Environmental Toxins and Men's Health

In a book about men's health, toxins may seem to be a topic of interest to a handful of readers—but in reality the information presented in this chapter informs many aspects of men's health, from prostate cancer to reproductive health and aging. These topics are only a selection from a wide field that plays an increasing role in many health scenarios, and as industrialization and worldwide pollution continue, an understanding of the impact of environmental toxins on men's health will prove to be increasingly relevant and necessary.

Where do environmental toxins come from?

During the early 1900's chemical manufacturing began to accelerate, and with the onset of World War II the need for the latest supplies and technologies supported the boom of this new industry. After the war, the world changed, and synthetic chemicals quickly became staples in our everyday lives. We welcomed the newest chemical products into our homes because they made chores cleaner and easier; eliminated those nasty odors; and left our hair and clothes smelling fresh. These chemical technologies have reshaped our society: we have eradicated diseases, escaped the dangers of infections, and become resistant to food scarcities by industrializing farming with pesticides, herbicides and fertilizers. Our economic growth expanded as a direct result of the chemical industry, where the newest polymers and coatings supported the most impressive technological developments from planes and rockets to computers and microchips. While advancements in science and technology brought many innovations, they also yielded dynamic changes in food production so that our water is now being delivered to us in plastic bottles and sold to us at 10,000 times its value and our food grown with pesticides, processed in factories, and packaged with preservatives and plastics. The net result of these many changes is that our children are now born with over 300 toxins in their bodies.

To arrive in our world of convenience has been a feat of intelligence, fueled by imagination and entrepreneurship. But in the process we forgot to ask whether the chemicals we were creating might impact the world in ways we didn't intend. As a result, the chemicals we now know of as pollutants and toxins, were indiscriminately added to our ecosystems, at unprecedented rates before we knew of their biological activity. We created these chemicals with ideas and math; relied on them for our engineering efforts, and we assumed, or perhaps chose to believe, that they were actually inert and therefore safe. As the chemist in the famous petrochemical promotional video of the 1950's demonstrated by pulling tires and Tupperware and toothbrushes from an Erlenmeyer flask—chemicals equal a luxurious life, and this maxim seems to have proven true. Today, curiously, the complacent attitude of the 50's remains—skin cream is just skin cream and pesticides only affect the insects.

In reality, the contamination is now ubiquitous. Our air is laden with particulate matter, imbalanced by the excessive burning of fossil fuels, and laced with nickel, lead, and mercury. The biogeochemical cycles that govern ecosystems have shifted, altering the abundance and diversity of species. The water we run from the tap has been treated to remove sewage and microbes, but the filtration only removes some compounds, while pharmaceutical drugs, heavy metals, and chlorinated compounds still make it into our homes and bodies. The water that comes from the showerhead still contains compounds that volatize with the steam and our shower curtains add to the toxic sauna by leaching phthalates into the air. In our yards we find the remnants of chemical waste because ,years ago a factory workers watched idly as chemicals drained from their pipes and disappeared into the soil, and now your children find it in the vegetables growing in your garden. We are exposed.

In some places the contamination is extreme enough to warrant government action, such as at US superfund sites, which require government mandated and supervised cleanup. The designation of the Hudson River as a superfund site has resulted in long-term dredging projects to remove sediments contaminated with polychlorinated biphenyls (PCBs) that were dumped

between 1940 and the 1960s and which still contaminate fish and make their consumption dangerous. This type of contamination occurs world-wide. Fish at the top of the food chain in oceans across the world demonstrate high levels of mercury and PCBs. In the Arctic, a region nearly free of industrial activity, air and water patterns coupled with bioaccumulation have resulted in some of the most highly contaminated fish and people in the world.

Slowly, over the last fifty years, we have begun to question the safety of these chemicals and the contamination that has resulted from our rapid and blind industrialization. We have found that many things can and should be considered toxic. But what does that mean? The answer to this question is complex, because toxins work through a variety of mechanisms to induce a variety of effects. Toxins act at the macro level, influencing organ systems and hormonal regulation; they also act at the cellular level by altering cell signaling processes and the behavior of biomolecules such as enzymes and membranes; they continue to act in the nucleus where they bind DNA, damagjng its structure and corrupting its data; they interact at the epigenetic level, tinkering with our nervous system that determines whether the DNA is read and transcribed into proteins.

There are neurotoxins which alter brain chemistry; there are endocrine toxins which interfere with hormone signaling; there are reproductive toxins that injure offspring and lead to malformations or pregnancy loss; there are immunotoxins that result in autoimmune diseases; there are nephrotoxins, liver toxins, and intestinal toxins. There are toxins that are carcinogens.

In short, toxins can interact with our bodies at every level and can produce or contribute to the progression of many of the diseases we encounter.

This information can be overwhelming. Although the picture is bleak, the information here is not intended to scare but to inspire in our readers the realization that our world is *chemically active*. There are many choices that can be made that will reduce toxic exposure and which may improve quality of life. But the field of toxicology is still uncovering new toxins and

how they work, and so doctors may be faced with environmentally caused symptoms and no clear toxic source. Anyone interested in using this information, must understand that identifying a toxic source may mean thinking like a toxicologist and sometimes imagining that something we have been told is inert, like plastic, is actually chemically active and capable of interacting with our chemistry.

Types of Evidence in Toxicology

In toxicology there are multiple types of evidence that can be used to identify a toxin, understand its mechanisms, and see its effects. The field is diverse and often in order to understand the full picture it is necessary to work with various types of evidence.

Most relevant to doctors are human studies which include epidemiologic studies and intervention studies. In general, epidemiologic studies collect information about people with certain diseases and suspected environmental toxins. They may evaluate clusters of cancer occurring in one town that point to a contaminated water source; they may assess the historical occupational exposures of individuals with a certain disease, such as farmers with prostate cancer; they may obtain samples from individuals and evaluate the influence of one or a number of parameters on that sample such as semen quality and number of cigarettes smoked per day. These types of studies are useful in establishing linkages between exposures and health effects.

