individuals with a body mass index (BMI) of 30 or higher who were exposed to BPA had a significantly increased risk of developing sex-related cancers (25).

To further investigate this mechanism, researchers evaluated whether 3T3-L1 cells could transition from pre-adipocytes to cancer-associated adipocytes (CAAs) when exposed to BPA. Their findings confirmed this transition, as evidenced by increased lipid accumulation and higher expression of cancer-associated markers, including leptin and adiponectin. Additionally, BPA-induced CAAs displayed reduced expression of *phosphatase and tensin homolog* (PTEN), a tumour suppressor responsible for downregulating the PI3K/Akt pathway and preventing uncontrolled cell proliferation. Concurrently, Akt pathway activity was elevated, facilitating migration and invasion of breast cancer cells. Finally, xenograft models in BALB/c mice demonstrated similar effects, as BPA-induced CAAs enhanced breast cancer cell growth in vivo, further supporting the role of BPA in tumour progression (25).

Additionally, some EDCs have been shown to interfere with cancer treatments. For instance, in vitro studies have demonstrated that BPA can reduce the therapeutic effect of tamoxifen's active metabolite, as well as that of cytostatic agents commonly used in breast cancer treatment (26)

Finally, a recent epidemiological study, analysed BPA levels in serum samples of breast cancer patients and healthy controls. First, they detected a significant higher concentration of BPA in cancer patient's samples compared to controls. Second, while they analysed BPA's role in oxidative stress on tissue samples, they found an increased ROS level in malignant tissues, along with significantly reduced levels of superoxide dismutase (SOD) and catalase (CAT). The study also highlighted altered expression of key breast cancer markers, including p53, WNT1 and ZEB1 genes. p53 expression was significantly upregulated at both the mRNA and protein levels, suggesting a potential link between BPA and oxidative stress-induced DNA damage. The study further correlated BPA exposure with lifestyle factors such as frequent consumption of canned foods and use of plastic bottles, emphasizing the environmental and behavioural dimensions of BPA's influence in breast cancer development (**Figure 13**) (27).

