How Organic Pollutants Poison Our Health

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Unsafe at Any Level

By

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TABLE OF CONTENTS

FOREWORD

You have seen the headlines, “ALS linked to pesticide exposure,” “EPA lags on Pesticide Action,” “Safety Debate on Cosmetics Splits Industry," “Pollution is linked to diabetes,” “Hyperthyroidism in cats linked to fire retardants,” “A Fertility Study’s Warning for Dogs.” We all know that chemicals in the environment are bad for us (and our pets) but few know exactly *how* they are bad for us: few know how the chemicals act once they are in our bodies. Even so-called “safe” levels of these chemicals can impose problems for all organisms-humans, pets, and wildlife. There are many reviews and white papers available that explain which chemicals are linked to which diseases, but these reports do not address the fundamental actions of the chemicals in our bodies. The purpose of this book is not to reiterate the diseases and causes, but to explain the specific ***mechanisms*** by which the carbon-based pollutants that inundate our planet threaten the very core of what it means to be alive. It is essential to understand the mechanisms of pollutant action in order to protect ourselves, our families, and the global community.

This book is written with as little jargon as possible so that readers with a modest scientific background can understand the basic mechanisms of pollutant poisoning at the cellular and molecular level. These mechanisms are simple in concept but do require some intellectual stretching. Neil deGrasse Tyson has for many years exhorted the public at large to increase their scientific literacy. “You can’t have people making decisions about the future of the world who are scientifically illiterate. That’s a recipe for disaster. I don’t mean just whether a politician is scientifically illiterate, but people who vote politicians into office.” He also said, “Scientific literacy requires sustained engagement.” People need to keep up with scientific discoveries. And the purpose of this book is to help in that endeavor.

Folks in general are uncomfortable with “scientific talk:” a recent ad on television belittles scientific knowledge by saying that “you don’t need to know that ‘science-y’ stuff to understand nutrition.” That is just not true-scientific literacy (“science-y stuff”) is necessary to comprehend and make sense of much of our world: our understanding of the world is becoming more complicated on a daily basis as scientists unravel increasingly more

complex scientific principles. In this Information Age, it is important that people become more aware of the implications and content of cellular biology, biochemistry, climate dynamics, astrophysics and many other scientific fields that have relevance to daily lives.

Individuals need to be conversant with scientific concepts in order to make the right choices for themselves and their families. Those choices include not only food and energy options but particularly include our political choices as suggested by Dr. Tyson. We currently have a US President who says that climate change is a "hoax." The folks who voted for him clearly did not know enough to understand that his view is dead wrong. One of the Senators from my home state still argues that climate change is based on "junk science." And when I confronted the other Senator from my state about clean water issues and why the government at that time had not revisited the clean water laws for forty years, he also claimed that all the water pollutant stuff was based on "junk science." These intellectual giants were elected because voters believed them about "junk science" or just didn't care. The electorate needs to be able to judge science on its own merits, to understand the long-term implications, and to elect leaders and representatives who understand and appreciate the scientific bases of most issues.

A citizen of the world might find it helpful to know about the basic concepts for which the Nobel Prize in Medicine and Physiology is awarded each year. One does not need to know how experiments were conducted or what chemicals were used to make the discovery but might try to understand the hypotheses for which the prize was awarded. In 2015, the award went to three scientists who had discovered new chemicals to treat parasites such as malaria and round worms. The remarkable fact about their discovery is that these new chemicals/drugs not only save lives, but that the "new" chemicals were not man-made: rather, they were isolated from fungi that normally live in the soil and from sweet wormwood (*Artemisia annua*) used in herbal remedies in China. Through these discoveries, we not only have new tools to combat deadly parasitic diseases, but moreover we see that the natural world *still* has secrets to reveal to us! With just a little effort, one can understand the fundamental concepts of science and become more scientifically literate.

The 2016 Nobel Laureate discovered unique mechanisms that control *autophagy (from the Latin-auto means "self" and phagy means "eat." Autophagy = self-eating)*. Autophagy is a basic cellular process for recycling cell contents. When some parts of a cell need to be degraded and recycled,

autophagy is the mechanism by which this occurs. By digesting cellular proteins, autophagy can rapidly provide fuel for energy or building blocks for renewal of cell components. Autophagy disruption has been linked to Parkinson's disease and type two diabetes. Disturbances in the autophagic machinery have also been linked to cancer. In 2018 the Nobel Prize in Chemistry was awarded to three scientists who were able to engineer proteins (and protein antibodies) that were more efficient. These engineered proteins can be used in everyday life to fight a variety of diseases (called antibody therapy). This engineering of proteins to change their three-dimensional shape and thus change their efficacy is directly applicable to some of the things that we will discuss in this book. Information about Nobel Prizes is always published in newspapers and magazines. These accounts are usually quite accessible to non-scientific readers to help the general public understand the reasons for awarding the prize. A reader hoping to become more scientifically literate might take advantage of these and perhaps follow up their reading with further research.

The ideas presented in this book will include protein synthesis and structure. We will examine why the *structure* of proteins is so fundamental to their functioning and why their functioning is simply an exercise in three-dimensional design. Every unique protein in your body–and there are trillions and trillions of proteins in a single individual-has a distinct shape. It is the shape or structure of each protein that determines the protein's function. Some proteins function as enzymes that perform all the metabolic processes in a cell. Some protein functions are structural, some proteins act as carriers of other proteins, and some proteins act as hormones (and hormone receptors). We will discuss protein functions in more detail in Chapter Four.

