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Environmental Pesticides and Heavy Metals — Role in Breast Cancer

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Additional information is available at the end of the chapter

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Abstract

The intent of this chapter is to provide an overview to the current thoughts and ideals regarding the involvement of pesticides and heavy metals in the progress of breast cancer. The history of pesticides encompasses a few millennia, but our understanding of the pesticide action and the health consequences has only begun to develop in the last 30-40 years. Interestingly, many of these pesticides have estrogen-like activity and may be involved in the development of breast cancer. A new category of estrogen-like compounds has been identified and studied in the last 30 years, the 'metalloestrogens'. Heavy metals, such as cadmium, which have estrogen like activity will be discussed. Finally, we will attempt to pull together the actions of pesticides and metalloestrogens as a possible synergistic mechanism by which these toxins may work to promote breast cancer development.

Keywords: organochlorines, pesticides, heavy metals, breast cancer

1. Introduction

Agriculture chemicals, otherwise referred to as "agrochemicals, " are a large family of chemicals that cover many pest issues associated with farming. For nearly 5, 000 years, crops have been protected by some form of "pesticide." Some of the earliest recorded use of "pesticides" was nearly 5, 000 years ago and involved the use of sulfur dusting in the area of modern-day Iraq and surrounding lands. Ancient Sanskrit hymns (Rigveda) allude to the use of various plant-derived compounds, some of which are poisonous, that can be applied to

crops, killing insects yet leaving the crops intact – the first insecticides [1]. There was little advancement in the field of pesticides for thousands of years. In the 1400s, the use of chemicals was tried by farmers to kill various crop-related insects. In most cases, the active ingredient of these chemicals was rooted in the actions of heavy metals (arsenic, mercury, and lead). Two hundred years later, the alkaloid, nicotine, was being investigated as a potential agent to eliminate insects from crops. The utility of nicotine prompted additional investigation into the use of natural products as insecticide/pesticide agents. Interestingly, two of the compounds which were developed, pyrethrum and rotenone, are still used today experimentally for various research-related purposes. The use of arsenic-based compounds prevailed for hundreds of years until the mid-1900s as not only agents for pest prevention, but also for poisonings [2]. A shift in the pesticide industry began to occur in the early- to mid-1900s. This began with the identification of dichlorodiphenyltrichloroethane (DDT) as a potent insecticide. DDT was originally developed 75 years earlier, but it was not until nearly 1940 when Dr. Paul Müller discovered that DDT was a very effective insecticide (later being awarded the Nobel Prize in Physiology or Medicine in 1948 for this discovery). Pesticides that contained chlorine were initially referred to as “organochlorines,” with DDT being considered the prototype organochlorine, which many subsequent agents were based on. Very quickly, considering the timeline of pesticide development and use over thousands of years, organochlorine pesticides were banned in the United States and replaced by newer pesticide derivatives. By substituting a phosphate for chlorine, the “organophosphate” class of pesticides was developed. In addition, the carbamates class was also developed and the organophosphates and carbamates effectively replaced organochlorine pesticides by 1975. Since then, pyrethrin compounds have become the dominant insecticide. Herbicides became common in the 1960s, led by “triazine and other nitrogen-based compounds, carboxylic acids such as 2, 4-dichlorophenoxyacetic acid, and glyphosate” [2].

In general, these pesticides can broadly include compounds/chemicals that can kill insects, invasive plants, invasive other organisms – generally anything that will kill pests that damage farmland. The drawback to the use of these chemicals is that they are all toxic and in most cases can enter the groundwater, or the food chain. Even if handled and stored properly, the chance of accidental exposure is greatest for those who live closer to the areas being treated. Normally, the release of toxic/dangerous chemicals into the environment is tightly regulated and controlled. The one unique aspect of pesticide use is that these are highly toxic chemicals and are voluntarily applied in the environment. By definition, a pesticide is a compound/chemical that will kill a variety of pests and the more specific name will indicate what is being killed, such as herbicides (weeds), fungicides (fungi), insecticides (insects), rodenticides (rodents), etc. In many instances, we can generalize and simply refer to any of these agents as a pesticide. Because of their widespread use, ease of accessibility, and, in some instances, low cost, the impact of pesticides on the environment has become profound and significant. This impact is no longer confined to agricultural fields, but can also include, homes, businesses, academic institutions, and some areas otherwise believed to be protected. The link between pesticides and cancer has long been a concern. Besides low-level accidental exposure, large-scale contamination can occur when there are industrial accidents or waste dumping of large quantities of raw agrochemicals. The production of agrochemicals is still big business, with

the top producers reporting revenue of nearly \$15 billion in 2013. Yet, in 2005 it was reported that the economic impact on pesticide use in the United States was nearly \$10 billion [3]. These studies broke down the pesticide cost on U.S. economy as follows: \$1.1 billion for public health, \$1.5 billion for pesticide resistance, \$1.4 billion crop losses due to pesticides, \$2.2 billion in avian losses, \$2 billion in groundwater contamination. The ratio of dollars spent on pesticides to dollars for crops saved is about 1:4 [4]. This ratio is essentially a return on an investment. You can expect nearly a fourfold return on your expenditures due to increased crop yield (due to reduced pest burden). A return this large is a significant benefit to the growers who can grow a more diverse annual crop, and will generate more crop output on an annual basis. This increased ability to generate goods would translate into a benefit for the consumer [5]. Alternatives to pesticide use have been explored and are currently being developed. Some of these alternative methods involve changes in cultivation methods, use of biologically derived pesticides (extracts or other compounds of organism that may kill the pest in question). There has been some indication that alternative methods can be equally efficacious to chemical pesticides and are safer to human populations also. The loss of crops and the economic impact on widespread damage resulting from pest infestation would be significant and catastrophic. Therefore, although agrochemical use is vital and necessary, it does come with a relatively high cost.

Although there have been significant inroads in the fight against breast cancer since 1994, there is still much to be done and even more that needs to be understood. Still, nearly 12% of women will experience invasive breast cancer in their lifetime – that is, over 20 million cases. It is understood that there are multiple factors that underlie the pathogenesis of breast cancer, either invasive or noninvasive. Key factors include 1) genetic predisposition, 2) environmental factors, and 3) lifestyle. None of the factors are exclusive, but rather inclusive of each other. Studies have demonstrated that there are numerous synthetic chemicals that can mimic the actions of estrogen. Exposure to estrogen-like compounds can come from many sources, including cosmetics and other household items [6]. Not all are detrimental, such as the phytoestrogens which are derived from plant sources. But there are compounds referred to as xenoestrogens, or “endocrine disruptors.” Regardless, they include numerous pesticides, including the broad class of organochlorine pesticides. These compounds have demonstrated adverse medical outcomes [7]. In addition, there are certain metals which are found in the environment that have displayed estrogenic activity [8]. Cadmium-based chemicals have been shown to be highly toxic and carcinogenic [9]. Ultimately, the goal for treatment and improved prognosis is the development of compounds which can counteract, or reverse, the effects seen after exposure to these xenoestrogen environmental contaminants. Many of the available compounds focus on estrogen-dependent tumors and the response to these drugs is favorable. But tumors lacking the estrogen receptor respond poorly to current treatments. The literature is lacking detailed studies on the coexposure of OCPs and metals and how these complex exposures may alter tumor development. Recent studies and reviews have only begun to shed light on this situation [10, 11].

The prefix “xeno” refers to something with a different origin. Therefore, the term xenoestrogen is a form of our endogenous estrogen hormone that imitates estrogen but comes from a

nonbody source. Xenoestrogens are also called “environmental hormones” or “endocrine disrupting compounds” (EDC). These forms of estrogen may be clinically significant because they can mimic the effects of endogenous estrogen and have been theorized to be involved in numerous pathologies. Xenoestrogens can be either synthetic or natural chemical compounds. Synthetic xenoestrogens are widely used industrial compounds (Table 1), such as PCBs, BPA, and phthalates, which have estrogenic effects on a living organism even though they differ chemically from the normal estrogenic hormones (estradiol, estriol, estrone). Naturally occurring xenoestrogens include plant-derived phytoestrogens. Because the primary route of exposure to these compounds is by consumption of phytoestrogenic plants, they are sometimes called “dietary estrogens.” Mycoestrogens, estrogenic substances from fungi, are another type of xenoestrogen that are also considered mycotoxins. An increasing amount of xenoestrogenic compounds have been deposited into the environment over the last century. The potential ecological and human health impact of environmental xenoestrogens has been of increasing concern over the last 20-30 years.