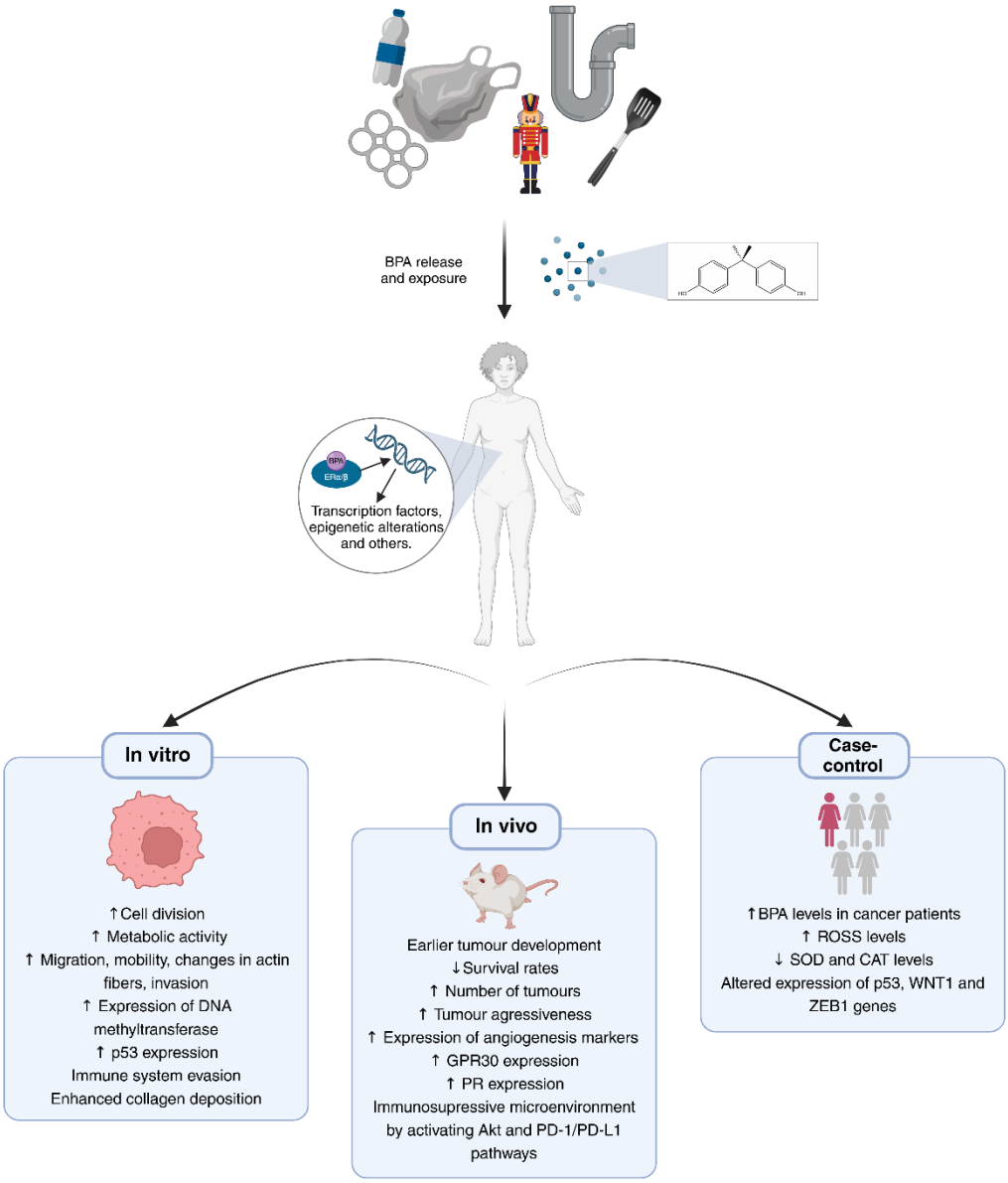


**Figure 13. Effects of bisphenol A exposure on breast cancer.** This EDC has demonstrated diverse effects in in vitro studies, in vivo studies, and case-control studies. BPA: Bisphenol A; CAT: Catalase; GPR30: G-protein coupled estrogen receptor; PD-1: Programmed death 1; PD-L1: Programmed ligand receptor 1; PR: Progesterone receptor; ROS: Reactive oxygen species; SOD: Superoxide dismutase. Created with BioRender.

## 4.2. Polychlorinated biphenyls

Since the prohibition of PCB production, many governments objective has been to eliminate or decontaminate PCBs from existing machinery that still contains or uses them. However, exposure to these compounds persists, and it remains of importance to study how they can affect the development of breast cancer.

A recently published study analysed data from the XENAIR case-control study, which is part of the E3N French prospective cohort study, revealed that the group with the highest PCB153 concentrations had a 69% higher likelihood of developing breast cancer compared to the group with the lowest concentrations (**Figure 15**) (28). However, a cohort study examining Japanese patients involved in the Yusho accident (1968)- who were exposed to high levels of PCBs and dioxins through contaminated rice- showed no significant positive association between the exposure to these contaminants and increased mortality from breast cancer (29).

In line with these findings, a cohort study that evaluated dietary intake of PCBs and dioxins across nine European countries and its correlation with breast cancer concluded that there was no significant association between reported dietary exposure to these EDCs and breast cancer incidence (30).

In addition to the existing knowledge about PCBs, a PCB quinone-type metabolite, named PCB29-pQ, was discovered to have the ability to promote aerobic glycolysis, an important marker of metabolic reprogramming in cancer progression. Qin and colleagues reported an up-regulation of the glucose transporter (GLUT1), along with other key enzymes involved in aerobic glycolysis when breast cancer MDA-MB-231 cells were exposed to PCB29-pQ. Moreover, PCB29-pQ increased cell migration and invasion in vitro and in female BALB/c murine models (**Figure 14**) (31).

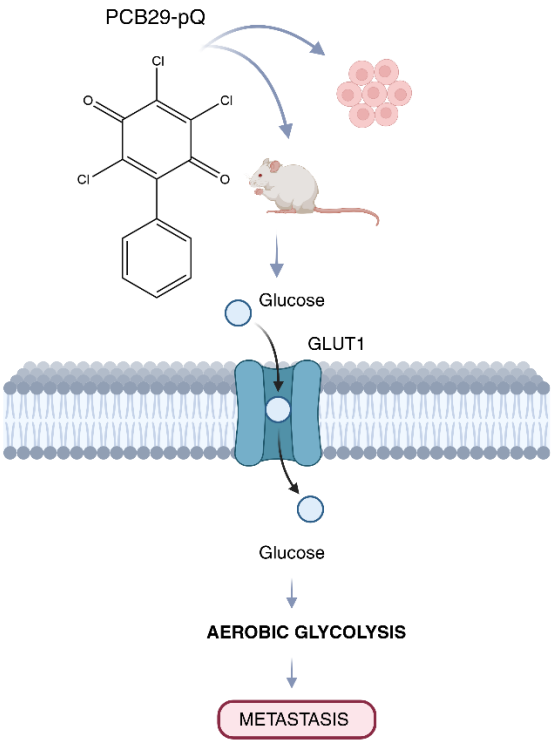

**Figure 14.** Diagram of PCB29-pQ, a PCB quinone-type metabolite that could promote aerobic glycolysis in BALB/c murine models and breast cancer MDA-MB-231 cells. GLUT1: Glucose transporter 1. Adapted from (30). Created with BioRender.