However, there are many limitations to human studies. Many studies utilize small sample sizes that may not accurately reflect larger trends in populations and therefore may produce conflicting results across studies asking the same or similar questions. Another limitation is that it can be difficult to control for other variables in human studies, and while epidemiology has designed various systems to control for these variations, such as matched controls, approaches towards establishing such controls may only rely on a few factors such as age, alcohol consumption, and family history of heart of disease. As a result, many human

studies have confounding variables that are not fully explored and which may make results unreliable.

Animal studies offer a more controlled format through which to assess the influence of a toxin on the body system as a whole and provide a way to observe whether ingestion of a toxin results in clinical disease such as bladder cancer or diabetes. Animal studies are useful tools for observing the effects of toxins across generations, an area of research which is receiving increasing attention as the science of epigenetics explodes and transgenerational effects of toxins are becoming increasingly evident. The main limitation is that rodent physiology is not a direct analogue for human physiology—mice have endogenous levels of vitamin C that make them resistant to certain toxins, are sensitive to stressful conditions and can be set off menstrual cycles through irregular light cycles or excessive handling. These differences coupled with the obvious genetic differences between humans and rodents prevent the systems from being directly translatable. This lesson was learned most painfully when DES (diethylstilbesterol) tested safe for pregnant rodents but resulted in devastating post-natal outcomes for the children of women who took it during pregnancy.

In vitro systems utilize immortalized human cell lines derived from a single donor. Cell lines may be derived from cells of a specific tissue type—such as bronchial epithelial cells infected with adenovirus 12-SV40, which immortalizes them so that the innate limits of cell proliferation which send cells into senescence after a certain number of passages do not apply, allowing longer experimentation than would otherwise be possible. Other cell lines are derived from tumor samples or from specific cancers. Cancer cells have already broken the barrier of senescence making them ideal candidates for long-term lab experiments.

In vitro experiments are useful because controlling numerous variables is possible, as is testing a wide number of toxins or potential toxins that may lack exposed human communities. *In vitro* testing is the first line of testing that occurs when a drug is being vetted for its safety and is often followed by some form of animal testing. While the system is widely used it also

presents a number of limitations. First, the cells are immortalized—meaning they cannot enter replicative senescence and in turn, a major part of their normal biology is in the "off" position. It just so happens that cancer cells are themselves usually immortalized, so it can be said that these cell lines are artificial and also partially biased towards the discovery of carcinogens. The process of cell culture may also represent inaccurate levels of exposure to a toxin. For example studies that maintain soluble compounds in the media are artificially creating an exposure that may be highly unlikely in a real-world setting because soluble compounds are rapidly cleared from tissues. Thus the dose is artificially elevated beyond an environmentally relevant level, thus the need for a relevant dose is an important concept in every toxicology study.

Dose makes the poison

Understanding dose is critical to understanding the toxicity of a substance. A primary dictum in the field concerns this topic: "the dose makes the poison." Without information about dose it is impossible to assess whether the substance is acting as a toxin.

This idea is most popularly understood through the concept of an overdose – a situation in which the prescribed amount of a drug is a quantity that the body can handle, but a larger amount of the same drug overwhelms the natural detoxification systems and leads to a toxic response. Here the toxin would be acting along a linear or curvilinear dose-response curve where increasing concentrations leads to an increasing response and the eventual crossing of a toxic threshold. This is the canonical response and may apply to a toxin such as thallium, but there are other patterns of response that exhibit U shaped response curves with high levels of stress response at low and high doses. The traditional notion that a large dose is required for a toxic response is no longer accepted, because we are learning that many compounds are more effective toxins at low doses. Low doses may not result in cell or organism death, but low doses can impact cell signaling pathways, DNA integrity, and epigenetic markers – and in fact low doses are more likely to lead to disease than high doses. High doses may inflict so much damage to a cell or an

organism that death will ensue, but it is only when the toxic insult is capable of being survived that something like a DNA mutation or an epigenetic change can be propogated and alter the identity and function of a cell to something more like a cancer cell.

Low doses then represent a danger in the context of chronic disease and diseases which take many years to develop such as cancer. Moreover many of the body's regulatory systems require endogenous chemicals at extremely low concentrations – they're called hormones. Bisphenol A is a chemical found in plastic that is a hormone mimic and as such, demonstrates efficacy in inducing responses at low concentrations . Evidence now points to a change in the paradigm where low doses over a long period of time may represent some of the most dangerous forms of exposure. Thus to fully understand the impact of dose we must also know the duration of exposure.

Duration of exposure

In the context of environmental medicine, duration is a critical component because for many toxins exposure is a life-long event. Contaminants in drinking water, ambient air (indoor leaching from paints, construction materials, and furniture), outdoor air (industrial pollution, car and plane pollution), food (pesticides, processing and packaging), and cosmetic and beauty products constitute daily sources of toxic exposure. These exposures occur everyday for years—and although no lab rat and no person will drop dead from using skin cream and no tumor will develop at the site of application, that doesn't mean something significant is not happening. Repetitive forms of exposure can chronically stress endogenous systems of maintenance and repair and they can also modify gene expression in favor of a profile that promotes carcinogenesis. Therefore an important question to keep in mind when evaluating toxicological studies is the following: are the dose and exposure time environmentally relevant? For example, studies that assess toxicity of a water contaminant based on a single exposure are irrelevant.

Moreover, in the real world, exposures to multiple toxins are occurring simultaneously — and while the toxicity profiles of many contaminants has been established, their effect has often only been evaluated on an individual basis. We have little information regarding the role of a mixture of chemicals; their interactions and their mutual support for each other in promoting toxic effects may not only be additive, but could also be synergistic.