Xenoestrogens have been implicated in a variety of medical pathologies, and over the last decade numerous studies have found evidence of adverse effects on human and animal health [12-15]. There is a concern that xenoestrogens may act as false messengers and disrupt the process of puberty and reproduction. The induction of cytochrome P450 isozyme, CYP1A, has been established to be a good bioindicator for xenoestrogen exposure. Another potential effect of xenoestrogens is on oncogenes, specifically in relation to breast cancer. The impact of xenoestrogens on breast tumor growth has been equivocal, leading to some speculation that these agents may not be involved in breast cancer progression. It does appear to depend on the form of cancer and the type of xenoestrogen exposure. However, there is growing evidence that xenoestrogens can increase breast cancer growth in tissue culture. One complication in drawing an accurate conclusion is that many studies have simply examined high concentrations/doses in short exposure periods. In most situations, exposures in this fashion would not occur unless exposed in a manufacturing or industrial setting. Also, the majority of the population will not be exposed to singular compounds. There will be “cocktails” of sorts, mixtures of many different xenochemicals with exposures to pesticides as well as heavy metals being likely. Additional work needs to be done before more accurate conclusions can be drawn regarding the effects of either the xenoestrogens or metalloestrogens (a form of xenoestrogen).

Estrogenic Class	Subclass	Representative Compounds
Phytoestrogens	Flavanones	Hesperetin
		Naringenin
		Liquiritigenin
		Pinocembrin
	Flavones	Acacetin
		7, 8-dihydroxyflavone
		Mirificin
	Prenylflavonoids	8-Prenylnaringenin
		6-Prenylnaringenin

Estrogenic Class	Subclass	Representative Compounds			
		8-Geranylnaringenin			
		6, 8-Diprenylnaringenin			
		Icaritin			
	Isoflavones	Daidzein	Glycitein	Peurarin	
		Daidzin	Ononin	Tectorigenin	
		Genistein	Pruentin	Biochanin A	
		Genistin	Formononetin		
	Isoflavanes	Equol (S-Equol)			
		Glabridin			
	Dihydrochalcones	Phloretin			
		Phlorizin			
	Coumestans	Coumestan			
		Coumestrol			
		Desmethylwedelolactone			
		Wedelolactone			
	Lignans	Enterodiol			
		Enterolactone			
	Flavonolignans	Silybin			
		Cinchonain – Ib			
	Flavonols	Quercetin			
Others	Deoxymiroestrol				
	Miroestrol				
	Resveratrol				
	α -Isosparteine				
Mycoestrogens	Zearalanone				
	Zearalenone				
	α -Zearalanol				
	α -Zearalenol				
Synthetic	4-MBC	DDE	Erythrosine	PBB	
	Alkylphenols	DDT	Heptachlor	PCB	
	Atrazine	Dieldrin	Lindane	Phthalates	
	BHA	Dioxins	Methoxychlor	Propyl gallate	
	BPA	Endosulfan	Parabens		
Metalloestrogens	Aluminum		Chromium (II)	Nickel	
	Antimony		Cobalt	Selenite	
	Arsenite		Copper	Tin	
	Barium		Lead	vanadate	
	Cadmium		Mercury		

4-MBC - 4-Methylbenzylidene camphor; BHA - Butylated hydroxyanisole; BPA - Bisphenol A; PBB - polybrominated biphenyl; PCB - polychlorinated biphenyl

Table 1. Grouping of xenoestrogens into four major classes

Below is additional description of synthetic xenoestrogens and their current use [16-18].

- Atrazine: widely used as a herbicide to control broad-leaf weed species and is the second largest seller in the United States. Atrazine is also applied to Christmas trees, lawns, golf courses, and other recreational areas.
- BPA (Bisphenol A): monomer used to manufacture polycarbonate plastic and epoxy resins used as a lining in most food and beverage cans in excess of 6.4 billion lbs/year. BPA can hydrolyze and the leaching of BPA has led to widespread human exposure.
- DDT (Dichlorodiphenyltrichloroethane): banned in 1972 due to its hazardous effects on the environment, it continues to be used in many parts of the world. DDT and its metabolites DDE and DDD are persistent in the environment and accumulate in fatty tissues.
- Dioxin: released during combustion processes, pesticide manufacturing, and chlorine bleaching of wood pulp. Consumption of animal fats is thought to be the primary pathway for human exposure.
- Endosulfan: is an insecticide used on numerous vegetables, fruits, cereal grains, and trees. Human exposure occurs through food consumption or ground and surface water contamination.
- PBB (Polybrominated biphenyls): used in computer monitors, televisions, textiles, and plastics foams to make them more difficult to burn. Production stopped in the mid-1970s, but due to the very slow degradation, PBBs can still be detected in moderate concentrations in the environment.
- PCBs (Polychlorinated biphenyls): also known as chlorinated hydrocarbons. PCBs were used as insulating fluids and coolants. PCBs were banned in 1979 but like DDT continue to persist in the environment.
- Phthalates: plasticizers which increase durability and flexibility to plastics. Both high and low molecular weight phthalates are used in a variety of consumer products which can include perfumes, lotions, cosmetics, varnishes, lacquers, and coatings including timed releases in pharmaceuticals.
- Zeranone: anabolic growth promoter for livestock, banned in Europe, but still present as a contaminant.
- There are over 25 other miscellaneous xenoestrogens that range from banned insecticides that may still be stored onsite [DDT, Dieldrin, endosulfan, methoxychlor], to “normal” household products like 4-methylbenzylidene [suntan lotion], to estrogen compounds like ethinylestradiol [birth control pills] plus a host of others. Collectively, even in small concentrations, these agents may have localized significant effects.

2. Pesticides

What is a pesticide? As defined by the Food and Agriculture Organization of the United Nations, a pesticide is: *“any substance or mixture of substances intended for preventing, destroying*

or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies" [FAO, <http://www.fao.org/docrep/x1531e/X1531e02.htm>]. By definition, classification of a pesticide is an exceptionally broad term and encompasses a multitude of different commercial and/or private uses. Due to the diverse nature of pesticides, they have been linked to a wide range of human health issues. The role of pesticides in the development of cancers, alterations in reproduction, as well as an array of symptomologies associated with acute, or short-term, exposures.

Comparison between short-term (acute) and long-term (chronic) exposure:

- Acute exposure: General irritation to the contact areas such as skin and eyes. If absorbed or inhaled, there may be peripheral nerve damage (neuropathies), or potential effects on the brain, which will result in headaches, dizziness, and fatigue. Exposure to significantly large quantities of a pesticide may result in a systemic poisoning that results in generalized organ failure and, potentially, death.
- Chronic exposure: Chronic exposure is occasionally the most difficult to diagnose and assess. Especially if the individual was exposed to low levels of the pesticide. Initially, there may be no symptomology, or visible signs that a person was exposed. With a slow degeneration of a particular physiological response, symptoms can become visible weeks, months, or even years after the exposure has ended. In some instances, pesticides that have been shown to bioaccumulate, can concentrate within the body and over time will elicit a toxic response. Pesticides can cause many types of cancer in humans. Some of the most prevalent forms include leukemia, non-Hodgkin's lymphoma, brain, bone, and breast, ovarian, prostate, testicular, and liver cancers.

Since the 1980s, increasing amounts of data have indicated there are both direct and indirect roles for pesticides in the disruption of the mammalian endocrine system. Alterations in normal homeostatic endocrine functioning due to pesticide exposure occurring from fetal development through adulthood can affect the delicate balance of hormonal systems involved with normal development. Within the larger universe of pesticides, a subset has been identified that exhibits abilities to alter mammalian (and nonmammalian) endocrine systems. Collectively, these agents are referred to as "endocrine disruptors." There are nonpesticide chemicals that are included on this list, such as the metalloestrogens, but pesticides on the list of endocrine disruptors include DDT, lindane, parathion, plus a multiple of others that are chemically and structurally similar.