The influence of PCBs on gene expression was also studied by Morin and her team. Their study analysed how PCB126 altered gene expression in primary benign breast epithelial cells (HBECs) and patient-derived explants (PDEs) (32). PCB126 significantly modified the expression of 144 genes. Among the upregulated genes, CYP1A1 and CYP1B1 stood out; these genes are regulated by the aryl hydrocarbon receptor (AhR) and play a key role in the detoxification and metabolism of xenobiotics. Likewise, interleukin 1 alpha (IL1A) and interleukin 1 beta (IL1B), which encode pro-inflammatory cytokines involved in immune signalling, and amphiregulin (AREG), a growth factor associated with cell proliferation and epithelial signalling, were up regulated (32). Nevertheless, a down-regulation was observed in matrix metallopeptidase 7 (MMP7), which is linked to epithelial homeostasis and tissue remodelling, and C-C motif chemokine ligand 28 (CCL28), which is related to immune regulation and lymphocyte chemotaxis (**Figure 15**) (32).

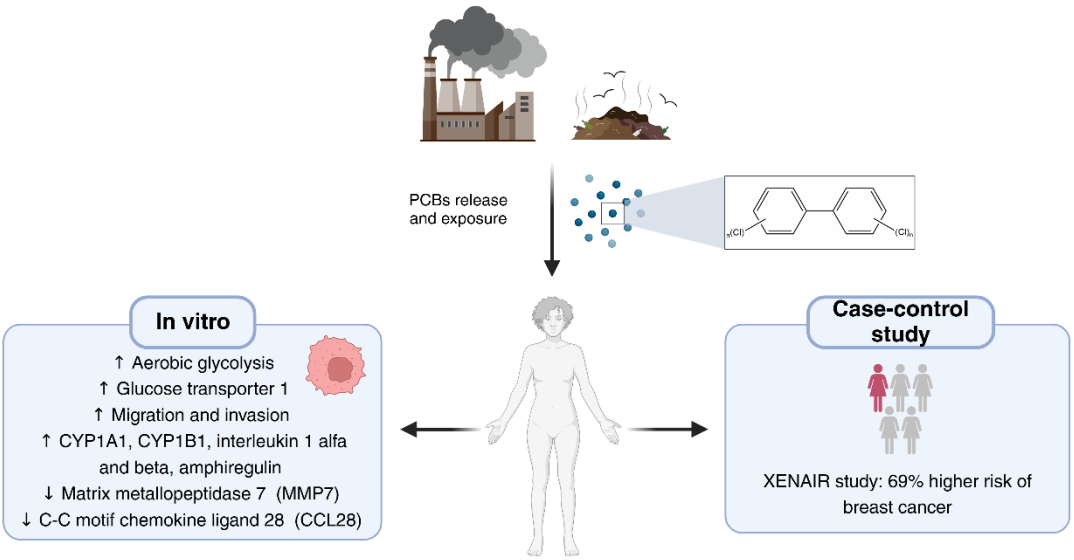


**Figure 15. Effects of PCBs on breast cancer.** This family of compounds has demonstrated a series of effects in vitro which are associated with the enhancement of breast cancer. Created with BioRender.

## 4.3. Parabens

Parabens are a significant focus of research due to their widespread and nearly unavoidable presence in daily life. In human skin, the small intestine, and the liver,

parabens undergo hydrolysis by esterases, converting them into p-hydroxybenzoic acid. The rate of this reaction increases proportionally with the length of the paraben's side chain. Consequently, methylparaben (MP), which has the shortest side chain, is the most abundant and remains largely intact in biological fluids. Intact parabens that evade first-pass metabolism by esterases can bind to serum albumin, which protects them from further metabolism and facilitates their distribution and bioaccumulation (**Figure 16**) (8).

Parabens can regulate ER-mediated gene expression in vitro. In fact, in silico simulations have shown that MP and ethylparaben (EP) interact with two key amino acids in ER's ligand-binding pocket. Moreover, this interaction is believed to be spontaneous (8). Additionally, it has been proven that all types of parabens exhibit similar affinities for ERs, when administered at certain doses, and among parabens administered at the same dose, those with longer alkyl chain induce the highest estrogenic activity (33).