Timing

Investigations that seek to link certain toxins with certain diseases may not find results even when the two are linked. For example, if a study assessed the effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on sperm health and did so by measuring the TCDD in semen, they would find no association. That's because TCDD can only affect sperm health during key developmental periods early in life, but when it does affect sperm health, it does so with a lasting impact. Timing is an important consideration for exposures that occur during sensitive windows of development such as *in utero* or during pre-pubescent periods of development. During these time frames there are critical windows of sensitivity where an inappropriate input can alter the epigenetic patterns and the developmental course of the cells. This type of change can lead to life-long consequences for health.

Mechanisms of Actions

Much like drugs, toxins can act through a variety of mechanisms. The impact of the toxin is dependent on many factors, including the type of toxin, the dose, the route of entry, the distribution of that toxin, and whether the toxin can reach its target molecule. The mechanisms of action for toxins are as vast as the biochemical interactions occurring in the body. Toxins may mimic substrates, inhibit enzymes, block calcium channels, overwhelm detoxification systems, alter DNA methylation, interfere with signaling cascades, inhibit transcription of key proteins or promote transcription inappropriately, inhibit DNA repair, damage DNA, and bind to hormone

receptors. Thinking broadly about these mechanisms is useful because it points to the fact that literally anything can be a toxin at the right dose, the right duration, and the right time.

Thinking for yourself

As you can see, assessing the toxicological activity of even a single substance is a complex task, requiring years of study before a full understanding of the mode of action can be ascertained. Furthermore, due to a lack of sufficient legislation, most chemicals are never tested for their safety. In 1976, the Toxic Substances Control Act (TSCA) was enacted with the intention of controlling risks from chemicals on the market. However, the thousands of chemicals already on the market were grandfathered in, under the assumption that they were safe. Most chemicals that currently enter the market undergo no hazard testing, and the EPA has no regulatory authority to demand such testing. Actions that seek to regulate chemicals can only do so with compelling proof of unreasonable harm—a standard that has been enforced so stringently that only 5 chemicals have been regulated in any way since 1976 (Denison 2009). In contrast to the testing batteries required for new drugs, these regulations are practically moot, although in reality there is little difference between a pharmaceutical and a chemical as both are ingested on a regular basis, be it in a pill or through air, water, or food: chemicals are chemicals.

Therefore, it is important to remember that simply because we lack definitive proof that a given substance is a toxin does not mean that it is not a toxin. Currently, there is a movement among some toxicologists to implement a precautionary principle wherein suspected toxins are treated with the same caution as proven toxins—so that while the necessary evidence is accumulated to ban a substance, unnecessary harm does not occur.

Toxins specific to Men's Health

Endocrine Disruptors

A major topic in this section will be the role of endocrine disruptors in prostate cancer and sperm health. These are by no means the only toxins relevant to the discussion, but they are a major concern and deserve attention by way of an introduction.

Many of the chemicals that have been released into the world since the 1940's are, or are suspected of being, the type that interact with hormone signaling systems. These chemicals are termed "endocrine disrupters" (ED) and have been the focus of much research in the field. The Endocrine Society defines an endocrine disrupter as a "a compound, either natural or synthetic, which through environmental or inappropriate developmental exposures alters the hormonal and homeostatic systems that enable the organism to communicate with and respond to its environment." (Endocrine Society 2010).

Many of the factors that we have already discussed influence the effect that an ED will have on the system. The age at exposure will strongly influence the impact because people are most vulnerable at times when the sexual organs are developing, when they are most sensitive to small alterations in the levels of circulating hormones. Critical time periods include *in utero*, in pre-pubescence, and the beginning of puberty. The effects of these exposures are not noticed until later in life, when problems involving fertility or cancer manifest. Because many relevant exposures occur during childhood, this latency period often makes it difficult to connect exposures to health outcomes. Additionally, dose response dynamics are distinct from those toxins where more means more toxic. Under normal circumstances, hormones act at extremely low levels, and therefore EDs can present dangerous outcomes at extremely small doses as well..

Mechanism of Action

Originally, the mechanism of action for EDs was thought to consist of an interaction between the nuclear hormone receptor and the hormone mimic, however current research shows that the interaction may actually include a broader range of receptors including nuclear steroid

HR, nonsteroid receptors, orphan receptors, and interactions with enzymatic pathways involved in steroid biosynthesis/metabolism.

One ED may interact with multiple receptors; while the focus here will be on interactions related to the androgen receptor and estrogen receptor, EDs may also interact with the glucocorticoid receptors, thyroid hormone receptors and other receptors which alter metabolism and promote the development of insulin resistance and obesity. Additionally, changes in hormone balance that occur with aging in men may be augmented by the influence of EDs.

Toxins affecting the Prostate Gland

Prostate Cancer

Despite the high prevalence, the mechanisms of carcinogenesis surrounding prostate cancer have yet to be fully elucidated, and its development appears to be a multifactorial process. While prostate cancers have a demonstrated sensitivity to androgens, and tumor growth often slows with androgen deprivation, circulating levels of androgen hormones lack consistent epidemiologic evidence supporting their role as a causative factor in prostate cancer. Similarly, there is also a lack of evidence supporting the role of circulating estrogen levels and the development of prostate cancers, despite the presence of higher estrogen levels in prostate cancers and the protective role of antiestrogens. Debate over the role of hormones continues because as a proliferative disease these cancers tend to respond to hormones with increased growth. However, the role of hormones may be more difficult to fully understand than simple correlations between circulating levels and prostate cancer outcomes. Hormone levels present *in utero*, during early childhood and in prepubescent growth may be most relevant to the development of prostate cancers. Within this area, questions of endocrine disrupting toxins and their role in male prostate development and health come to bear on the discussion. While the evidence is not definitive, there is a substantial weight to suggest, at minimum, the involvement of toxins in prostate cancer progression and

severity. In turn those looking to avoid prostate cancers should exercise caution and reduce their exposure to endocrine disruptors until their safety can be proven.