We will also see how small, hydrocarbon chemicals can interfere with the structure of a large protein by attaching to tiny pockets in the protein and, thereby, changing its shape and its function. It was originally thought that the chemicals identified as Endocrine Disrupting Chemicals (or EDC's) exerted their disruption by mimicking hormone action through attaching to proteins that function as hormone receptors. This assumption was primarily due to the discovery of the effects of a man-made drug called diethylstilbesterol (DES) which was originally synthesized as an estrogen-mimic that worked through the estrogen receptor (this will be discusses in Chapter Two). In recent years, however, basic research in drug discovery labs and many basic research labs have shown that there are numerous ways in which pollutants can interrupt cellular biology through other pathways. In fact, it appears that there are no systems, endocrine or

otherwise, that are safe from disruptive effects of the chemicals in our environment. Moreover, these chemicals can disturb cellular protein components at concentrations way below the "safe" levels determined by the United States Environmental Protection Agency (EPA) and Food and Drug Administration (FDA). The question we have to ask ourselves is, what level of metabolic disturbance do we feel comfortable as labeling safe?

The chemical interactions that will be described in this book are more concerned with the three-dimensional shape of molecules than with electron distribution-more concerned with the flexibility of molecules than with oxidation and reduction equations. The chemistry that will be presented here is very simple and no equations have to be balanced. Rather, the interactions between pollutants and proteins presented here are more closely related to a picture puzzle than classical chemistry. Have you ever been working over a picture puzzle and needed one key piece-with a "pocket" over here and a "tab" over here and a "pointy piece" down here? You see a piece that might fit and with a gentle push it fits but when you step back to look at it, it is the wrong piece. It ruins the picture by introducing a nonsense component to the picture. This is the type of chemistry we will be discussing-molecules with three dimensional structures that fit together like puzzle pieces to accomplish important cellular, biochemical results but when mismatched pieces are plugged-in, a muddle ensues.

At the end of each chapter there are references to research publications. Reading these papers may be a stretch for some folks, but give them a try. They are included to reassure the reader that, in fact, the research to back-up the assertions in this book are peer-reviewed and published in reputable journals and to give extra information to those who enjoy a deeper study. Many of the references are actually review articles where an individual researcher has looked at all the peer-reviewed published work on a given topic and condensed the work to a manageable size. Some simple, cartoon-like illustrations are included in the reviews to help with the understanding of some of the concepts. Included in the additional reading sections are a few "White Papers" by the Endocrine Society of America-the most prestigious endocrine society in the world-that were written with the stated purpose of trying to educate politicians and the EPA on the severity of the problem of EDC's. Give the articles a try. Most of them can be obtained on Google-Scholar using either the regular or advanced search.

List of Abbreviations

ACTH – adrenocorticotropin hormone
AFP – Alpha Feto Protein
A, T, U, G, C – adenosine, thymidine, uridine, guanine, and cytosine nucleotide bases
ATP – adenosine triphosphate
BDE - brominated diphenyl ether
BPA - bisphenol A
cAMP – cyclic adenosine monophosphate
CoA – coactivator A
Da – Dalton
DDT – Dichlorodiphenyltrichloroethane
DES – Diethylstilbestrol
DHT - Dihydrotestosterone
DNA - Deoxyribonucleic acid
E_2 – estradiol, estrogen
EDC – endocrine disrupting chemical
EGW – environmental working group
EPA – environmental protection agency (US)
FDA – Food and Drug Administration (US)
FSH – follicle stimulating hormone
GH – growth hormone
GnRH – gonadotropin releasing hormone
HDL - high-density lipoproteins
LBD – ligand binding domain
LDL – low-density lipoproteins
LH – luteinizing hormone
MIH – Mullerian duct inhibiting hormone
NH – neurohormone
NT – neurotransmitter
PAH – polycyclic aromatic hydrocarbons
PCB - polychlorinated biphenyl
PRL – prolactin hormone
RBC – red blood cell

RNA – ribonucleic acid (mRNA – messenger ribonucleic acid)
T – testosterone
T_3/T_4 – thyroid hormones

CHAPTER ONE

INTRODUCTION: BIG PROTEINS AND SMALL POLLUTANTS

I flipped the light switch as I entered the lab. The soft hum of the florescent lights as they came on added to the buzz and purr of the equipment. I had come in early to begin a series of experiments with a new antibody I had received from a biotech company in Boston. Antibodies are big proteins produced by living organisms to protect themselves against bacteria, viruses and other harmful things introduced into the body from the environment. Antibodies are extremely specific-they "recognize" and "attack" specific targets in an exceptionally precise manner. When you get a vaccination for a specific virus like mumps, the vaccination "primes" your body to be able to make antibodies very quickly to that particular mumps virus should you become infected and it protects you from full blown infection. Antibodies are good at detecting large invaders (like viruses) but with extremely small molecules like pollutants, they are not so good.

It is to the everlasting glory of science that it has been able to use the precision of antibodies to measure and identify compounds in tissues and fluids from a variety of organisms. Moreover, immunotherapy (the use of antibodies to fight certain diseases like multiple sclerosis and cancer) is being used more frequently and has become the therapy of choice for many disorders. The antibody that I had ordered from Boston was one that bound specifically to a common growth-factor protein that I wanted to identify in a regenerating tissue my lab was studying. In order to use the antibody in an experiment, I first had to validate its specificity and precision using a series of control experiments. This would take all day, so I had come in early to begin.