To highlight parabens' potential estrogenic and antispermatogonic activity, a study in which MP and propylparaben (PP) were administered to adult zebrafish revealed testicular malformations and alterations, such as impaired germ cell development and multinucleated monocytes. These results suggest that parabens may play a significant role in up-regulating ERs and down-regulating androgen receptor (AR) in zebrafish (**Figure 16**) (34).

Recognizing the effects of parabens on breast cancer by their proven affinity to ERs, Wafaa and colleagues further investigated MP's role in promoting the tumorigenic behaviour of MCF-7 cells. Their results showed that MCF-7 cells exposed to MP had an increased viability in a concentration- and time-dependent manner, a dose-dependent Ki-67 expression increase, a remarkable increased E2 secretion, particularly at high doses, a significant increase in early apoptosis at various concentrations, reduced lipid peroxidation (based on MDA levels), an increased total antioxidant capacity (TAC), and a significantly decreased oxidative stress index (OSI), which showed a strong negative correlation with cell viability (35). In contrast, a recent study conducted in zebrafish showed that PP and MP decreased the expression of various genes related to oxidative stress (**Figure 16**) (34).

A similar study analysing the effects of MP and PP in mice found that, although the number of new tumours did not reach statistically significant levels compared to the control group, they caused a qualitative -but not significant- increase in tissue invasion. Moreover, treated mice developed cancer phenotypes associated with advanced pathological stages in human breast cancer, including a significant increase in total mammary tumour volume over time and an increased number of pulmonary metastases per week, that did not differ significantly from the total of pulmonary metastases from the control group (**Figure 16**) (33).

A different approach was taken by Dairkee and colleagues to demonstrate the effects of EDCs in promoting breast cancer. Their study involved volunteers following a regimen

of reduced xenoestrogens exposure -focusing on parabens and phthalates- which they called REDUXE. Later, they analysed the differences in breast tissue characteristics before and after the REDUXE regimen (36). Interestingly, results from the REDUXE intervention showed that genes typically associated with cancer, such as toll-like receptor 4 (TLR4), myeloid differentiation primary response 88 (MYD88) and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), exhibited reduced expression levels returning to profiles characteristic of normal tissue. Meanwhile, increased levels of minichromosome maintenance complex component 7 (MCM7) and suppressor or mothers against decapentaplegic homolog 4 (SMAD4) genes were observed, suggesting a possible protector role. Moreover, when comparing gene expression profiles between E2 controls and REDUXE samples, six genes were significantly downregulated, while eleven genes were upregulated. All seventeen genes were associated with the E2-modulated pathway (**Figure 16**) (36).