Farming

The strongest link between environmental exposures and prostate cancer can be found in the occupational exposure of farmers and their families, who are exposed to high levels of pesticides throughout the lifetime. Numerous human studies have demonstrated an increased risk for prostate cancer among farmers in North America. There are conflicting reports between studies, with some reporting a modestly increased association and others reporting no association. Difficulties in attaining consistent results may occur because farmers are exposed to a large group of chemicals that likely vary in dose, duration, and mixture across studies. If only a few are highly active with respect to the prostate, then conflicting results are expected.

Farming has been associated with an increased risk of mortality from prostate cancer (Morrison 1993), an increased risk of prostate cancer for those with a family history with exposures to clorpyrifos, ,coumaphos, fonofos, phorate and permethrin, butylate (Alavanja 2003; Mahajan 2006); and an increased risk of prostate cancer for Caucasians (Meyer 2007). Epidemiologic studies examining prostate cancer's relationship to single pesticides have yielded more concrete findings, including a direct link between the fungicide methyl bromide and prostate cancer risk (Alavanja 2003). Here the mechanisms of action are unknown, but it is suspected that such exposures interfere with hormone metabolism in the body, leading to abnormal steroid levels. The pesticides chlorpyrifos, fonofos, coumaphous, and phorate are suspected of interfering with the functioning of p450 enzymes which metabolize hormones in the liver. The disruption of these enzymes would result in increased levels of circulating estrogens.

Toxins affecting Tumor progression

Some EDs may help prostate cancers to form and/or progress. Hormonally dependent tumors deprived of endogenous hormones may appear to have entered hormone-independent states of growth, and be deemed androgen independent, when in reality they are utilizing toxins with endocrine activity to stimulate growth. The following section provides evidence suggesting that various toxins work by stimulating hormone-mediated growth in prostate tumors. Clinically, these findings may be relevant for individuals who have already been diagnosed with prostate cancer or for men who are looking to take preventive steps.

Bisphenol A

Bisphenol A (BPA) is an environmental estrogen capable of interacting with the estrogen receptors, ERα,ERβ and GPR30 as well as with mutated forms of the androgen receptor (AR). *In vitro* studies have found that BPA is capable of interacting with mutated ARs found in prostate cancer cells and that such interactions may promote the therapeutic resistance of some forms of prostate cancer to androgen deprivation. It has been shown that tumor-derived mutant forms of AR are responsive to the noncanonical ligand binding with BPA (Wetherwill 2005). In this study, environmentally relevant doses of BPA stimulated prostate cancer cells with mutated receptors to proliferate and bypass cell cycle check points, facilitating androgen-independent growth and suggesting that tumor recurrence in patients receiving androgen deprivation therapy may be facilitated by exposure to BPA (Wetherwill 2005; Wetherwill 2006).

BPA exposure typically occurs via hard plastic water bottles and the epoxy resins found in tin can linings. See Tip #4 for exposure reduction information.

Cadmium

Cadmium may also play a role in the progression of tumor development. An *in vitro* study with prostate cancer cells demonstrated that cadmium treatment can lead to the development of androgen independence and an estrogen hypersensitivity marked by increased

levels of estrogen receptors and hyperproliferation in response to E2(Benbrahim-Talla 2007). In rats, oral exposure to cadmium induced prostatic tumors and preneoplastic lesions and also promoted the growth of pre-existing tumors (reviewed in Waalkes 2000).

Cadmium exposure can occur via cigarette smoke and contaminated food sources.

Arsenic

Arsenic can also interact via the estrogen receptors. Epidemiological evidence from both the US and Taiwain (Chen 1988; Lewis 1999) demonstrates that exposure to arsenic may increase prostate cancer mortality. Increased rates of mortality may be due to the progression of prostate cancers towards androgen-independence via arsenic exposure. *In vitro* studies involving arsenic transformed prostate cells support this hypothesis and demonstrate that arsenic can promote androgen independence, which may be linked to activation of Ras signaling that enables activation of the AR in the absence of androgens (Benbrahim-Talla 2007).

Arsenic exposure occurs via drinking water. Certain regions of the United States, such as Vermont, are known for higher levels of arsenic in water. Patients may be advised to test their drinking water to determine levels of arsenic, utilize a reverse osmosis filter, and increase consumption of methyl donating supplements, folate, and B12 to aid in arsenic metabolism and excretion.

PCBs

Polychlorinated biphenyls (PCBs) are a group of synthetic chemicals that were banned in the 1970's but whose high persistence maintains PCBs in active circulation in estuarine environments. Primary human exposure to PCB's occurs through the consumption of contaminated fish. Exposures increase generally with respect to fish size, as larger fish have the opportunity to bioaccumulate more PCB's over their lifetime. PCBs are highly lipophilic and the highest concentrations of PCBs are found in the fatty tissues of fish.

Many PCBs demonstrate estrogenic, anti-estrogenic, and dioxin-like properties depending on the specific congener, where congeners vary based on molecular orientation and level of chlorination. Several human studies have a found an increased risk of prostate cancer with exposure to PCBs. One study evaluated the levels of different persistent organic pollutants in adipose tissue of individuals with prostate cancer and found an association between levels of PCB153 and trans-chlordane and the occurrence of prostate cancer (Hardell 2006). Another study evaluating serum PCB levels found an association between PCB180 and prostate cancer (Ritchie 2003).

Exposure to PCBs primarily occurs through the consumption of seafood. See Tip 5 for information about selecting seafood with lower levels of PCBs.