The young and elderly are particularly sensitive to the actions of pesticides. In particular, children under the age of 15 exhibit significant risk due to the continuing development of the central nervous system. In addition, the child's developing respiratory, gastrointestinal, hepatic systems can be likely targets for pesticide toxicity. Compounding the potential problem is the child's close proximity to where the pesticides are located. In most cases, pesticide storage will be at ground level. Also, application of pesticides to crops, plants, grasses, etc., puts the

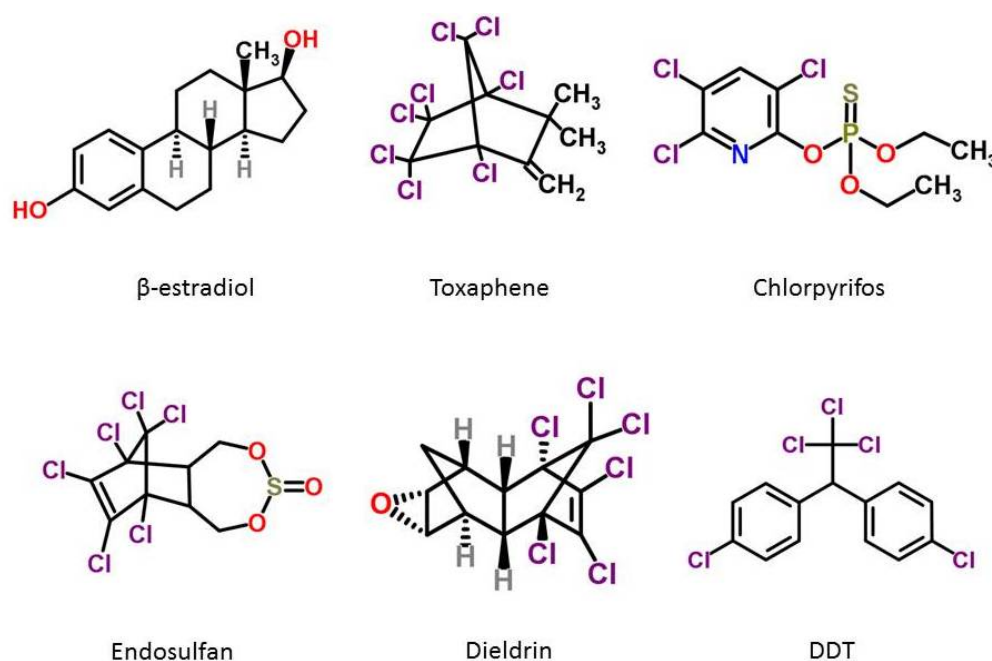
pesticide at locations where the child may physically come into contact with the pesticide through normal activities. This potential elevated risk factor, coupled with the child's inability to eliminate or neutralize the pesticide can result in numerous physiological deficits observed during development, suggesting that children are suffering disproportionately from the impact of pesticide exposure.

Pesticides can be classified by target organism (herbs/weeds, insects, fungi, rodents), chemical structure (organic, inorganic, synthetic, or biological/biopesticides), and physical state (powder, solid, liquid or gaseous). Plant-derived pesticides, or "botanicals," have been developing rapidly. These include the pyrethroids (plant-derived pesticides), rotenoids, nicotinoids, and a fourth group that includes strychnine/scilliroside. The broad classes of chemical pesticides can include:

1. **Organophosphates (OPs):** affect the nervous system by disrupting the enzyme acetylcholinesterase, which is responsible for the breakdown, or degradation, of acetylcholine. Inhibition of this enzyme will lead to increased parasympathetic tone, due to increased activity at both muscarinic and nicotinic cholinergic receptors. They were developed during the early 19th century, but their effects on insects, which are similar to their effects on humans, were discovered in 1932. Some are very poisonous. Individuals exposed to high levels of OPs can develop what is referred to as "acute cholinergic syndrome," which is characterized by a variety of symptoms including rhinorrhea, salivation, lachrymation, tachycardia, headache, convulsions, and death [19]. However, they usually are not persistent in the environment so the threat of bioaccumulation is not as great compared to organochlorine pesticides. The more toxic organophosphates have been replaced by the less-toxic carbamates.
2. **Carbamates:** affect the nervous system by disrupting the enzyme acetylcholinesterase, which is responsible for the breakdown, or degradation, of acetylcholine. Inhibition of this enzyme will lead to increased parasympathetic tone, due to increased activity at both muscarinic and nicotinic cholinergic receptors. The enzyme effects are usually reversible. There are several subgroups within the carbamates family including the thiocarbamate and dithiocarbamate subclasses.
3. **Organochlorines:** A major physiological action of organochlorines is the disruption of the sodium/potassium balance of the nerve fiber. Altering the resting membrane potential may have two effects; one of which is the ability to turn an excitable cell into the "on" position. In the instance of organochlorine compounds, that results in constant action potentials traveling down the nerve pathways. In general, the organochlorine group has a wide range of toxicities with some agents being relatively nontoxic, yet other compounds eliciting a high level of toxicity and cellular damage. Collectively, the ban on organochlorines is near universal, due to the fact that these agents can persist in the environment for significant lengths of time.
4. **Pyrethroid:** They were developed as a synthetic version of the naturally occurring pesticide pyrethrin, which is found in chrysanthemums. They have been modified to

increase their stability in the environment. Some synthetic pyrethroids are toxic to the nervous system.

5. **Sulfonylureas:** The sulfonylurea class prototype was originally developed and used to treat Type II Diabetes, but many of the analogs in the sulfonylurea family can be found commercially available to control weeds. Most of the sulfonylurea agents are broad spectrum and work by inhibiting the enzyme acetolactate synthase which is vital for weed growth. The sulfonylureas are generally safer to humans compared to the other classes of agents, and only a fraction of the sulfonylurea agent is needed compared to other herbicides. Due to the highly toxic nature of many of these compounds, most have been discontinued in the United States, yet, there are significant stock piles that are in need of disposal, or are still extensively used outside of the United States. For this chapter, most of the discussion will focus on the actions of the organophosphate and organochlorine agents.



Chlorpyrifos: CSID:2629, <http://www.chemspider.com/Chemical-Structure.2629.html> (accessed 19:43, Feb 6, 2015)
 β -Estradiol: CSID:5554, <http://www.chemspider.com/Chemical-Structure.5554.html> (accessed 19:47, Feb 6, 2015)
Toxaphene: CSID:66611, <http://www.chemspider.com/Chemical-Structure.66611.html> (accessed 19:48, Feb 6, 2015)
Endosulfan: CSID:3111, <http://www.chemspider.com/Chemical-Structure.3111.html> (accessed 19:49, Feb 6, 2015)
Dieldrin: CSID:10292746, <http://www.chemspider.com/Chemical-Structure.10292746.html> (accessed 19:51, Feb 6, 2015)
DDT: CSID:2928, <http://www.chemspider.com/Chemical-Structure.2928.html> (accessed 19:53, Feb 6, 2015)

Figure 1. Chemical construction comparison between β -estradiol and pesticides with known or suspected estrogenic activity. Figures all obtained from ChemSpider [www.chemspider.com]

2.1. Environmental impact

In addition to the desired effects of pesticides, such as killing invasive weeds, crop-damaging insects, etc., most pesticides have demonstrated the ability to persist in the environment for

extended periods of time. Ground applications can result in a leaching effect into the ground-water, and eventually into the water supply. These agents are accumulated and concentrate in the sediment found in lakes, rivers, and streams. This allows introduction into the food chain when ingested by fish. This cycle can then continue in a forward-moving manner. These characteristics of pesticides have made the assessment of the environmental impact very difficult. Years, even decades, after discontinuing the use of a particular pesticide, it may still be detected in the environment at levels that could pose a direct risk to humans or other mammals. There are also potential indirect risks associated with pesticide bioaccumulation. High levels of toxic pesticides can damage the soil, alter the quality of the soil, and potentially reduce crop yield or quality. By impacting food production, certain pesticides may elicit detrimental effects, both direct and indirect, for significant periods of time.