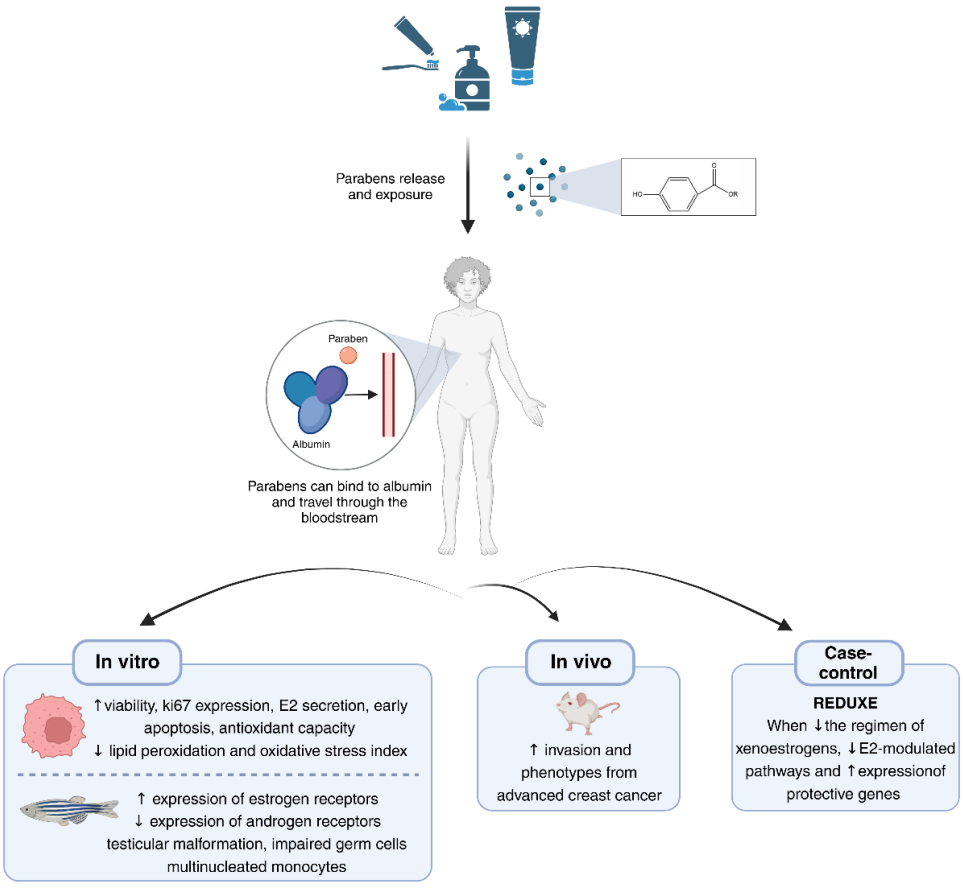


**Figure 16. Effects of parabens on breast cancer.** Research has found the influence these compounds have in various pathways associated with the disease. E2: 17β-estradiol. Created with BioRender.

## 4.4. Dioxins

Dioxins are highly toxic environmental pollutants that accumulate in the food chain, primarily in animal adipose tissue. To exert their toxic effects, dioxins require the activation of AhR and cytochrome P450 enzymes. This family of compounds are mainly by-products of industrial processes, such as waste incineration and chemical manufacturing. Due to their persistence and bioaccumulation, dioxins pose serious health risks and have been classified as Group I carcinogens by the IARC, given for their proven ability to cause cancer in both humans and animals (37).

The WHO organisation introduced the term TEF (toxic equivalency factor) to express the toxicity of dioxins, furans, and PCBs. These values were revaluated in 2022, but while new results were proposed, some remain unreliable due to lack of data. However, it is estimated that applying the newly proposed TEFs would reduce dietary EDC exposure by 50% (38,39).

Since primary source of human exposure are food products, Shi and colleagues conducted a study to assess dietary dioxin exposure and its association with cancer risk correlation and disease burden on the Chinese population. Results showed that levels of dioxins in food have decreased between 2000 and 2020. However, in specific regions, dioxins remain at high concentrations. Notably, the highest exposure group was children aged 0–14 years, who also exhibited the highest associated cancer risk. Moreover, the cancer burden attributable to dietary dioxins was higher in males than in females, with prostate cancer being the most affected type in men and breast cancer in women (**Figure 17**) (40).

Similarly, a prospective cohort study in non-occupationally exposed U.S. women revealed a significant association between ambient dioxin emissions from municipal solid waste incinerators (MSWIs) -located from 3 to 10 km from residences- and an increased risk of invasive breast cancer, including both ER+ and ER- subtypes (**Figure 17**) (41).

In the same line, another prospective cohort study was conducted to determine the influence between living near dioxin-producing industrial facilities and breast cancer incidence and mortality. Over an 11-year follow-up period, 2,670 new cases were diagnosed, most of them ER+. While living closer to these facilities was associated with a higher risk of developing breast cancer, these participants also tended to present additional risk factors, such as higher BMI and smoking habits. Moreover, breast cancer risk decreased with increasing distance to these facilities, likely due to the relatively short half-lives of certain dioxins (**Figure 17**) (42).

From a molecular perspective, Meriem Koual and her team studied how breast cancer MCF-7 cells, in co-culture with hMADS preadipocytes, responded to TCDD exposure in their microenvironment. Under these conditions, cells presented reduced adhesion,

enhanced expression of ALDH1A3, CYP1A1 and CYP1B1, lower mRNA expression of E-cadherin and significant morphological alterations, such as extended lamellipodia (cytoskeletal projections on the edges of a cell) and the formation of giant multinucleated cells. All these new characteristics contribute to a pro-metastatic phenotype (43). Interestingly, when they replicated the same conditions in zebrafish xenograft models, the number of brain metastases in the TCDD-exposed groups significantly increased compared to the control group (**Figure 17**) (43).