I was pouring gels when Charlie came in. He waved at me across the lab and began his own work. Charlie was a Freshman work-study student who washed glassware for the entire lab. He also provisioned all the work stations in the lab with latex gloves, disposable pipette tips and pure water

for mixing reagents. He was from a small, rural town and a bit shy. He came in early each day so that the lab was ready by the time the graduate/undergraduate research students came in. I smiled at him and waved back, then returned to my work. I did a series of experiments with "control" growth-factors to confirm that the antibody worked in my hands. By the end of the day the antibody had not reacted with any of the controls. It was a complete dud!

The next day, I repeated all the experiments but still got no positive results. I called the company in Boston to complain and they assured me that it worked every time in their hands. They sent me new control growth-factors and a bottle of their buffer-in case my buffer ingredients were bad. Remarkably, the experiments worked like a charm with their control factors as well as my own. The question was, what was the matter with my buffer? After poking around the lab and asking questions of everybody, it turned out that the matter with my buffer was Charlie!

One of Charlie's duties was to fill a large storage container with distilled water for rinsing the lab glass-ware. The lab had a substantial still in which distillation of tap water took place. There was one stopcock to drain the distilled water into the storage container for dish washing. Another outlet carried distilled water to a series of filters. At the end of the filters there was a second outlet to drain the final product, the purified (or "nano-pure") water. There are many volatile organic compounds in tap water-and most of them have boiling points below that of water. Pesticides and herbicides and other volatile hydrocarbon chemical compounds found in tap water boil off before water boils, and stay with the distilled water during the condensation phase. In order to get rid of the volatile organic compounds, the distilled water has to be further treated by serial filtration. It is essential to have the purification/filtration step linked to the distillation step to produce really pure water. In our lab, we called this pure water "nano-pure water" and it was used at all the research work stations to mix buffers and reagents. Unfortunately, the nuanced differences between distilled and nano-pure water were entirely lost on Charlie and he had been filling all the nano-pure glass bottles at all the work stations with plain, distilled water. When I mixed my antibody buffer with the nano-pure water, the control reactions worked exactly as they had with the Boston buffer!

I tell this long, "just-so" story to illustrate two important facts. First, it is difficult to produce really pure water. Second, and the main focus of this book, is that small, organic molecules found in all tap water and even in

distilled water can interact with large proteins (like antibodies and growth factors) and render them ineffective. Proteins-such as antibodies, enzymes, hormones, and carrier proteins (like hemoglobin, which carries oxygen)-are large organic molecules with intricate three-dimensional shapes. The shapes of the proteins are integral to the function of the protein. Proteins are architecturally complex molecules and they often have pockets of hydrophobic (lipid-loving) amino acids. Small organic molecules are lipid-like and can attach to the hydrophobic areas on a protein. Charley helped demonstrate that a small organic molecule that remained in the distilled water was capable of binding to the antibody and/or the growth factor to change their shapes and functions so they would not recognize their proper binding partner. The pollutant could have bound to the growth-factor and rendered it unrecognizable by the antibody or to the antibody so that it shape could not recognize the growth factor. Irrespective of which way it acted, the pollutant somehow inhibited the normally precise antibody: growth- factor interaction.

Organic Pollutants

The Industrial Revolution was revolutionary in more ways than one. Not the least because it began a rapid transformation of the Earth's environment. With industrialization came industrial waste. And the world has been filling with waste for well over a hundred years. Much of the waste is man-made, petroleum-based organic chemicals which are totally "unnatural" to living organisms. In other words, compounds synthesized from industrial, man- made petroleum-based precursors for use in industry are compounds which living cells rarely or never produce themselves. Since human cells don't synthesize these small compounds, they have limited ability to efficiently deal with them. Some compounds, but not all, are altered in the human body to make them more water soluble so that the kidneys can remove them from the blood. Some of the compounds, but not all, are detoxified by special proteins produced by the liver. Most of these industrial organic compounds are small and oil-soluble; once they get past the body's innate defenses, they can be deposited and stored in fat tissue or they can nestle into fat-loving (hydrophilic) pockets found in most proteins in the body.

Organic compounds are molecules composed of carbon atoms bound to other atoms such as oxygen, nitrogen, and sulfur. If you have ever had an Organic Chemistry class, you will remember that organic molecules can be synthesized in the lab, but we tend to associate organic molecules

with living organisms and the word "organic" simply refers to compounds made up of carbon and other atoms. We are a carbon-based species-as is all life on earth. Some science fiction movies propose that other types of life are possible based on other atoms such as silica but life on earth is pretty much restricted to carbon building blocks. "Organic molecules" should not be confused with "organic food." Organic food-as is all food-is made up of carbon molecules, but the term organic food as it is popularly used refers to food that has been grown without the addition of fertilizers, herbicides, fungicides etc. Pollutants such as fertilizers and insecticides (which make up a great proportion of our pollutants) are constructed of carbon molecules also, but these small chemicals are for the most part synthesized in labs, not by living cells.

In her book, "Silent Spring" Rachael Carson stressed the *toxic effects* of various man-made organic chemical insecticides (especially DDT) on wildlife and humans. What I hope to show in this book is that the effects of these man-made organic chemicals can be much less obvious than a toxic end-point such as death or cancer. Other end points might be small interferences of metabolic events, immune system disturbances, behavioral problems or a reduced quality of life. It is suspected that many of the chronic diseases that we see today may be environmentally exacerbated by the uptake and storage of these unnatural chemicals. Some diseases can be explained (in part) by the inactivation of antibodies (as suggested above) which could lead to illness and cancer. I suggest in this book, that all non-natural organic chemicals in the environment whether they are present at high or at very low levels have the potential to bind indiscriminately to functional proteins and are thus a hazard to a fully healthy life.