There are thousands of chemicals that are available, yet only a few hundred have undergone toxicity testing. Legislation has been recently introduced to update the Toxic Substance Control Act and increase the testing of these chemicals. The Environmental Protection Agency has published its list of pesticide active ingredients that are to be screened under the Federal Food, Drug and Cosmetic Act [20]. This final list only contains 67 compounds that are considered either active or inert ingredients in pesticide preparations – a number that falls significantly short of the total number of compounds that are currently available. Accompanying this lack of information is the uncertainty as to whether chemicals can be causative agents in the development of breast cancer [21]. The broad class of organochlorine compounds includes organochlorine pesticides (OCPs). Although most OCPs have been banned from use in the United States, with dichlorodiphenyltrichloroethane (DDT) being the prototype compound in this class, there are a few still in use. Endosulfan has been linked to numerous pathologies, and is in the process of being phased out by 2016. Lindane and Dicofol are still used in the United States. Although the number of OCPs is dwindling, there is still the potential that stored OCPs may be used. In addition, OCPs have been shown to persist for extended periods of time in the environment [22] and in the body [23, 24]. Collectively, these data would suggest that OCPs may be a persistent health risk for decades to come with regard to long-term toxicity. Synthetic organochlorine agents can be used in the production of plastic (polyvinyl chloride, PVC), chloromethanes (solvents – carbon tetrachloride; and anesthetics – chloroform), insulators (polychlorinated biphenyls – PCBs) on electrical wire, but these have been phased out. You may still find PCBs in older homes, thus still making PCBs a potential risk factor. With the increased production of hazardous chemicals like heavy metals for other industrial uses as well as the production of these compounds as by-products of industrial processing has created a series of unique challenges for the disposal of these compounds. A variety of heavy metal combinations that were released into waste effluent has demonstrated a synergistic lethality on aquatic organisms [25]. Based on this synergism, the conventional mechanisms of determining lethality (LD₅₀, etc.) may not be accurate. Reductions in toxin content to 1/3 or 1/6 of LD₅₀ for a single toxin may not be a large enough dilution to adequately reduce toxicity in aquatic environments [25]. There have been numerous reports of contamination worldwide, land-based and aquatic-based in the food chain. In the United States, marine mammals near Alaska (walrus, beluga whale, bowhead whale, and rigged seal) have shown increased levels of a variety of toxins such as DDT, PCBs, mercury, and cadmium [26]. In addition, chlordane

and toxaphene have been detected in increasing concentrations. The elevations in these compounds, and their estrogenic nature, may interfere with the reproduction of these species, thereby endangering their future. A combination of pesticides and heavy metals has been shown to induce larval abnormalities in Pacific oysters, reducing their ability to grow and proliferate. A trend, if allowed to continue, will result in the loss of this oyster species permanently. On the east coast of the United States, elevated levels of organochlorine pesticides, PAHs, and heavy metals have been found in oysters collected in the marshland [27]. These agents will then enter the food chain and be consumed by other animals or humans further bioaccumulating. Elevated levels of organochlorine pesticides and heavy metals have been found in a species of eel off the coast of Belgium [28]. A variety of edible species in China have been reported to have elevated levels of organochlorine pesticides and heavy metals [29]. Other reports have substantiated these findings and extended them to other parts of the globe [30, 31]. Organochlorine pesticides and heavy metals have become a significant worldwide hazard contaminating numerous species of marine life, posing a threat to the survival of that species as well as becoming an increasingly significant threat for entering the general human population through their consumption. As a result, increased vigilance needs to be implemented to prevent the loss of these species and the further contamination of the human population.

Increased incidence of contamination is not confined to the aquatic environment. Over the past two decades, there has been an increased awareness of potentially toxic compounds entering the food chain. This could be through the spraying of pesticides to maintain crop integrity, or through groundwater contamination following industrial contamination. The presence of pesticides, hormones, food additives, and other chemicals has increased the public's concern over the status of their food supply. Food safety is a major public concern worldwide and food consumption has been identified as the major pathway for human exposure to certain environmental contaminants. "Chemophobia" (the fear of chemicals in foods) is the major reason for individuals to choose organic food – believing that these types of foods would be free of synthetic pesticides. Generally, organic farms avoid the use of chemically derived pesticides, fertilizers, and other additives, but instead favor more "natural" means of removing pests and improving crop yield. Many believe that "organically" grown crops are "better" health-wise. In some instances, this is the case, but even without some of the usual additives, there is an increased contamination when contaminated groundwater is used for irrigation [32]. Even with the differing farming systems, and the public's perception that organically grown foods are healthier, increased surveillance and screening must be done to monitor the amount of potentially harmful chemicals that are being ingested on a daily basis [33]. In Europe, a continent-wide study involving the World Health Organization found that there were dangerous levels of pesticides and heavy metals in foods which were previously believed to be safe [34]. The major difficulty in analyzing field samples for contamination is that multiple other chemicals and compounds can be identified. The complexity of these chemical-chemical interactions within a biological system cannot be understated. It is the complexity of these interactions which obscure the ability to definitely determine and predict the effect of the sample on a biological system. Mansour and Gad [35] have developed an ecological testing system using *Daphnia magna* Straus, which can be performed rapidly and easily by a field technician. One advantage is that it will test the entire sample, without regard for the number

of contaminants. It can also be performed without large-scale and expensive equipment. The drawback is that the individual toxin cannot be distinguished [35].

Over-the-counter “natural products”, herbal preparations that have reported therapeutic benefits, including herbal medicine such as Saint John’s Wort, Ginseng, Gingko, etc. generate significant revenues and their use is increasing, especially in the United States [36, 37]. Spending on these natural products in the United States has more than doubled in the last 10 years to nearly \$15 billion in sales [37]. One group reported in the late 1990s that some natural products such as curcumin and isoflavonoids can act as an estrogen receptor antagonist and block the effects of estrogen overstimulation [38]. They found that phytoestrogen-related compounds significantly attenuated the effects seen following exposure to the pesticide DDT, and the pollutants 4-nonylphenol and 4-octylphenol [38]. Several studies have shown that Chinese herbal medicines and other botanical supplements may be contaminated with heavy metals, and in some cases at toxic levels [39-41]. There are differences between the preparations of natural medicines. There is the “raw” form – which usually will take the form of the root, nut, bean, etc. Most concern has been directed toward proprietary blends. At this time, it is believed that the external toxins are introduced [42]. Interestingly, some known toxins, such as arsenic and other forms of the arsenicals, lead and mercury, are intentionally added for their purported medical value [43-45]. Contrary to public perception, heavy metals have been detected in raw preparations and, in fact, some species of plants are known to concentrate (bioaccumulate) heavy metals [42]. Heavy metal contamination has also been found in some supplements sold in the United States, but at levels that were not deemed to be hazardous [46]. Since most over-the-counter preparations and those sold over the Internet, fall under the category of “supplement,” there are no extensive guidelines for the limits of heavy metals and pesticides that can be contained in the preparations. Collectively, it appears that consumption of certain phytoestrogens may have a therapeutic benefit in ameliorating the effects observed following exposure to some pesticides and/or environmental toxins, but with some possible contamination hazard of their own. Although the levels of pesticides and heavy metals that would be ingested by consuming raw, or formulated, herbal preparations would be low in the vast majority of the preparations, care must be used and stringent monitoring must be utilized to prevent accidental intoxication of individuals who are ingesting over-the-counter herbal preparations.

2.2. Pesticides and the link to cancer

While agriculture has traditionally been tied to pesticide-related illnesses, over half of the commonly used pesticides are linked to cancer. The true burden of environmentally induced cancer is greatly underestimated. Chemicals can trigger cancer in a variety of ways, including disrupting hormones, damaging DNA, inflaming tissues, and turning genes on or off. Many pesticides are known to cause cancer, and virtually everyone in the United States is exposed to them on a daily basis. In animal studies, many pesticides are carcinogenic (e.g., organochlorine, creosote, and sulfallate), while others (notably, the organochlorine agents DDT, chlordane, and lindane) are tumor promoters. Some contaminants, such as arsenical compounds, in commercial pesticide formulations also may pose a carcinogenic risk. In humans,

arsenic compounds and certain insecticides used occupationally have been classified as carcinogens with organochlorine insecticides being linked with cancers of the lung and breast [47]. With the development of new technologies, the casual association of pesticides with cancer has been strengthened. Most of the human studies have involved retrospective studies examining the incidence of particular cancers like breast cancer and their exposure to pesticides. Now, there is a great deal of research and focus on the identification of biomarkers that can be used to identify these associations between pesticides and cancer. One technology that may be invaluable for biomarker development will be the use of toxicoproteomic-based data [48]. Regardless of the direction that the technology takes us in the future, there is a significant amount of work that still needs to be done to more fully understand how pesticides can be affecting the development of breast cancer as well as other chronic human disorders [49].

Farmers in many countries, including the United States, have lower overall death rates and cancer rates than the general population. Although death rates may be lower, in-depth analysis of the incidence of specific diseases (cancer) indicate that there may be a higher rate of certain cancers among farmers and agricultural workers. Additional work attempting to correlate the types of disease with the chemical exposure has not been unequivocal and there have been many conflicting reports either supporting or refuting the cancer–pesticide link. Similar to what was discussed earlier, the developing young, from fetus to mid-teen, are some of the highest risk groups for disease resulting from pesticide exposure. Young females who may be exposed to an endocrine disrupting agent (such as DDT) have been reported to have a higher incidence of breast cancer compared to populations which were not exposed. Interestingly, this same risk exists if the parents are exposed prior to conception, suggesting that either the pesticide, or changes in the maternal system, can be passed onto the fetus. Collectively, these rural populations of agricultural works and their families tend to be exposed at a higher rate and at higher concentrations than the general population, and as such may experience a higher incidence of particular cancers (breast) than the general population [6].