Reproductive Toxins for Men

Over the last 50 years there has been a global decline in sperm counts occurring at the rate of about 1% per year and largely impacting developed regions such as North America and Europe (Swan 2000). Trend analyses of this process has led to controversial statements about the role of environmental toxins in the decline. The purpose of this section is to highlight the direct link between toxins and sperm health, which can be measured both by the number of sperm cells, and by indices influencing pregnancy rates, including sperm motility and DNA damage to sperm. There is a substantial amount of epidemiologic evidence that links concentrations of particular toxins found in urine and seminal fluid to particular changes in sperm health – these studies will be reviewed here and collectively point to the growing, yet still under-recognized importance of men's preconception health. Living in an industrial world results in a panoply of environmental exposures which may influence sperm health and that can act through a variety of mechanisms to do so. Some toxins may be endocrine disruptors, while others may influence sperm health *in situ* as they alter the environment of the seminal fluid, creating adducts and DNA damage or leading

to increased oxidative stress. These effects can influence the process at multiple stages, including increasing time to pregnancy, increasing spontaneous abortions, and even leading to birth defects and childhood cancers.

From a clinical perspective, the toxins outlined here are relevant to those couples having difficulty conceiving or seeking reproductive counseling.

EDs that impact sperm motility, sperm count, and sperm health

A note about criteria:

The WHO reference values (WHO 1999) are typically used to evaluate sperm health, though there is some debate as to whether these values are appropriate (Nallela, 2006; Cooper 2010). One complaint about the WHO reference values is that they cannot be used to clearly demarcate fertile men from infertile men, because sperm populations are considered heterogeneous and often even normal sperm demonstrate some abnormal parameters. In a study designed to evaluate the WHO parameters (Nallela 2006), WHO reference values were compared to group means for individuals who were clearly identified as fertile with proven conception, as infertile due to male factor infertility (MFI), and as infertile with or without known female factor infertility. The study demonstrated that motility and concentration were better predictors of fertility than sperm morphology under both the WHO reference values and Tyberg's strict criteria. Motility and concentration had more distinct means with less overlap between the groups and the percent of MFI individuals with high motility was much smaller relative to sperm concentration and morphology. In general, sperm motility has proven to be a strong indicator of fertility in *in vivo* and *in vitro* fertilization scenarios and as a biomarker for normal development of both the seminal fluid and the spermatozoa occurring at multiple stages, including in the testis and epididymis. That said, there is also clear evidence that it is not the only predictor of fertility because some MFI cases demonstrate high levels of motility (Nallela 2006), while other studies have indicated that normal morphology is also a critical component (Coetzee 1998).

Another relevant criteria to assessing sperm health is assessing the level of DNA damage. Chromatin in sperm is highly compact due to the replacement of histones with protamines, which facilitates the formation of a unique chromatin structure consisting of toroidal forms. These forms are then protected with disulfide cross-links that stabilize, compact, and ultimately protect the DNA. A variety of factors can lead to DNA damage including endogenous damage, defects in DNA packaging, and high levels of oxidative stress. Infertile men often exhibit about two times as much DNA damage relative to fertile men (Zini 2001) and such damage may influence reproductive outcomes, via spontaneous miscarriages or idiopathic infertility (ASRM 2008). At this time there is no definitive method for using levels of DNA damage to assess infertility, but many human studies support the association (Kodama, 1997; Zini 2001; Spano 2000). At a mechanistic level, the association is further confirmed by an *in vitro* study in which sperm were exposed to various doses of radiation which consistently produces dose-dependent double strand breaks in the DNA. DNA-damaged sperm were found capable of fertilizing the embryo but the damage resulted in a low rate of embryonic development and high early pregnancy loss; researchers found that the oocyte has the capacity to repair the DNA damage so long as it is below the experimentally determined threshold value of 8% of DNA (Ahmadi 1999). Thus DNA damage in spermatozoa is a potential source of pre-zygotic DNA damage.

Endocrine Disrupters

Phthalates and sperm motility/concentration

Phthalates are a group of chemicals used to make plastics more flexible and durable and are used as solvents in personal care products. Phthalates have a demonstrated effect on semen parameters, including sperm motility and semen concentration and are suspected of impacting male testicular development *in utero*, an effect that has been documented extensively in animal models where the long-term impacts include reduced testosterone levels, testicular malformations, and reduced sperm counts (Howdeshell 2007; Noriega 2009; Howdeshell 2008). Phthlates are

anti-androgens that act through a variety of mechanisms and which are known to inhibit multiple enzymes in the testosterone synthesis pathway including CYP17A1, HSD3B, and HSD17B3 in rat models (Foster 1983;Ye 2011). Androgen signaling is highly conserved between rats and humans, suggesting that phthalates may impact human male development and testicular function.

Because phthalates can impact testosterone production in the testes, they may negatively impact spermatogenesis. Several epidemiologic studies have found evidence to support this conclusion. A fertility clinic in Massachusetts found an association between monobutyl phthalate (MBP) and sperm that was below WHO reference values for sperm motility and sperm concentration (Duty 2003; Hauser 2006). They determined that there were dose response relationships between urine levels of MBP and sperm motility and concentration; as well as for monobenyzl phthalate and sperm concentration (Duty 2003). Clinically, reducing body burden of phthalates may improve sperm counts and motility. See Tips 2 and 4 for ways to reduce exposure.