Further research also investigated how dioxins were linked to the AhR, a ligand-activated transcription factor that regulates multiple target genes and is also known as a xenobiotic receptor. In this context, when activated by dioxins and other pollutants, AhR has been shown to play a critical role in breast cancer progression, promoting immune tolerance, tumour microenvironment remodelling, and other alterations that drive chemoresistance and metastasis (44). Specifically, AhR activation induces nuclear factor-kappa B (NF-κB) pathway, which promotes the expression of pro-inflammatory chemokines, such as Interleukin-8 (IL-8). Additionally, it regulates chemokine ligand 1 (CXCL1), chemokine ligand 2 (CXCL2) and chemokine ligand 5 (CCL5) genes, which promote cellular migration and macrophage recruitment to the tumour microenvironment. Moreover, AhR activation increases amphiregulin (AREG) production, leading to tumoral proliferation and macrophage infiltration (**Figure 17**) (44).

Shan and colleagues further investigated the effects of mixed exposure to EDCs on human breast cancer cells. MCF-7 cells were exposed to TCDD or mono-2-ethylhexyl phthalate (MEHP), resulting in different outcomes. While MCF-7 cells had an increased cell migration and invasion when exposed to a single EDC, co-exposure results showed a reduced cell migration and invasion. Moreover, the co-exposure conditions led to lower CYP1A1 mRNA levels, that were increased when exposed to a single EDC. Furthermore, AhR expression in BALB/c nude mice was also decreased in the co-exposure group. These results suggest a competitive antagonism between TCDD and MEHP in their inhibition of MCF-7 cells (**Figure 17**) (45).