Yet, to say that there is a significant correlation between pesticide exposure and breast cancer incidence would be an overstatement. Retrospective studies have yielded conflicting results. Most of the studies recognize shortcomings, such as small population size, difficulty in assessing pesticide exposure, and correlating blood pesticide levels to the progression of breast cancer. Pesticides that had widespread use, and were widely popular and commercially available, DDT, DDE, and dieldrin, are the best examined. Since many of these pesticides have exhibited severe health-related adverse effects, older pesticides have been banned, or their use restricted. Yet, due to bioaccumulation, persistence in the environment, and the need to dispose of older stockpiles, they remain an environmental and health concern. As the conflicting evidence has come forward, it appears that one factor that may be important for the toxicity to DDT/DDE/dieldrin is ethnic background. This suggests that genetic polymorphisms between ethnic groups may predispose individuals to cancer risk [50]. A post hoc meta-analysis by Ingber et al. [51] concluded that there was no definite correlation between DDT/DDE exposure and breast cancer. This was done via a PubMed and Web of Science search of nearly 500 cases. Although there were slight elevations in the levels of DDT and the incidence of breast cancer, none of their correlations were statistically significant. Of course, the analysis

may have missed some ethnic groups or other populations where a positive correlation existed, but offers a strong conclusion that these compounds alone may not be enough to promote breast cancer development. In an Indian population from Jaipur, women with higher levels of DDT and its metabolites, dieldrin and heptachlor, were correlated with the incidence of breast cancer [52]. A study examining women of Caribbean descent aimed to correlate pesticide exposure with the incidence of both prostate and breast cancer. Due to relatively high organochlorine pesticide use on the island of Martinique, and what appeared to be an elevated frequency of prostate and breast cancers, the study intended to draw a correlation between pesticide exposure and cancer. They report that there is a positive correlation in the exposure to pesticides and the incidence of breast cancer [53]. Contrary to these studies, research into the causal relationship between pesticides and breast cancer in the United States has not been as convincing and in some instances mixed [50]. An east coast study examined a population of women from Long Island, New York, and found that there was some evidence for a positive correlation between exposure and cancer. It was not a strong correlation, and involved other factors, but would warrant further investigation [54]. Yet a west coast study, the "California Teachers Study" cohort showed no correlation between pesticide exposure and the incidence of breast cancer [55]. Clearly, there is a need to correlate all of the different factors involved in the pathogenesis of breast cancer with the exposure to suspected carcinogens.

It has been known for some time that select pesticides interfered with the action of estrogen. This term was loosely referred to as an "Endocrine Disruptor." This category has since been expanded to include many items such as detergents, disinfectants, plastics, and an increasing number of pesticides. There are three main actions of an endocrine disruptor. First, the pesticide can mimic the actions of estrogen (or testosterone), thereby causing an increase in estrogen-related physiological responses. Second, the pesticide can act like an antagonist and block the actions of estrogen at its receptors. This will prevent the normal physiological responses associated with estrogen stimulation of its receptor. Third, the pesticide may have a broader effect and interfere with the synthesis, transport, metabolism, or elimination of estrogen. This may have a variable effect with either an increase or decrease in estrogenic effects being observed. Regardless, the normal homeostasis of the system will be disrupted.

Of the broad classes of pesticide – the organochlorine class has been the most extensively studied and has shown to be the most potent as an endocrine disruptor. In the 1990s, studies were done in cell culture model systems which demonstrated that organochlorine pesticides were more potent at activating the estrogen receptor, whereas organophosphorus pesticides were relatively ineffective at modifying estrogen receptor activity [56]. Although the focus of this chapter is breast cancer, other sites of estrogen activity, such as the uterus, also demonstrated that organochlorine pesticides can stimulate receptors on the uterus leading to the development of uterine leiomyoma [57]. The preponderance of data has focused on the ability of these compounds to act directly on estrogen receptors. Initial screening, if originally negative for estrogen receptor activity, does not necessarily mean that organochlorine pesticides are devoid of cancer-stimulating properties. There are indirect mechanisms by which pesticides can influence the proliferation and function of breast tissue. One possible indirect mechanism can be through the binding to tubulin and arrest of the G2/M cycle [58].

A recent study [59] has substantiated the ability of pesticides to influence cellular proliferation. Ventura et al. [59] demonstrated that chlorpyrifos induces a redox imbalance altering the antioxidant defense system and inhibition of cellular proliferation of ERK1/2 phosphorylation. They concluded that the effect on ERK1/2 phosphorylation was not a direct effect but an indirect effect as a result of the changes in the redox state of the cell. Also, most exposures will involve mixtures of compounds. Recent work has begun to explore these effects of mixtures on proliferation of breast tissue. In addition to direct effects of the constituents on the estrogen receptor, the mixtures may also interfere with cellular proliferation [60], inhibition of androgen activity [60], and the upregulation of protein kinase genes associated with tumor development [61]. Not all effects on breast cancer cells are mediated by the genomic actions of estrogen receptors. Activation of CaMKIV pathways is structure-dependent, with estrogen being the most potent activator [62]. Similar results were observed with the activation of PI3-K, MAPK, and PKC. Select phytoestrogens such as resveratrol displayed minimal activity [62]. This would suggest that there is a structural requirement for non-estrogen compounds to elicit effects on intracellular kinase systems. Collectively, the summation of these effects would have a detrimental on cellular function.

The majority of the data in the literature has examined the effects of pesticides on either the estrogen α or β receptor (ER α or ER β). Structurally, these pesticides can be highly diverse with little resemblance to β -estradiol, the most potent of the various forms of the estrogen hormone (estrone and estriol being the other two). Yet, even with these structural disparities, the pesticides can still function as an endocrine disruptor. In the mid-1990s it was reported that methoxychlor and DDT bound to estrogen receptors and that it was potentially through this action that binding to estrogen receptors in the fetal brain, neural development was altered and as a result, the territorial behavior in male mice was affected [63]. This substantiated an earlier report using cell cultures that pesticides such as DDT, chlordecone and toxaphene stimulated estrogen receptors endogenously expressed in MCF-7 cells [64]. Interestingly, not all agents stimulate the receptor, some function as antagonists. More intriguing is that some agents are an antagonist at one subtype but an agonist at the other. It appears that the ER α is most sensitive to the actions of a variety of compounds. Pesticide exposure in mammary cell tumors that express both ER α and ER β has been shown to most effectively reduce the mRNA for ER α alone – with virtually no effect on ER β mRNA expression (with the exception of chlorpyrifos). These effects were reversed when estradiol was added to the incubation media [65]. This report was further advanced by identifying prochloraz as a potential candidate for estrogenic-like effects. In a manner similar to β -estradiol, prochloraz downregulated the expression of both ER α and ER β mRNA expression as well as reduce the expression of the ER α protein itself in the MCF-7 cell line [66]. An advancement used for the screening of compounds against actions at estrogen receptors is the use of reporter cell lines. These cells express both ER α and ER β mRNA, and express function ER α and ER β receptor protein. Kojima et al. [67] report the use of Chinese hamster ovary (CHO) cells which have been transfected with both ER α and ER β cDNA. They screened nearly 200 pesticides – and nearly 25% demonstrated activity at ER α receptors, with nearly 15% exerting activity on the ER β receptor [67]. Interestingly, they screened these compounds against the expression of androgen receptors (AR) – and their findings were that about 15 pesticides had both ER and AR activity, which

would result in endocrine disruption in both males and females. Using HeLa cells with an estrogen reporter luciferase vector into two cell lines one each with the ER α or ER β constructs permitted simultaneous screening of multiple compounds for agonistic or antagonistic activity [68]. Several pesticides (DDT, transnonachlor, chlordane, fenvalerate, and Toxaphene) were able to stimulate both ER α and ER β [64, 68]. Only a few compounds demonstrated antagonistic properties at the ER α receptor (carbaryl, pentachlorophenol, 2, 4, 5-trichlorophenoxyacetic acid), yet significantly more pesticides were shown to be able to block the activity of the ER β receptor (chlordecone, methoxychlor, carbaryl, endosulfan, endrin, dieldrin, and Aldrin) [68]. The negative effects of pesticides can be offset by interactions of the anticancer agents, 4-hydroxytamoxifen and trilostane. Both agents will block the estrogenic activity of compounds (estrogen and pesticides) at the estrogen receptor. In addition, these compounds will tend to downregulate protein kinase genes that were upregulated following exposure to pesticides [69]. Resistance may develop to the actions of 4-hydroxytamoxifen, which can be overcome with trilostane administration. In MCF-7 breast cancer cells, trilostane exposure was shown to increase the expression of ER β [69]. Upregulation of ER β may then provide additional ameliorative effects on the proliferation of breast cancer cells following the development of tolerance to first-line therapy. Collectively, the potency of these compounds was significantly less than that of β -estradiol, but continuous exposure to low levels of these compounds, or multiple compounds, may result in yet unknown consequences.