PCBs and sperm motility

PCBs as previously discussed are synthetic halogenated aromatic compounds that are lipophilic and highly persistent in marine life. There are numerous studies that have demonstrated the presence of PCBs is correlated with decreased sperm motility and sperm count (Dallinga 2002; Ritchoff 2003). The consistency of this association varies depending on which congener is tracked: often an individual congener is chosen because it represents a reliable biomarker for total PCB exposure. Therefore in some cases, studies present null or insignificant findings in the trend when tracking certain congeners (Hauser 2003; Rignell-Hydbom 2004), but the association is often upheld when PCBs are assessed as groups and with some individual PCBs such as PCB-138 (Ritchoff 2003). In Taiwan in 1979, large-scale exposure to PCBs occurred through contaminated rice oil that was used extensively in cooking. Twenty years later a human population study assessed the impact of the *in utero* exposure to PCBs and PCDFs on the semen parameters of young men between the ages of 16-18 years old. Those exposed *in utero*

demonstrated increased abnormal morphology and reduced motility (Guo 2000). In another study conducted in this population, men that were sexually mature at the time of exposure were evaluated to assess any long-term damage that may have resulted from the exposure. Exposed men aged 37-50 had sperm motility and counts similar to their age-matched controls, but had significantly reduced capacity to bind and penetrate hamster oocytes, suggesting that long-term damage likely occurred (Hsu 2003). This human case-study demonstrates the concept that the timing of the exposure is critical in assessing its effect.

Exposure to PCBs can be reduced through changing fish consumption habits – see Tip 5.

Dioxins alter sperm count and motility

Dioxins include a broad range of compounds with the most toxic form being 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) whose toxicity is mediated through the aryl hydrocarbon receptor (AHR).

Epidemiologic evidence supporting dioxin's role in impacting male fertility comes from a chemical plant explosion that occurred in Seveso, Italy in 1976 where high levels of TCDD massively contaminated the surrounding environment. In a study conducted 22 years after the explosion, sperm counts were assessed of young men who were different ages at the time of the explosion, and researchers found that the impact of TCDD on sperm count and motility depended on the age at the time of exposure (Mocarelli 2008). Those who were 1-9 years of age (infancy/prepuberty) had reduced sperm count and motility, while those that were 10-17 (puberty) had an inverse effect of elevated sperm counts and motility relative to age-matched controls from nearby uncontaminated areas. Exposure in both age groups resulted in decreased levels of estradiol and follicle stimulating hormone – an effect that occurred at 68ppt, a dose that is within one order of magnitude of the dose found environmentally in the industrialized world. Numerous rat studies have also been performed to evaluate the impact of TCDD exposure that occurs *in utero*, and while the results are at times inconsistent, there is sufficient evidence to support the

idea that exposure *in utero* in rats is associated with decreased sperm counts and that the interaction is likely occurring through epidydimal function (Foster and Maharaj-Briceno 2011).

Non-persistent pesticides and semen quality

Non-persistent pesticides are considered "contemporary" pesticides and exist in contrast to persistent pesticides of the organochlorine variety, including DDT. The three main groups of non-persistent pesticides include organophosphates, carbamates, and pyrethroids. Studies assessing occupational exposure to pesticides in farmers and their semen quality have had mixed results, with some studies finding a strong association (Kaimajajima 2004; Padungtod 2000; Abell 2000) and others finding weak (Juhler 1999) or no association (Larsen 1998; Whorton 1979). In the general population, pesticide biomarkers have been associated with reduced sperm concentration and motility (Swan 2003; Meeker 2004). Variation in study results may be attributed to variations in exposure constituents, where the mixture of pesticides varies from study to study. This may be an example of the importance the mixture of toxins present, where changes in the composition of the mixture alters toxicity.

Other toxins that may impact sperm counts

The toxins included in this section are only a selection of the ensemble of toxins that may impact spermatogenesis. There are number of anti-androgens that may impact spermatogenesis and testes development by inhibiting testosterone. Testosterone inhibition *in utero* can potentially alter male reproductive abilities throughout the lifetime, and interfere with the continued testosterone production that occurs *in utero* and in the Leydig cells. These toxins are classified as anti-androgens due to their ability to bind the AR in rat models or inhibit testosterone synthesis in rat models (Ye 2011). The high homology between the AR signaling system in mammals suggests that these toxins may have similar effects on humans.

Methoxychlor (MXC), an organochlorine pesticide, interferes with testosterone production in rodent Leydig cells (Akingbemi 2000). Organotins, chemicals used as agricultural fungicides and rodent repellents, inhibit multiple enzymes in T biosynthesis (Ohno 2005; McVey 2003; Lo 2003). 1,2-Dibromo-3-chloropropane (DBCP), a pesticide used to control plant worms, has resulted in male infertility in exposed workers (Potashnik 1979 Israel) and inhibits testosterone production in rats (Kelce 1990). Benzophenes are synthetic chemicals that block UV light and are used in food packaging printing and sunscreens (benzophonene-3/oxybenzone). Most benzophonenes demonstrate *in vitro* ability to inhibit enzymes involved in testosterone production with BP-1 being the most potent and active in rodent models (Nashev 2010).

As we previously mentioned, BPA is largely considered an estrogenic compound because it binds the estrogen receptors, but it is also an anti-androgen due to its binding of the androgen receptor and has potency on the level of flutamide, which is typically used as a negative control in experimental studies for androgen antagonists (Sohoni 1998). BPA has been shown to inhibit T production *in vitro* and *in vivo* and has been shown to inhibit human enzymes involved in T production including CYP17A1 and HSD3B (Ye 2011; Niwa 2001).

Toxins in the seminal fluid

In addition to EDs, numerous other toxins can impact the reproductive process. Many types of toxins have been found in the seminal fluids including lead, cadmium, mercury, nicotine (and cotinitine its metabolite), as well as chlorinated solvents such as trichloroethylene. These toxins work through a variety of mechanisms and can alter spermatogenesis, sperm morphology and motility, and can induce DNA damage that may be passed on to the developing embryo and result in abortion of the fetus or congenital birth defects.

Toxins in the seminal fluid may act via oxidative stress. Oxidative stress is implicated in many diseases, is a condition that occurs across all cell types, and is a major driver for aging and cancer. A cell's antioxidant defense systems are comprised of reactive oxygen species (ROS),

which scavenge molecules like glutathione and catalase. Oxidative stress occurs when these systems become overwhelmed by excess generation of ROS, which may be prompted by exposure to certain toxins, stress, or environmental factors such as heavy metals that engage in Fenton reactions. Once oxidative stress overwhelms these scavenging systems, ROS can lead to multiple types of cellular damage, including damaging DNA, proteins, and lipid peroxidation, a chain reaction that can severely damage membranes.