In the 15th century Paracelsus (a Swiss alchemist and physician) stated that, "The dose of the substance makes the poison," implying that toxic substances could be harmless in low doses. Toxicity, however, in his view was either death or extreme sickness. The end game in Carson's book was also a "toxic" effect (either death or cancer). These are, of course, terrible outcomes but there are other consequences to being exposed to pollutants in addition to death or cancer. These other outcomes are subtle and insidious. They are harder to detect but deleterious nevertheless. What if some of the antibodies in your body that were generated to the influenza shot you got last Fall were rendered inoperative by a chemical absorbed from your lawn pesticide? Your immunity to influenza might be rendered less effective due to the effects of the pesticide. Death is not the outcome, but rather a mild case of the flu. You might blame your winter flu on a bad batch of flu shots when in fact it was due to your spraying your lawn. Your

life was never at risk, but the quality of your life was somewhat diminished. Risk assessment says that this diminution is acceptable, but the increases of certain diseases that have occurred since the onset of the industrial revolution, argues against that conclusion.

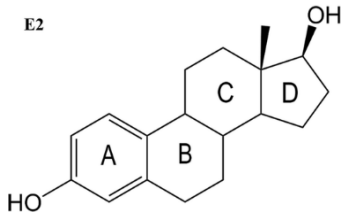

Figure 1-1. Estrogen (E_2). This steroid hormone consists of four ring structures A, B, C, and D, and two -OH groups at either end.

It is difficult to predict which of the hundreds of thousands of compounds already in the environment will interact with human proteins. The ones that do interact, tend to be small molecules and they often have ring-structures like steroid hormones. Fig. 1-1 shows a small steroid molecule with four "ring" structures and two -OH groups at either end. This molecule is estrogen (beta-estradiol or E_2: the "2" in E_2 refers to the two - OH groups on either end of the molecule). Estrogen is a natural steroid hormone found in many animals. Some environmental pollutants are said to be "estrogen-mimics." By mimicking estrogen, they are able to disrupt normal estrogen physiological reactions and pathways. Thus, such pollutants are called Endocrine Disrupting Chemicals or EDCs. The structures of some organic EDC pollutants and their similarity in shape to estrogen will be given in the next chapter.

The origins of pollutant chemicals are varied and arise from different sources in different parts of the world. Many of the pollutant chemicals found in the environment are derived from petroleum processing. The treatment of crude oil to produce gasoline generates many small carbon molecules that are subsequently used in the manufacture of a wide variety of products such as plastics, fertilizers, herbicides, fire retardants, pharmaceuticals, solvents, lubricants, textiles, electronic, and cosmetic products. Pollutants leach out of these products and are found ubiquitously in all environments. Some have short life-spans, but others can hang around in the environment for years. There is no uncontaminated water on the planet. Even polar bears have petroleum pollutant chemicals stored in their fat.

The ways that pollutants get into living organisms are multifold. Many are eaten with food which has been sprayed with insecticides, fungicides, and/or herbicides. Also, chemicals can seep into food from plastic beverage containers or the plastic linings of metal cans. Many are inhaled and absorbed into the blood system in the lungs and some are absorbed through our skin through the many personal care products we use every day (It should be unnerving to you that Vaseline® is sometimes called petroleum jelly). Pollutants can be introduced from the mother to the fetus across the placenta during gestation or to the newborn in mother's milk after birth. Small, organic molecules can cross the placenta and affect the embryo as it is developing. This route can disrupt the development of fetal organs and the expression of adult behavior. There are so many junctures at which foreign chemicals from the environment can affect humans that it is impossible to know for certain where any given entry point may occur.

Endocrine Society White Papers

The Endocrine Society White Papers (2009, 2012, and 2014) report that people and animals are exposed to ever-changing patterns of chemicals: exposure to pollutants is rarely to a single pollutant. Most exposure is to mixtures of chemicals and because of the types, patterns, and dosages of these chemicals are so varied, it is important to take into consideration the cumulative effects of the pollutants. These effects can be additive, synergistic or antagonistic. Additive is self-explanatory: when a number of folks try to lift a log by using their hands, back, and leg muscles the net effect is *additive*. *Synergistic* means that some lift the log with their muscles while others help to lift the log using a rope thrown over a tree limb. Both groups help to lift the log, but in different ways. *Antagonistic* is someone sitting on the log to make it impossible or harder to lift. Thus, a pollutant can have the same effect as another pollutant (additive), it can have the same effect through a different pathway (synergistic), or it can have a completely opposite effect from another pollutant (antagonistic). With multiple chemical exposures on a daily basis, it is extremely difficult to parse out individual effects.

Many carbon-based, petroleum-derived compounds are present in the environment in forms that are variously persistent. Jobs that are associated with some types of chemicals (such as painters, fire-fighters, farmers, paper manufacturers, coal miners, steel, rubber, and chemical workers) have higher burdens of chemical exposure and higher rates of a variety of cancers than those that are not so exposed. For example, farmers are at

greater risk for non-Hodgkin's lymphoma than non-farmers. Over 70,000 chemicals were reported by manufacturers as being in commercial use in the USA as of February 2001. Unfortunately, the EPA and the FDA have no idea exactly how many chemicals are used in consumer products, nor what products they are used in. Of these thousands of chemicals produced worldwide only a few of them have been suggested to have effects in humans and wildlife. Fewer still of these chemicals have actually been tested for disrupting effects.