In addition to screening for estrogen receptor activity, other methodologies are being developed to further understand the actions of these endocrine disruptors. Estradiol hydroxylase activity has been examined by numerous investigators as a potential predictor of carcinogenic activity. In particular, estradiol 2-hydroxylase has received much attention as a potential predictor. Yet, the results have not been clear and the interpretation of these findings has been complicated, leading to the conclusion that estradiol 2-hydroxylase is not the best predictor of carcinogenic activity [70]. The enzyme "aromatase" is vital for the conversion of testosterone to β -estradiol. A logical extension would be to examine pesticides for their ability to inhibit aromatase (or CYP19 aromatase) activity similar to estrogen [71]. Under physiological conditions, estrogen would negatively feedback onto the aromatase enzyme reducing activity and reducing the amount of estrogen being synthesized from testosterone. Similar to the results observed in the estrogen receptor assays, fungicides such as prochloraz and imazalil inhibited aromatase activity to a greater extent than 4-hydroxyandrostendione. Other pesticides did inhibit aromatase activity but at significantly reduced efficacy and potency. Nearly 33% of the compounds tested exhibited some form of aromatase inhibition [72]. More recently, these findings were substantiated by Sanderson et al. [73]. They report that many of the compounds which may exhibit weak aromatase inhibition did so at concentrations that were cytotoxic to their cell system, R295R cells [73]. They did describe similar effects with fungicides and their effect on inhibiting aromatase activity, but it was suggested that these effects may not be through direct inhibition of aromatase activity, but through inhibition of phosphodiesterase activity [73]. Changes in human CYP19 aromatase expression may then lead to a predisposition to cancer development. One study examined a polymorphism in the CYP19 gene in a Greek population and linked this polymorphism, the population exposure to pesticides, and the incidence of breast cancer [74]. This study did not find a strong association between the short

tandem repeat polymorphism, pesticide exposure, and breast cancer development. It is obvious that there are many avenues that can be traveled for the development of breast cancer and that many of the compounds that were viewed to be promoters of tumor formation may in fact function through multiple pathways, and with mixtures of agents, the potential interactions may go up exponentially. A recent report by Sitgaard-Kjeldsen et al. [75] substantiated these conclusions. Using mixtures of pesticides, they report that the observed outcomes are mediated by alterations in ER α , ER β , androgen receptors, and aromatase [75]. Where understanding the effects of single compounds is important, more fully elucidating the combined actions of pesticides on the development of breast cancer is tantamount to completely understanding the actions of these compounds.

3. Heavy metals/metalloestrogens in the environment

Many heavy metals are naturally occurring and are used in a variety of industrial settings. Metals cannot be created or destroyed, but can change form, altering their biological availability and toxicity. Metals used in industry many times wind up in the food supply, groundwater, drinking water, and soil. Metals are found in many consumer products as well. These metals include copper, cobalt, nickel, lead, mercury, tin, chromium, cadmium, aluminum, vanadate (metal anion), antimony, barium, selenite, and arsenite. Cadmium is found in many farm fertilizers and can make its way into soil and water. Some of the other main sources of cadmium include cigarette smoke, rechargeable batteries, certain cosmetics, bread and other cereals, potatoes, root crops, and vegetables. Cadmium is a widely distributed metal used in manufacturing and present in a number of consumer products. It is used as a metal alloy, in paint, batteries (Ni-Cd), pigments, metal coatings, plastics, welding, and battery manufacture. Numerous heavy metals have been implicated in the development of a variety of cancers (arsenic, beryllium, cadmium, nickel, and hexavalent chromium, plus others). There have been an increasing number of studies which have shown that cadmium may facilitate breast cancer development. Nearly two decades ago, the presence of heavy metals as a potentially toxic addition to inorganic pesticide mixtures was addressed [76]. They report that multiple metals such as cadmium, cobalt, copper, and zinc can be found in relatively high levels as impurities in pesticide preparations. In other preparations, higher levels of lead, nickel, iron, and manganese were observed [76].

Metalloestrogens are a class of inorganic xenoestrogens which can affect the gene expression of human cells responding to estrogen. There have been numerous reviews on the subject of heavy metals as potential endocrine disruptors or metalloestrogens [77-80]. The most extensively studied of all of the metalloestrogens is cadmium. Cadmium is found in the air (ambient, occupational, and cigarette smoke). Under normal conditions, the concentration of cadmium in the air is relatively low. Occupational exposure limits to cadmium have been lowered 50-100-fold in the last few decades as the toxic effects of cadmium were better understood. Cadmium can also be found in the soil and in groundwater. Examining sources of cadmium contamination, the greatest source of human exposure is phosphate fertilizers, followed by fossil fuel combustion (automobile exhaust), iron and steel production, natural source, cement produc-

tion, cadmium-containing products, and finally incineration. Over $\frac{1}{2}$ of all of the cadmium exposure is through exposure to phosphate fertilizer and automobile exhaust. Cadmium has been found in higher concentrations in males compared to females and can affect steroid function in both genders by interfering with the biosynthesis of androgens, estrogens, and progesterone [80]. The majority of the published literature has focused on estrogen effects versus androgen effects. Dyer [79] and Byrne et al. [81] did review some of the published androgenic effects of heavy metals on testicular function. The effects of metalloestrogens are related to the physiologic function of estrogen because metalloestrogens have shown affinity for estrogen receptors. Because they can mimic estrogen, thus activating the receptor, they are considered harmful and potentially linked with breast cancer [7]. Heavy metals including copper, cobalt, nickel, lead, mercury, tin, chromium, and vanadate exhibited estrogenic activity when subjected to a variety of tests, resulting in a two- to fivefold increase in the number of human breast cancer cells [82]. In another study, two different screening systems were used to test for “estrogenicity” in heavy metals, ultimately verifying the estrogen-like activity of these metals. Antimony and barium were also implicated as being estrogenic [8]. In the Choe et al. [8] study, the potency of cadmium in eliciting estrogenic effects was second only to n-butyltin. In vivo studies which utilized a concentration of cadmium that could be considered “environmentally-relevant,” elicited numerous changes in female rats [83]. Changes were the most pronounced in estrogen-dependent tissues such as the mammary glands. In general, there was an observed upregulation in the development of the glands, increased milk production, increased receptor density (estrogen and progesterone). Collectively, all of these changes could lead to the overdevelopment of breast tissues, and the development of particular cancers. Part of the effects elicited by cadmium resembles those of effects attributed to estrogen, which has led to cadmium being considered a “metalloestrogen”. Similar to the effects of pesticides on fetal development, the exposure of the female fetus to cadmium can also result in the accelerated growth and development of breast tissue [83]. In sum, all of these changes have been associated with an increased risk for developing breast cancer.

3.1. Metalloestrogen link to breast cancer

The exposure to metalloestrogen metals has increased dramatically over the last half century. This has occurred through an increase in environmental release of these metals and an increased contamination with the food and water supply and through smoking of tobacco products. It has been suggested that these agents can stimulate estrogen receptors in the absence of estrogen, leading to a potentially negative health impact in postmenopausal women. Further compounding their health hazards is the extremely long biological half-life (cadmium is 10 to 30 years) and the ability to bioaccumulate [81, 84]. Cadmium and other heavy metals have been shown to be estrogenic, and have the ability to activate the estrogen receptor, similar to the effects of estradiol [82, 85]. In vitro assays examining estrogen responsiveness using various metals demonstrated potencies of 25-100% of estradiol [82, 86]. In vivo assays demonstrated that acute administration of metals (including cadmium) elicited classic estrogenic responses with an increase in uterine weight, hyperplasia, and hypertrophy of the endometrial lining, increased expression and density of progesterone receptors and increase in mammary tissue density [82, 86]. A recent study focusing solely on mammary developing