Investigations on the role of oxidative stress and sperm health have been occurring since 1943, when John Macleod demonstrated that spermatozoa incubated in an oxygen rich environment demonstrated a significant and rapid loss in motility that was reversed by the addition of catalase to the system (MacLeod 1943). Since then, several forms of male infertility have also demonstrated high levels of ROS, and it is generally accepted that high levels of ROS result in DNA damage and lipid peroxidation in spermatozoa. DNA damage in spermatozoa has been linked to infertility, impaired embryonic development, pregnancy loss, birth defects, and in some cases to childhood diseases such as cancer and autism (Gharagozloo 2011). Although many studies point to the importance of oxidative stress and male sperm health, clinical attitudes toward this practice remain controversial (Practice Committee of American Society for Reproductive Medicine, 2008). The debate on this subject is centered around the lack of consistency in clinical trials to demonstrate that oxidative stress is a marker of male infertility and the challenges associated with clinical treatments using antioxidants.

While appealing in theory, the use of antioxidant therapies presents several challenges in practice. First, it is difficult to attain the correct level of antioxidants needed to reduce the load of ROS to improve sperm function, but to not completely eliminate ROS. Sperm require ROS at some level for their signal transduction mechanisms and ROS are critically involved in the rate of hyperactivation and the ability to undergo the acrosome reaction, which are important steps in penetrating the *zona pellucida* of the egg and achieving fertilization (Sharma 1996). Second, at higher concentrations and in certain combinations, some antioxidants such as vitamin C and E in

high doses co-administered can go on to have pro-oxidant effects, which may further compromise sperm function (Donnely 1999).

Trichloroethylene

Trichloroethylene (TCE) is a chlorinated solvent used extensively in the 1950's as a dry cleaning agent and is currently used as a metal degreaser. TCE exposure can occur via contaminated drinking water; in a survey of eight states conducted by EPA, 46% of people were found drinking water with TCE.

Most evidence points to the fact that TCE is not a reproductive toxicant. However, there are limited studies on this topic, and there is some evidence indicating an association between exposure to TCE and time to pregnancy (Sallmen 1998). An occupational study conducted on workers from an electronics factory where TCE was used as degreaser employed extensive medical examination along with a questionnaire. There were no differences found between the two groups with the exception of a dose-dependent increase in hyperzoospermia, which in some cases is related to infertility (Chia 1996). Animal studies indicate that TCE does alter sperm viability in a dose-dependent manner and effectively reduces the ability to fertilize oocytes in unexposed females (DuTeax 2004)

Air pollution

Air pollution is comprised of a number of elements that varies based on location, time of year, and other environmental and industrial factors. Exposures occurring from air pollution may include exposure to metals such as lead, nickel, cadmium; gases including nitrogen oxide, carbonium oxide, and sulphur oxide; and a variety of particulate matter including particles less than 2.5 microns in size (PM 2.5). Thus the effect of air quality on changes in sperm health may vary depending on the abundances of different toxins and their relative concentrations over time.

Occupational studies have utilized tollworkers as study populations because their exposure to vehicle generated air pollution is concentrated and consistent. Motorway tollgate workers were found to have significantly lower sperm motility and kinetics relative to their age-matched controls (De Rosa 2003) as well as increased DNA fragmentation and chromatin damage (Coagero 2011). Concentrations of lead and nitrogen oxide were inversely correlated with the sperm motility parameters, suggesting that increases in their concentration have a negative impact on semen quality (De Rosa 2003). The impact of air pollution may not be limited to those with occupational exposure, as PM 2.5 levels have been correlated with sperm motility in residents of Salt Lake City (Hammoud 2010). Interestingly, this correlation occurred with a temporal delay – where the relevant exposure occurred three months prior to the changes in sperm motility, suggesting that exposures which occur during spermatogenesis are most relevant to sperm health.

Smoking

Smoking exposes men to a large number of carcinogens, including cadmium, lead, and benzo-a-pyrene. One cigarette contains 0.6 – 2.0 μg Pb^{2+} and 1.0 - 4.5 μg Cd of which about 1/10 is inhaled. Smoking on the order of 20 cigarettes to 1 pack of cigarettes produces a measurable increase in the level of seminal Cd (Oldereid 1994; Benoff 2000), as well as a significant decrease in the level of semen zinc. Cadmium can increase the level of oxidative stress leading to DNA damage, a process that may be augmented by zinc deficiency, which plays a role in protecting DNA via chromatin compaction and stabilization.

The evidence linking smoking to male factor infertility is strong – smokers have been associated with sperm possessing lower rates of respiration (Chohan 2010), increased BPDE-DNA adducts (Zenzes 1999), increased DNA fragmentation (Sepaniak 2006), increased incidence of meiotic spindle dysfunction leading to anueploidy (Rubes 1998; Robbins 1997), lower sperm concentration (Ramlau-Hansen 2007; Kunzie 2003; Chen 2007), less motility (Kunzie 2003; Chen 2007), and increased abnormal morphology (Chen 2007). Smoking then leads to various

forms of genetic damage that impair sperm function, reducing chances of fertilization but also decreasing genomic stability in sperm and thereby increasing chances of unsuccessful pregnancies. For example, BPDE-DNA adducts can be passed onto embryos even when only the father is a smoker (Zenzes 1999). This genetic damage to embryos in the form of DNA adducts and aneuploidy may impact embryonic development and lead to failed implantation or loss of pregnancy. It may also explain the observation of some studies that link smoking with elevated rates of spontaneous abortion in smoking couples (reviewed in Zenzes 2000). Most frightening is the association between paternal smoking and childhood diseases. In a human study conducted in China, paternal smoking occurring during the preconception period was associated with an increased risk of childhood cancers, including acute lymphocytic leukemia, lymphoma and brain tumors (Ji 1997). The rate of occurrence rose in accordance with pack-years of paternal smoking for acute lymphocytic leukemia, lymphoma and all other cancers.