In 2016, a new but anemic law was passed to reinforce the power of the US Environmental Protection Agency (EPA) in slowing the release of new chemicals into the environment until certain safety standards were met. Unfortunately, this new law was "repealed and replaced" by the current administration. The repealed law, itself, however, did little or nothing to clean up the toxic super sites that exist all over the US. The most recent administration has cancelled even that small effort and pulled out of the Paris Accords which would have made cutting back on pollutants mandatory. With the new administrative changes, the future of the EPA and its ability or sympathy to clean up any of the environment is now in grave doubt. Without federal and global commitment to clean water and air, it is difficult to see any change in our environmental situation. But things are not hopeless. There are many things we can do to protects ourselves and our society by becoming more aware of potential sources of pollutants and working to get rid of them. We need to focus on local treatment of water by water districts: but the tragedy in Flint, Michigan leaves us skeptical about that resource. Citizens have to become active in local water and run-off water treatments plans by local governments. Plus, there are things we can do at home, at our "Point-of-Use" (POU), to protect ourselves. These tactics will be described in the last chapter.

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CHAPTER TWO

EDCS, BPA, AND DES: A TOXIC ALPHABET SOUP

Since the 1990's, many pollutants have been identified as "endocrine disrupting chemicals" or EDCs. EDCs interfere with or impede the biosynthesis, metabolism, or action of hormones which are naturally-occurring organic chemicals produced by endocrine glands or tissues and found in the bodies of all animals from humans to earthworms to jellyfish. Hormones are released into blood (or body fluids) and are carried to their targets which are usually at some distance from the gland or tissue that produced and released them. Human endocrine glands include the thyroid gland, pituitary, ovaries, testes, pancreas, and adrenal glands (we will review some of these endocrine organs and their systems in later chapters).

The evolution of endocrine systems has resulted in hormones that have very specific effects on very specific "targets." The target for a given hormone is defined as a cell (or tissue) that contains "receptors" for the hormone. Hormones carry out their effects by binding to specific receptors located at their target cells and EDCs exercise their influence by disrupting those specific effects of hormones at the receptor or at other points of control.

History of EDCs

In the days between WWI and WWII a great deal of important organic biochemical research was done regarding isolation and chemical characterization of hormones (which are organic molecules composed of carbon, hydrogen, oxygen, and other atoms). In 1929 the first structure of the human female steroid hormone estrogen was published in the U.S. and Germany: it is a ubiquitous molecule, produced and used by both humans and many other animals. Estrogen is made up of three, six-carbon rings and one five-carbon ring (see Figs. 1-1 and 2-1, top). Hydrogen atoms and oxygen atoms complete the molecule. Estrogen is quite small: it is

synthesized from cholesterol which has a molecular weight of 387 Da and estrogen weighs less at 272 Da. (Don't worry about the "Da" which stands for Daltons. The Dalton is named for Sir John Dalton-1766-1844-whose atomic theory entirely changed the field of chemistry. Da is merely a way of designating the weight of molecules. It is so small that we won't worry about it! But we will use it as a way to compare the sizes of pollutants, hormones, and proteins.) The average human protein is also an organic molecule, but with an average weight of over 30,000 Da. The take home message here is that proteins are often 100X larger than cholesterol and steroid hormones. This becomes important later as we look in more depth at proteins in later chapters.

The path leading to the discovery of EDCs was paved with good intentions. In the 1930's E.C. Dodds, an organic chemist in the United Kingdom, began synthesizing compounds that would "mimic" the newly discovered female steroid hormone estrogen. He was hoping to fabricate a compound that would be easier to synthesize than the steroid itself. Ease of synthesis was crucial to drug mass production and such an "estrogen-mimic" drug would have vast therapeutic possibilities. In those days, hormone receptors were poorly understood but it was known that hormones had targets and triggered specific physiological events. Dodds synthesized a large number of compounds that looked somewhat like estrogen. He then measured the ability of each compound to mimic estrogen in a very simple "bioassay:" he injected the newly synthesized compounds into female mice. If the compound succeeded in inducing the onset of a reproductive cycle in the mice (which is under the control of estrogen), the compound was scored as an estrogen-mimic. The strength of the response was scored on how concentrated the injected compound was and how long it took the mice to enter a reproductive cycle. The lower the dosage and the shorter the interval, the more potent was the score of the compound.

Estrogen and bisphenol A

The research group in UK synthesized a large number of compounds and most of them had no effect in the mouse bioassay, but some of the compounds did have some "estrogen-mimic" activity. It should be pointed out that none of the compounds were naturally occurring compounds. These compounds were all synthesized in the laboratory. The structure at the top of Figure 2-1 is estrogen. It should be noted here (since you will see many similar structures) that such a line drawing is a shorthand for organic molecules. Each line represents a bond between two carbon atoms.

Carbon atoms can form circular, ring-like structures as seen here. You don't need to worry about this except to note that estrogen is an organic molecule which means it is composed of carbon atoms. Life on earth is formed from organic (carbon) atoms. Petroleum (and coal) are merely the products of decomposed bodies of long-ago carbon-based animals (from dinosaurs to diatoms). The fuel that is obtained from these animal-remains is also carbon-based and when burned, the single-carbon CO_2 that is released as the final end product is the last stage of the carbon saga. As you are well aware, it is the release of CO_2 into the environment that is responsible for a good deal of the climate change that we see today.