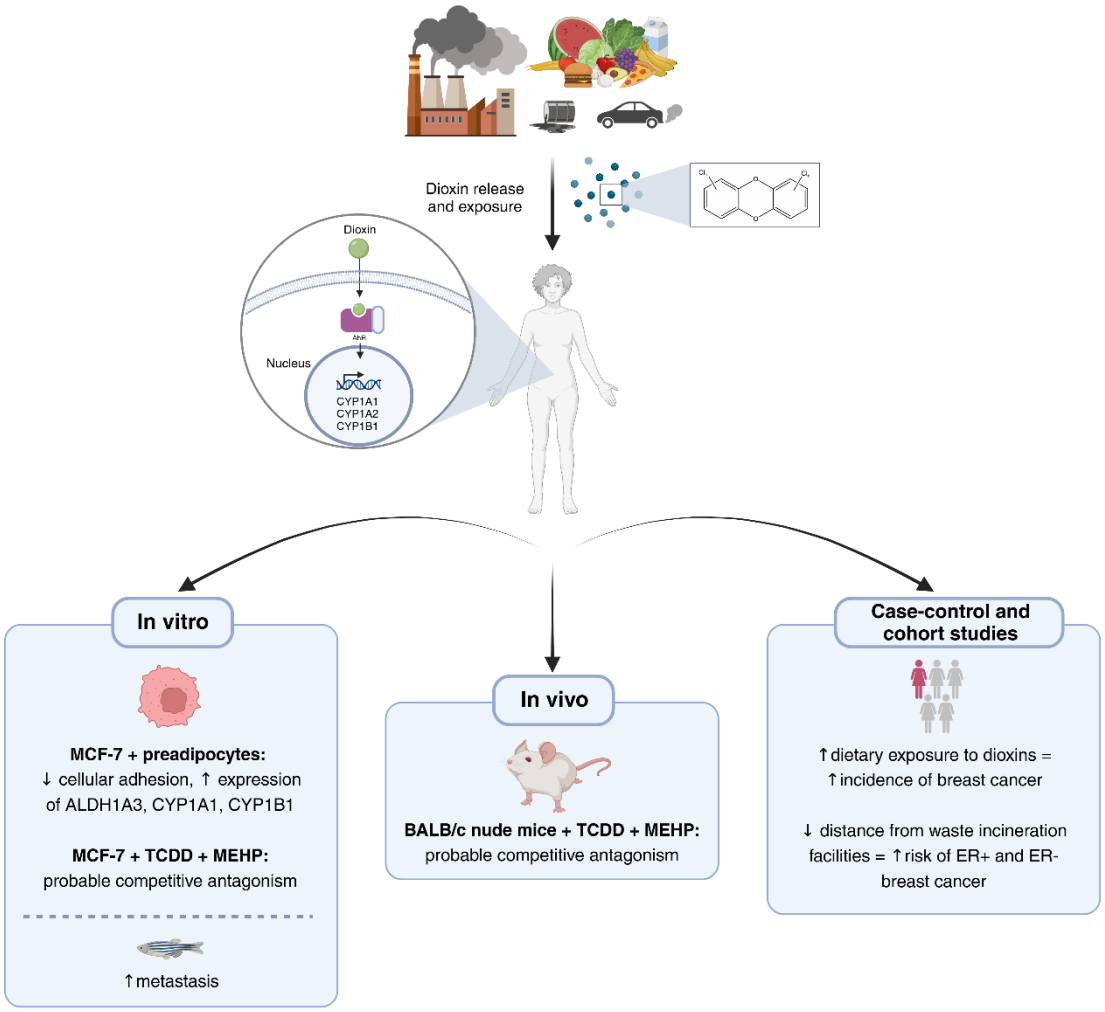


**Figure 17. Effects of dioxins in breast cancer.** Once inside the body, dioxins bind to the AhR in the cytoplasm, forming a ligand-receptor complex. This complex translocates to the nucleus, where AhR functions as a transcription factor, inducing the expression of CYP450 gene. AhR: Aryl hydrocarbon receptor; ALDH1A3: Aldehyde dehydrogenase 1 family member A3; CYP1A1: Cytochrome P450 family 1 subfamily A member 1; CYP1A2: Cytochrome P450 family 1 subfamily A member 2; CYP1B1: Cytochrome P450 family 1 subfamily B member 1; ER+: Estrogen receptor positive; ER-: Estrogen receptor negative; MCF-7; Michigan Cancer foundation-7 (breast cancer cell line); MEHP: Mono(2-ethylhexyl) phthalate; TCDD: 2,3,7,8-Tetrachlorodibenzo-p-dioxin. Created with BioRender.

# 5. DISCUSSION

The impact of EDCs on human health, particularly in the development and progression of cancer, has been in the spotlight for many decades. Although research is ongoing, it remains insufficient to bridge existing knowledge gaps, given the increasing severity of the issue and the continued exposure to EDCs in daily life.

In the present study, various types of data were analysed to gain a better understanding of how EDCs participate in breast cancer and to assess the extent of our current knowledge. This section aims to summarize the key effects of the studied EDCs on breast cancer, discuss the limitations of this type of research, highlight the importance of EDCs exposure on public health and examine current measures to prevent exposure and future proposals.

Numerous studies have demonstrated that EDCs can influence breast cancer development and progression through multiple mechanisms. These compounds can mimic endogenous ligands or interfere with molecular pathways, primarily estrogen and androgen signalling, leading to disruptions in cellular homeostasis. Moreover, EDCs induce epigenetic modifications, such as altered DNA methylation and histone modifications, which affect gene expression and genomic stability. Additionally, EDCs promote tumour proliferation and progression by enhancing cell cycle activity, metabolic reprogramming, and resistance to apoptosis. Furthermore, many EDCs contribute to increased migration and invasion of other tissues, facilitating metastasis by altering the cytoskeletal structure and modifying the expression of adhesion proteins. Furthermore, EDCs also modulate the tumour microenvironment by promoting chronic inflammation and suppressing the immune system's protective role, creating conditions that allow cancer cells to expand and proliferate more easily.

Although these compounds were first mentioned in the 20th century, when various researchers began noticing changes in the wellbeing of humans and other forms of life, it seems that not enough is being done to mitigate the harm caused by EDCs. While EDCs are not yet fully understood and further research is needed to fully comprehend their toxicity, the conclusions of this work suggest that key factors influence EDC exposure and could be assessed through government restrictions and policies. For instance, research has shown that living within 10 km of municipal waste incinerators or constant exposure to polluted air from industrial facilities can have an impact on the development and progression of breast cancer. A good example of this is the 2020 Zaldívar landfill fire in the Basque Country, which caused severe environmental pollution, releasing high levels of toxic dioxins and furans into the air. These persistent pollutants pose serious health risks, including hormonal disruption and an increased cancer risk. In addition, people who consumed higher amounts of canned food or plastic-wrapped foods face an

increased risk of developing breast cancer due to EDCs present in these materials. Importantly, these two factors have been found to strongly correlate with an individual's socioeconomic status.

On the other hand, research has found that vulnerable individuals aged 0 to 14 are at a higher risk of exposure to EDCs than other age groups raising another important area of study: how these compounds may also impact children's growth and the development of other diseases.

Finally, it has been proven that finding fewer toxic congeners of these compounds might not be the solution. In a study where the effects of bisphenol S (BPS) and bisphenol F (BPF), structurally related replacements for BPA, on breast tissue were tested, researchers found that even these alternatives had the potential to induce changes in cell's organoid architecture and proteome (46). Moreover, in a previous study conducted in a zebrafish model, bisphenol AF (BSAF) showed higher toxicity and estrogenic activity than BPA, which was presumed to be more harmful (47).