following endocrine disruptor administration showed that cadmium can alter normal mammary tissue development, gene expression, and that early exposure may lead to a predisposition toward breast cancer development [21]. Cadmium can mimic the action of estrogen and appears to recognize the estrogen binding site on both the ER α and ER β receptors. Some of this action is via the estrogen receptor mediated by the GPR30 receptor [85]. Via the GPR30 receptor, cadmium stimulation can activate MAPK, raf-1, and ERK1/2 [85]. The expression of estrogen receptors is also affected and results in the levels of estrogen receptor being greatly reduced [87], again mimicking the effects of natural estrogens. These, and other, *in vitro* assays have shown that cadmium is estrogenic and can mediate estrogen receptor action, as well as the mitogenic effects of estrogen receptor stimulation. Yet, there have been mixed results in other experimental systems suggesting more work needs to be performed [88, 89]. The regulation of expression and activity of estrogen receptor plays an essential role in the growth, differentiation, and prognosis of human breast cancer. Thus, the effects of the metals in living organisms have also been found to change the breast anatomy in specific ways, making it more susceptible to cancer, including an earlier onset of puberty, an increase in epithelial area, and an increase in the number of terminal end buds in the mammary gland [83]. Aluminum has been found to interact with DNA, binding strongly to the phosphate backbone of the structure under neutral conditions [90]. This suggests that aluminum could serve as a possible source of DNA damage, increasing the chances of DNA mistakes and promoting the growth of the damaged cells. It has also been shown that aluminum can interfere with cell growth regulatory processes through many pathways, including altering gene expression [77]. The correlation between breast cancer and heavy metals is not confined to a few metals, but numerous metals have been identified in breast biopsies in significant concentrations. This is a correlation not observed when compared to biopsies from healthy breast tissue [91, 92]. Similar to what has been reported regarding the correlation of pesticide exposure to breast cancer, there are some questions regarding the function of cadmium as a metalloestrogen which can promote breast cancer proliferation [93]. In the majority of *in vitro* assays, the data are fairly convincing regarding the metalloestrogen effects of cadmium. The actions of cadmium have provided the more compelling evidence of a correlation with breast cancer proliferation [84]. The affinity of cadmium for the estrogen receptor (ER α) is comparable to that for estrogen (0.4-0.5 nM), yet the actual binding site of cadmium to the estrogen receptor is yet to be determined [84]. One current belief is that ER α is sequestered in the inactive form – low estrogen or no estrogen. Once activated, the receptor undergoes a conformational change and enters the active state. Increasing the number of active ER α receptors will increase estrogen responsiveness [81]. Cadmium can block the action of estrogen suggesting that at least parts of its effects are mediated through the estrogen binding site on ER α . Molecular studies have further supported this hypothesis examining various mutations and the ability of a selective estrogen ER α antagonists to block estrogen effects in transfected COS-7 cells through interactions at the hormone binding domain on ER α [94]. Through mutation studies, potential interaction sites have been identified as cys381, cys447, glu523, his524, and asp538 [81, 94]. The actions at estrogen receptors, the activation of various protein kinases, and the increased proliferation of breast cancer cells have been replicated by a variety of investigators in a variety of assay systems. In humans, these findings are not quite as clear. Whereas some studies have suggested a correlation between cadmium content and breast cancer [91, 92], Silva et al. [93] review numerous studies where the data are inconclusive. As with pesticide exposure, the

discovery of biomarkers specific for cadmium exposure is necessary. Although the data are not definitive for being a causative agent, cadmium can be considered a risk factor for breast cancer development. Another drawback to interpreting *in vitro* data is that these studies usually involve acute exposure. In humans, exposure to cadmium results in years if not decades of low-level exposure that results in an increasing bioaccumulation and increased cadmium burden on the patient. Acute *in vitro* studies can examine receptor and nonreceptor-mediated intracellular effects in the relative short term. Real-world exposure would involve a very gradual change in intracellular signaling systems, leading to changes that may lead to indirect changes that are not being observed in the current *in vitro* experimental paradigm.

4. Combination of metalloestrogens and pesticides

There has been extensive work done examining the individual actions of heavy metals or pesticides in the environment. There has been virtually no work done examining the combined effects of heavy metals and pesticides in the environment. This relative dearth of information has left a significant void in our understanding of the actions of these compounds and their potential role in the development of breast cancer. Many of the compounds discussed in this chapter have demonstrated the ability to interact with many of the pathways associated with tumorigenesis. Interactions with the function of a variety of caspases, Akt/mTOR pathway, p53, ERK1 and 2 signaling pathways, etc. have created a clear need for extensive work in these areas. In many instances, there are no clear direct interactions, but may in fact act through various steps in one or more of these cell cycle cascades. It is quite clear that pesticides and heavy metals do coexist in the environment. There have been numerous reports outlining the effects/coexistence of these compounds in various ecosystems and at various levels of the food chain.

Current knowledge is lacking regarding the collective effects of estrogen and all of the various estrogen-like compounds. Only in the last two decades has there been increasing amounts of work examining the estrogenic effects of naturally occurring compounds as well as synthetic compounds such as pesticides and metals. What is unknown is what overall effect all of these agents will have on a person. These effects have been shown as early as following *in utero* exposure [95] or through prolonged exposure to trace levels of contaminants leading to endometriosis [96]. The basic physiological responses for estrogen are to control secondary sexual characteristics, influence metabolic activity, central nervous system effects, effects on bone turnover, and, through aromatase, interplay with testosterone. Estrogen can exert these effects by a receptor-mediated mechanism – through estrogen receptor alpha [ER α], estrogen receptor beta [ER β], and through GPR30. Once stimulated, these receptors will work through intracellular mechanisms leading to nongenomic and genomic responses, and with improving technology, we know that estrogen can regulate hundreds of genes – with the majority (~70%) being downregulated [97, 98]. Since complete pathways have not been elucidated for each of the main classes of exogenous estrogenic compound, it is not known or it is unclear whether there are additive, synergistic, or potentiating effects of these compounds when humans are coexposed. This is one of the troubling aspects of our current knowledge. We have little idea of what effects polyexposure will have on a human, and at what level or threshold will we

begin to see these effects. An enormous amount of work still needs to be done in these areas to determine the safe exposure levels, not just for the known compounds, but also for combinations of the compounds [99, 100].

Not only are the potential pathways taken by each of the estrogenic compounds highly complex, but the process of carcinogenesis is also very complex. These processes can be highly involved with many steps and processes working in concert to finally yield a malignant transformation [11]. Cancers may be single-cell in origin and with the mutation of a few genes, errors are expressed that lead to errors in replication and/or growth. In addition, these malignant/mutated cells, dependent on the environment (pollution, toxins, etc.) can cause the conversion of otherwise “normal” cells to cancerous [11]. These “gene–environment interactions” (GEI) are broadly defined as interactions between environmental exposures and specific (risk) genotypes. The term GEI refers to the joint influences of genetic and environmental factors on health and disease. Environmental exposures affect gene regulation and/or act as additive risk factors in conjunction with a particular allelic form of a gene (genetic polymorphism), influencing disease initiation and progression. GEI also entails the different effects of a given environmental exposure on individuals and the different effects of a genotype in people with different histories of environmental exposure. For an excellent review of GEI and the mechanism(s) by which GEI can lead to cancer formation, refer to Tabrez et al. [11]. Kiyama et al. [98] reported a comprehensive listing of various genes which are regulated by estrogen and reviewed their activity. They focused on the classical “endocrine disruptors,” by focusing on their cell signaling. Their studies first examine gene expression profiles, followed by cell signaling responses. The signaling pathways identified could be used as candidate toxicity pathways to monitor and evaluate endocrine disruptor action [98].

In addition to in vitro studies, there have been human studies which have addressed the concerns associated with the combinations of pesticides and heavy metals. There is a considerable burden of in vitro and in vivo immunotoxicity evidence regarding the detrimental actions of pesticides and heavy metals. Yet, as evidence and information mounts, the findings are still far from unequivocal. Between differences in study design, test subjects, data analysis, and model systems used, etc., it has been virtually impossible to develop a clear correlation between these environmental agents and incidence of disease [99, 100]. Making the correlation between low-level exposures in animal studies to the immune system altering effects observed in humans has been difficult. Also, the effects on human health of the synergistic interactions between natural, medical, dietary, and environmental estrogens have not been fully elucidated yet. There are several factors which need to be accounted for when examining the effects of environmental estrogens: 1) immune status (immunocompromised would have larger response) of the individual, 2) gender (females more responsive than males), 3) status of pollutant exposure, and 4) duration of the exposure. Exposure to the metalloestrogen arsenite in utero altered mammary gland development prior puberty [95]. There was an overgrowth leading up to puberty and a densifying of the breast tissue. After puberty, there was a clear upregulation in the density of estrogen receptor-alpha ($ER\alpha$) due to the increased and altered response of the $ER\alpha$ transcripts [95], which may lead to the increased risk of developing breast cancer. The large “ENDO Study” examined 22 trace elements and found that 19 of the 22 (86%) of the elements (mostly heavy metals) that were examined were not correlated with endome-

triosis [96]. Yet, the remaining three that did appear to correlate with endometriosis were cadmium, chromium, and copper – 3 metals that are known metalloestrogens. Additional work will need to be done to substantiate these findings and support their conclusions.