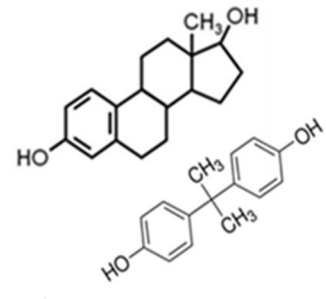

Figure 2-1. Comparison of the shapes of estrogen (top) and BPA (bottom).

One of the earliest estrogen-like compounds that the Dodds's lab produced had only two ring structures (compared to the four rings of estrogen) but it did have two "-OH" groups on each end of the molecule as does estrogen (compare the two compounds in Fig. 2-1). The new estrogen- mimic resembled the structure of estrogen but was a bit smaller. The compound at the bottom of Fig 2-1 is called 4,4'-(propane-2,2-diyl) diphenol (also known as bisphenol A or BPA). BPA was a good estrogen-mimic and it is easy to see how it could look like estrogen sufficiently to mimic the hormone. But BPA was soon abandoned when another compound with a thousand-fold more estrogen-mimic activity was found. The rejected estrogen-mimic BPA was promptly exploited by other synthetic organic chemists and became an important component in plastics manufacturing. Today BPA has many industrial uses: in 2011, an estimated 10 billion pounds of BPA were produced in the US for manufacturing polycarbonate plastic and for the lining of metal cans, making it one of the main chemicals produced worldwide. A chemical that was first synthesized to be a hormone mimic is now routinely used in most plastic products on the market. Some estimates state that human

consumption of BPAs from the coatings of metal cans alone to be over 6 micrograms per person per day. Human exposure to the estrogen-mimic BPA is “ubiquitous and nearly continual.” 93% of Americans examined tested positive for BPA in their blood. The United States-Food and Drug Administration (FDA) maintains that "BPA is safe at the current levels occurring in foods" based on extensive research, including two more studies issued by the agency in early 2014. One of the jobs of the US FDA and EPA is to establish “Safe Levels” of most organic chemical pollutants. A “Safe Level” varies from chemical to chemical, but always assumes that there are levels of contamination below which no damage is done to humans and wildlife. European agencies, however, remain concerned about the levels of BPA that might be safe. In 2016, France announced that it intends to classify BPA as a “substance of very high concern”. For more information about human consumption of specific pollutants, see the Endocrine Society’s white papers for 2009, 2012, and 2014 listed at the end of the previous chapter.

Diethylstilbestrol-DES

The estrogen-like organic compound from the Dodds’s laboratory in the UK that proved to be a 1000X-better than BPA in the mouse bioassay was called 4-4’-dihydroxy- a:b diethyl stilbene (diethylstilbestrol or DES). In 1937, when DES, was first synthesized, the research that produced it was funded by the UK Medical Research Council (MRC), which had a policy that did not allow the patenting of drugs that were discovered using public funds. DES was not patented and, therefore, was quickly produced by more than 200 pharmaceutical and chemical companies worldwide. The new DES drugs were marketed under various names in a number of countries including France, the Netherlands, Great Britain, and the US. Because of its structural similarity to estrogen and its ability to mimic estrogen in some bioassays, DES was called an estrogen substitute and could act as a drug to treat various human female reproductive problems. In 1964, Prof. Dodds was knighted for his work in synthetic organic chemistry.

As it turned out, however, DES was to become infamously known as the first widely-recognized example of an endocrine disrupting chemical (an EDC). From the 1940s until the late 1970s, DES was FDA-approved in the US as an estrogen-replacement therapy for estrogen-disordered states such as menopause and miscarriage. DES had multiple FDA-approved uses including treating prostate cancer. In 1960, DES was found to be very effective in the treatment of advanced breast cancer in

postmenopausal women. In 1977, the FDA approved another estrogen-like drug (tamoxifen) with efficacy similar to DES but with fewer side effects. (Side effects of DES and all other drugs are due to the ability of the drug to enter all cells in the patient's body in addition to the target cells and to interact non-specifically with proteins in some cells and to disrupt metabolic functions.) This "side-effect" phenomenon will become particularly important as we look at pollutants more closely. DES is a man-made compound, not produced by the human body (nor by any organism that also synthesizes estrogen). In Fig. 2-2, one can see the similarities between estrogen (on top) and DES (on bottom). Both have ring structures and the characteristic "– OH" groups on either end. DES has extra "arms" in the middle of the structure which limit the interaction between DES and the estrogen receptor, the significance of which we will discuss later.

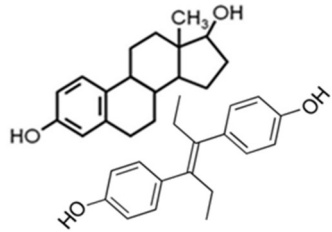

Figure 2-2: Comparison of the shape of estrogen (top) to the shape of DES (bottom)

From the 1940's to the 1970's, doctors all over the Western world prescribed DES to many millions of pregnant women who were having trouble carrying fetuses to birth. DES was thought to block spontaneous abortion and promote fetal growth. DES was used widely to help prevent miscarriages which were thought to be due to hormonal irregularities during pregnancy. In 1953, however, a randomized, controlled study showed that women prescribed DES *did not* have healthier pregnancies. Marketing campaigns by drug companies nevertheless persisted. And physicians continued to prescribe DES for miscarriages until the 70's. There was no observable effect of DES on the mothers that were treated with it, and the effect on the babies they were carrying was not actually recognized until the babies reached adulthood.