Taken together, these findings highlight the need for more restrictive laws that limit the exposure and emission of EDCs, as well as the implementation of preventive measures to prevent more incidents that significantly increase population exposure to specific EDCs. In Spain, various regulations exist regarding the sampling and analysis of PCBs and dioxins in food (48). The European Commission has emitted multiple recommendations on the acceptable levels of these EDCs in food, which vary depending on the type of food but are always based on the ALARA (As Low As Reasonably Achievable) principle, as stated in *Reglamento (UE) 2023/915* (49). In contrast, there is only one emission limit value for PCBs and dioxins- 0,1 ng/$Nm^3$-, which applies to the total amount of these EDCs released from waste incineration facilities (50).

Alternatively, the regulations that limit the concentrations of parabens in cosmetic products have established a maximum concentration of 0,4% for short-chain parabens, such as MP and EP, and a maximum concentration of 0,8% when used in combination. In contrast, the limit for long-chain parabens, such as PP and BP, is stricter, with a maximum concentration of 0,14% allowed in these products. Moreover, the European Commission banned the use of several parabens in cosmetic products in 2014 due to their detrimental effects on human health (51).

In contrast, the regulation of bisphenols has undergone significant changes recently. On January 3$^{rd}$ of this year, a new regulation, adapted from a directive issued by the European Commission, was approved, and later took effect on January 20$^{th}$. This regulation bans the production of all products containing BPA and its potentially harmful derivates in materials intended for contact with food and beverages. However, products which are already on the market will remain available until January 2029 (52). Exceptions will apply when no suitable alternative is available to ensure microbiological safety. Despite these regulatory efforts, it is important to note that the health effects of these

EDCs may still manifest in individuals long after the ban. However, these measures will help protect younger and future generations.

Despite these regulations, a key takeaway from this project is that only a limited number of EDCs were studied while humans are exposed to thousands of these chemicals daily. For the most part, the effects of these mixtures on human health remain unknown, not only in relation to breast cancer but also other pathologies. Additionally, new EDC candidates continue to be identified. For instance, in February of this year, France proposed resorcinol, a phenolic compound widely used in the cosmetic, pharmaceutical, and industrial sectors, as a potential EDC (53). In conclusion, safer alternatives must be developed to replace these compounds, as even those with low endocrine-disrupting activity contribute to a cumulative exposure, which may still pose health risks. Furthermore, when complete avoiding exposure is not possible, efforts should focus on protecting workers regularly exposed to these chemicals, as well as people living near emission sources.

To conclude, this study has faced various limitations. One of the primary challenges is the complexity of the mechanisms underlying breast cancer development. Multiple biological pathways interact, making it difficult to isolate the specific role of a single EDC. On the other hand, this project focused on the disruptive activities of the studied EDCs when administered either alone or, at most, in combination with one additional toxic compound. However, humans are simultaneously exposed to numerous EDCs. Which may interact in a competitive, synergistic, or unpredictable ways that remain largely unstudied. On the same line, many of the mentioned studies were conducted on cell models, typically analysing one or two EDCs at a time. In contrast, epidemiological studies, provide data on the correlation between exposure to a single EDC and the incidence of breast cancer in a specific population. However, as previously discussed, future research should focus on studying mixtures of EDCs in, preferably using in vivo models, as these have demonstrated greater reliability and informative value. Finally, another limitation of this study is that many of the available publications for review were considered outdated according to the chosen scientific rigor criteria, particularly those concerning PCBs. This raise concerns that scientific interest in finding solutions to prevent exposure to these chemicals has declined over time, despite their continued widespread presence in daily life.

## 6. CONCLUSIONS

In conclusion, this study highlights the significant role of EDCs—including bisphenols, PCBs, parabens, and dioxins—in the development and progression of breast cancer, although the supporting evidence differs in strength across compounds. BPA promotes cell proliferation, migration, epigenetic alterations, tumour microenvironment modifications, and immunosuppression. PCBs disrupt gene expression and cellular metabolism, although their association with breast cancer risk remains inconclusive. Parabens exhibit estrogenic activity, contributing to cell proliferation and oxidative stress, while dioxins, classified as Group I carcinogens, promote inflammation, metastasis, and treatment resistance. Epidemiological studies suggest that high exposure levels, influenced by environmental factors, diet, and cosmetic use, may increase breast cancer risk. Given their persistence in the environment and human adipose tissue, further research is crucial. Additionally, stronger regulations and preventive measures are needed to minimize exposure and mitigate potential health risk.

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