5. Conclusions

Over the past 20-30 years, it has become increasingly evident that pesticide use can have unwanted physiological effects beyond the acute exposure. Many pesticides have been banned in numerous countries as these health effects have been described. Even with banning some pesticides, there are stockpiles that need to be disposed of, and some are still used frequently in developing nations. Initial toxicology analysis has focused on the toxicity of individual compounds but this method of assessment may significantly underestimate the risk associated with these compounds when found in mixtures. Many health organizations are now calling for retesting of these agents, but with new guidelines for assessing the potential risk to human and animal life [101, 102]. Through a variety of complex mechanisms, many of these agents have been shown to interact at the estrogen receptor, both $ER\alpha$ and $ER\beta$. These effects can occur in the absence of estrogen and can potentiate estrogenic effects in many mammalian tissues, such as breast and uterus. These interactions lead to the hypothesis that particular pesticide agents – such as organochlorine pesticides – may disrupt the natural endocrine function (i.e., “endocrine disruptors”) of the organism. Interference with the reproductive systems of aquatic- and land-based wildlife may lead to dwindling populations of species of fish, shellfish, and mammals, potentially leading to their extinction. In humans, studies to establish the correlation between pesticide exposure and breast cancer has not been clear and absolute. There has been evidence of positive correlation within some ethnic populations, whereas other studies have yielded negative correlations. In most instances, these were retrospective studies and the design, subject inclusion, and the number of subjects has limited the ability to draw strong conclusions. Obviously, the effects of long-term human exposure needs further study with strict guidelines. There also needs to be a strengthening of the toxicity testing of pesticide mixtures to avoid underestimating the potential toxic effects.

Select heavy metals have been shown to have estrogenic properties and have since been referred to as “metalloestrogens.” Of these, cadmium has been the most extensively studied and appears to be the most potent metalloestrogen at stimulating the estrogen receptor. Both the affinity and inhibitory constant at the $ER\alpha$ receptor is approximately 0.5 nM, which is in order with the affinity of estrogen for its receptor. In vitro studies have shown that cadmium, and some other metalloestrogens, can elicit estrogenic effects resulting in elevation of both $ER\alpha$ and $ER\beta$ receptor densities, increased expression and activity of intracellular protein kinases, increased density of progesterone receptors, and the increased size of the uterus as well as increased development and proliferation of breast tissues. Collectively, all of these responses in the presence of metalloestrogens led to speculation that metalloestrogens may be correlated to the incidence of breast cancer. Most of the data currently available have been anecdotal, and have involved in vitro assay systems and breast cancer cell lines (such as MCF-7). In these systems, it is clear that cadmium is the most potent of the metalloestrogens at stimulating tumor development through direct actions at the estrogen receptor, as well as

intracellular effects which may be indirect but involve the activation of many signaling systems implicated in tumor development. In human studies, these correlations are not as clear. In a few studies, there was a higher concentration of cadmium in the breast tissue compared to controls, but the direct relationship with breast cancer progression was not clear. Other studies have shown no relationship between cadmium exposure and breast cancer development. One shortcoming of the in vitro studies is that they are relatively acute exposure, for a short duration. This makes it extremely difficult to draw comparisons to human exposure. The potential that low-level, decade-long exposure to cadmium (remembering that cadmium will bioaccumulate, so exposure could be just from the body burden) may result in subtle changes which increase the predisposition to breast cancer development. Many groups are now calling for additional investigation into the mechanisms by which metalloestrogens exert their effects. This additional work is critical to better understanding the actions of metalloestrogens at the estrogen receptors (through NMR or X-ray crystallography), and the interaction of metalloestrogens on intracellular signaling systems.

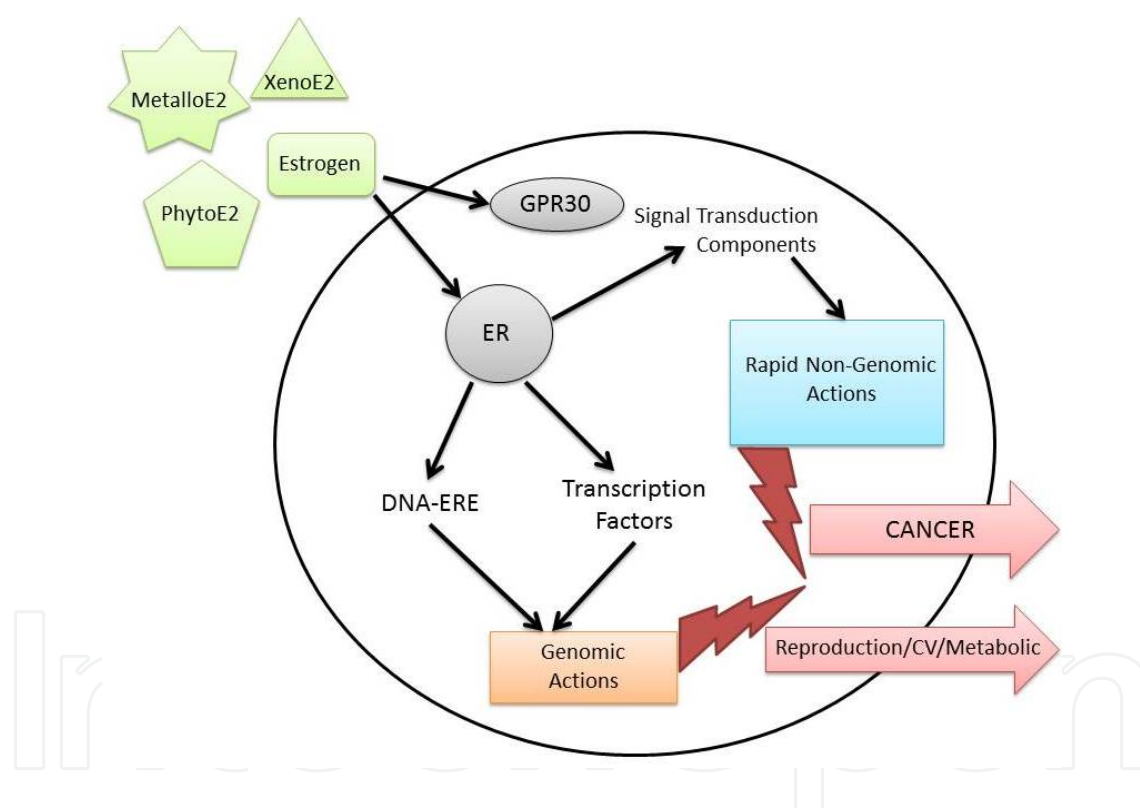


Figure 2. General schematic of the effects of estrogen, xenoestrogens [XenoE2], metalloestrogens [MetalloE2], and phytoestrogens [PhytoE2] on cellular function and the resulting physiological/pathological outcomes. Modified from Darbe, 2014 [97] [PhytoE2 = isoflavones, etc.; XenoE2 = pesticides, etc.; MetalloE2 = heavy metals like cadmium, etc.].

Lastly, to believe that an individual would be exposed to only one agent would be naïve. For example, a smoker working in the pesticide industry would undoubtedly have elevated cadmium levels due to the tobacco smoke, and possible passive exposure to the pesticides through dermal absorption or inhalation. The combination of these compounds may have a great additive effect on the development of breast cancer. Currently, there are few studies that address these types of combination exposures and virtually none involving the human

population. Collectively, as our understanding has grown regarding the negative health effects of pesticides and metalloestrogens, a significantly greater number of questions have arisen and has shed light onto obvious gaps in our understand. As much work that has been done in the last 30 years or so, even more work needs to be done in the next decade or two to assist in our understanding of the pathogenesis of diseases like breast cancer. By better understanding the root causes and foundations for breast cancer development, we will be able to focus our toxicological investigations onto those causes. Also, as we improve our understanding, both in the development of breast cancer, and the involvement of pesticides and metalloestrogens, we will be able to develop better biomarkers. It may not be impossible to completely eliminate exposure, but a viable biomarker that will predict with a high degree of reliability will help with therapeutic interventions at a much earlier time, thereby reducing the morbidity and mortality of this disease.

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