In the 1970's a cohort of young women whose mothers had been treated with DES in the 40s began presenting to physicians with a rare type of cervical/vaginal cancer. As a result of these cases, physicians began to systematically examine women and men whose mothers had been

prescribed DES while pregnant. Offspring of DES-treated mothers were diagnosed with altered or deformed reproductive systems when compared to patients whose mothers had not been treated with DES. In 1971, the FDA issued a bulletin to all U.S. physicians advising against the use of DES in pregnant women. The FDA also removed prevention of miscarriage as an indication for DES use and added pregnancy as a contraindication for use. On February 5, 1975, the FDA ordered 25 mg and 100 mg tablets of DES withdrawn.

The number of persons exposed to DES *in utero* during 1940–1971 is unknown, but may be as high as two million in the US. One-third or more of women exposed to DES *in utero* ("DES-daughters") have some identifiable genital abnormality. These include clear cell vaginal cancers, cervical irregularities, and abnormalities of the uterus. The uterus of many DES-daughters is smaller and the internal space inside the uterus is reduced in volume and has a narrowed aspect (in the form of a "T" rather than the larger urn-shaped space in the normal uterus). Men exposed to DES *in utero* ("DES-sons") have an increased incidence of testicular cysts and an increased risk of other genital abnormalities. Some of these anatomical differences included malformed external genitalia. The male urethra also shows reductions in diameter and sperm from these males has various malformations which would make them unlikely to fertilize an egg. Studies exploring the relationship of *in utero* exposure to DES and testicular cancer have yielded mixed results. Some DES daughters and DES sons demonstrate behavioral abnormalities; atypical behavior relating to sexual identity and gender dysphoria have been reported in this cohort of people. Some couples trying to start families were given the news that they would probably never be able to have natural children and the reason that these couples were infertile was because they themselves were exposed to DES years before while *in utero*.

Since reproductive system development *in utero* is under hormonal/growth factor control, DES was, therefore, the first recognized Endocrine Disruptor Chemical (EDC) because its effects were on the reproductive system. DES has become the "Proof of Principle" that exposure to unnatural estrogen- like compounds (aka EDCs) during fetal development could alter the function and pathology of various hormonal targets much later in life relative to exposure. Exposure of embryos/fetuses to DES has led to a new term, "the fetal/developmental basis of adult disease." Endocrine systems other than the reproductive system (e.g. thyroid, pancreas, pituitary, and metabolic systems) can also be affected by exposure to common EDC pollutants during fetal development.

Thalidomide

In the 1950's and 60's, another synthetic drug, thalidomide, was prescribed as a sedative and as a cure for "anxiety, insomnia, gastritis, and tension." It was used to lessen nausea and to relieve morning sickness in pregnant women. When the drug was used in pregnant women, some newborns showed profound developmental malformations. Many babies whose mothers had been prescribed thalidomide while pregnant were born with missing or deformed legs and arms. Correct development of limbs during embryonic development is a complicated process that involves hormones and growth factors that must correctly interact in a systematic manner with receptors. In the case of thalidomide, the disruption in the fetus was to normal limb development in contrast to disruption of reproductive development as seen with DES.

These *unplanned experiments* using humans, has demonstrated that drugs given to the mother can cross the "placental barrier." These tragic results signaled to the scientific community that non-natural compounds can cross the placenta and interact with developmental systems of humans to disrupt normal fetal development.

Tamoxifen

DES was used to treat advanced breast cancer until 1977, when the US- FDA approved another selective estrogen receptor "modulator" with efficacy similar to DES-this new drug was called tamoxifen. In Fig 2-3 the shape of tamoxifen (on bottom) is compared to estrogen (on top). The similarity in shape to estrogen is evident, but tamoxifen lacks the "-OH" group on the right-hand ring. However, the -OH group on the left-hand side of tamoxifen is absolutely necessary for the molecule to bind to the estrogen receptor. It also has a rather clumsy-looking arm (a ring-with-side-chain) extending down from the center of the molecule. The side-chain apparently results in an ungainly fit of tamoxifen for the estrogen receptor and will be discussed further in Chapter Ten. Tamoxifen inhibits the estrogen receptor: that is, it prohibits the native hormone from binding to the receptor and is incapable of binding to the receptor like the native hormone, it thereby inhibits receptor stimulation without mimicking the effect of estrogen. The receptor has no way of differentiating between an inhibitor or the native hormone. The receptor simply binds to any molecule that "fits," and that single -OH group at the left-hand side of the rings seems to be an essential part of this molecule that fits into the estrogen receptor.

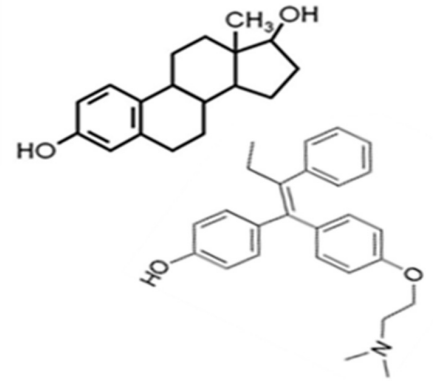

Figure 2-3: Comparison of the structures of estrogen (top) and 4-hydroxytamoxifen (bottom)

Non-human EDC disruptions

Scientists working on non-human animals during the 1980's and 90's, began publishing field observations of strange things that were happening to the reproductive systems of organisms in the wild. Most vertebrate wildlife rely on estrogen and testosterone to regulate reproductive system development and function. Phenomena were being observed in some animals that pointed to significant adverse effects of pollutants on endocrine-controlled developmental events. In the 1990's it was reported that alligator populations were dropping in Lake Apopka (Florida). This drop was attributed to significant changes in the levels of circulating estrogens and testosterones in the Apopka alligators when compared to control alligators in other lakes. Levels of DDT and other chemicals were high in the lake water in which these animals lived. The source of the chemicals was traced to an upstream factory that released a wide range of waste chemicals into the stream that fed the lake. Penises of the adult males in the contaminated lake were under-developed. These alligators with small penises were unable to successfully copulate and this resulted in a decrease in the numbers of alligators in the population. It appeared that the sexual development of the alligators *while still in the egg* was disrupted in the presence of the lake pollutants. About this same time, others reported that a widely used herbicide called atrazine when applied to tadpoles, acted to stimulate the enzyme that converts testosterone to estrogen, thus increasing estrogen in mature African clawed frog. Because of the increased estrogen levels, many of the frogs grew both testis and

ovaries which resulted in a decreased ability to reproduce, and finally in reduced populations of frogs. A reduction in reproductive ability in any animal species is a truly existential problem.

The early observations of the effects of DES and other synthetic compounds and the evidence from natural populations of animals, strengthened the argument that exogenous chemicals/hormone-like compounds could 1) cross the placental barrier during fetal development, 2) have adverse effects on developing fetuses (or in the case of alligators and frogs the effects were on eggs and juveniles), and 3) have effects that might not express themselves until later in life as decreased reproduction potential. The control of sexual development that is so profoundly disrupted by DES will be discussed later, leave it for now that all human embryonic and juvenile development is under hormonal/growth factor control.

EDCs and Humans

There is a growing record of peer-reviewed publications that has emerged over the past 20 years, that reports the testing of various compounds for their ability to disrupt endocrine functions. Not all of the EDCs are classified as estrogen-mimics, but a large number of them are. It has been predicted, based on the high degree of similarity between steroid hormone receptors that all such receptors are potential EDC targets. Here is a small sampling of chemicals that are identified as human EDCs:

PCBs and Dioxins: Synthetic chemicals (109 related chemical substances) used as industrial solvents/lubricants (and byproducts-biphenyls PBB's), and for bleaching paper products**.** Prior to 1970, polychlorinated biphenyls (PCBs) were extensively used in industrial manufacturing. They have been subsequently phased out of use, but remain a problem since they can remain intact in the environment for up to ten years. And they can stay in the human body for very long periods of time. The majority of Americans have measurable levels of PCB's in their blood. **BPAs**: We discussed this estrogen-mimic above. Bis Phenol A is a plastics additive. Also used in epoxy resins (can linings) and thermal paper. **PAH**s: Polycyclic aromatic hydrocarbons-1,3-Dimethylbutylamine used as fillers for dietary supplements. **Plasticizers**: phthalates used to strengthen plastics used in flooring material and many other uses. **Pesticides and Herbicides:** methoxychlor (MXC), chlorpyrifos, dichlorodiphenyl trichloroethante (DTT, Aldrin, Dieldrin, Endrin, Endosulfan II, Chlordane), and related compounds. **Fungicides**: vinclozolin is used on crops, in textiles and to

clean water towers. **Certain pharmaceuticals**: Such as diethylstilbestrol (DES) discussed above. **Chlorobenzene compounds:** Penta- and Hexa-chlorobenzene (HCB). **Organotins** (*Tributyl tins-TBTs*): TBT variants are registered for use in the United States as active pesticide ingredients and are used in marine paint to reduce biofouling. They are also used to control schistosomiasis in various parts of the world. **Perchlorates**: Oxidants used in rocket fuel, fireworks, airbags, and ammunition. **Flame retardants**: Penta-, octa- and deca- brominated diphenyl ethers used in polyurethane and electronics (BDE's such as Firemaster 550). Most of these EDC compounds can be isolated from human blood.

The structure of some EDC pollutants is shown in Figure 2-4. Compare their structures to that of estrogen given above. These EDCs are all organic compounds, synthesized in laboratories from petroleum-derived precursors. Some have chlorine atoms attached. The individual molecules are small enough to move into cells, similar enough to attach to steroid receptors, and hydrophobic enough to be attracted to hydrophobic regions of proteins other than receptor binding sites.

The Environmental Working Group (EGW) reports, "Most of these chemicals are perfectly legal under the Safe Drinking Water Act and most state regulations, but well above levels authoritative scientific studies have found to pose health risks. What's more, the Environmental Protection Agency has not added a new contaminant to the list of regulated drinking water pollutants in more than 20 years. This inexcusable failure of the federal government's responsibility to protect public health means there are no legal limits for the more than 160 unregulated contaminants detected in the nation's tap water." There are naturally occurring cellular and serum enzymes in humans that can metabolize some of these pollutants, but it is not clear how effective they are in protecting the body from the onslaught of so many different EDCs. The normal substrates for these pollutant-metabolizing enzymes are unknown and the fact that they can metabolize pollutants is accidental.

EDCs have been linked in either a causal or an epidemiological relationship with many human disease states. The number of EDCs per body weight (called "body burden") of new mothers correlates with 1) lower IQ scores, 2) attention deficit disorder, and 3) some motor deficiencies in offspring. In females, the accumulation of EDCs has been associated with or linked to female tract malignancies, abnormal reproductive structures, endometriosis, miscarriages, precocious puberty (including premature breast development), uterine fibroids. Some children