Lead

Lead exposure occurs via inhalation and ingestion as well. It is highly prevalent in the ambient air due to combustion of petroleum products and can be ingested through food products or water contaminated by lead-lined pipes. Because lead resembles calcium, it is often incorporated into bones where it remains bound until mobilization of calcium and phosphate occur. It can also interfere with calcium transport in cells, which is integral in maintaining many normal cellular functions.

The role of lead exposure remains controversial and has been extensively reviewed (Benoff 2000), with the controversy ranging from conflicting results in human studies likely resulting from a host of confounding variables, as well as a lack of clarity regarding the appropriate way to assess levels of lead (in seminal fluid or in spermatozoa). A recent human study has a found a significant correlation between the percentage of immotile sperm and seminal

plasma levels of lead and calcium (Mendiola 2011). Further, seminal lead concentrations negatively affect outcomes of artificial insemination (Benoff 2003).

Clinical Tools

Minimizing exposures: Implementing a precautionary approach through lifestyle choices

The evidence presented here demonstrates that exposures to numerous toxins occurs on a daily basis and are *not* benign in nature. In some cases, evidence may be lacking to support a clear mechanism or outcome, but historically many cases of weak evidence have later become definitive evidence. Therefore, a precautionary approach is advisable until potential toxins are definitively shown to be non-toxic.

We are exposed unwittingly and unknowingly to a vast array of toxins found in nearly every aspect of modern life. The task of minimizing these exposure is admittedly daunting. While there are some cases where we have little control over our exposure, as with ambient air pollution, there are cases where we can make choices that will significantly mitigate our exposures.

One major resource people can use while working to minimize exposures is the Environmental Working Group (EWG) website. This nonprofit offers a number of easy-to-use internet tools that link scientific research on toxins and useful consumer information. They have also conducted their own surveys on toxic loads in drinking water and beauty products that provide a reliable assessment from a third party.

Tip # 1: Eat certified organic foods

Basic idea: Conventional foods are often contaminated with a number of pesticide residues that cannot be removed with washing or peeling (Lu 2010). As discussed, even modern pesticides that are non-persistent can have deleterious effects on men's health, including risk and outcome

of prostate cancer as well as sperm health. Men experiencing difficulty conceiving may benefit from changing their diet.

Evidence: Changing from a non-organic diet to an organic diet can reduce exposure to pesticides. In an intervention study with 23 elementary school children, organic foods were substituted for non-organic foods for 5 days during a fifteen day study in which urinary excretion of pesticides was measured in the morning and evening. Children enrolled in the study were consuming strictly non-organic diets that included regular consumption of fresh fruits and vegetables, fruit juices, and wheat-containing foods. Prior to the intervention, all the children had metabolites of organophosphorous pesticides, including metabolites of malathion and chlorpyrifos in their urine. Immediately after switching to organic foods, detection of the pesticides decreased to undetectable levels and returned to the original levels when the non-organic diet was resumed (Lu 2006).

Resource: The Environmental Working Group (EWG) provides a Shopper's Guide to food consumption utilizing data from the Pesticide Data Program to provide a comparison of the load of pesticides on commonly consumed foods. The data used to establish their shopping guides is derived from 51,000 tests for pesticides conducted between 2000 and 2009 in which nearly all of the foods had been rinsed or peeled prior to testing. Their "Dirty Dozen" contains the most contaminated foods and includes apples, celery, strawberries, peaches, spinach, nectarines (imported), grapes (imported), sweet bell peppers, potatoes, blueberries (domestic), lettuce, and kale/collard greens. Their "Clean 15" include foods least likely to test positive for the presence of pesticides and includes onions, sweet corn, pineapples, avocado, asparagus, sweetpeas, mangoes, eggplant, cantaloupe (domestic), kiwi, cabbage, watermelon, sweet potatoes, grapefruit, and mushrooms. These lists may be useful for individuals looking to reduce pesticide exposure but unable to afford all organic produce: www.ewg.org/foodnews/summary.

Tip #2: Assess the safety of personal care products

Basic idea: Personal care products such as shampoos, conditioners, moisturizers, deodorants, shaving creams, after shave gels, and fragrances often contain long lists of ingredients. These ingredients are largely unregulated – such that manufacturers can put nearly any chemical into personal care products and are not required to test its safety. Furthermore, labeling regulations for these products allows incomplete or misleading labeling of ingredients – so avoiding specific compounds listed on the label may not mean that you are not being exposed to them anyway under the guise of a vague ingredient such as "fragrance."

Evidence: Many personal care products test positive for the presence of endocrine disrupting compounds. The evidence linking these compounds to reproductive dysfunction and prostate health has been presented in this chapter. According to the EWG, personal care products often contain phthalates such as DEP and di-butyl phthalate, which are linked to adverse changes in sperm health. Many personal care products often contain parabens which are estrogen-mimics and are widely used as a preservative in personal care products.

Resource: The EWG has established a comprehensive database in which they have independently tested over 69,000 products for the presence of numerous chemicals and ranked them with hazard scores: www.ewg.org/skindeep. Individuals looking to reduce their exposure can query this database to find the safest products available.

Tip #3: Drink clean water

Basic idea: Drinking water represents a source of exposure to numerous toxins including metals, chlorinated compounds, industrial chemicals, and pharmaceuticals. Due to the vast array of contaminants found in drinking water and the resulting health effects, it is advisable to obtain a high quality water filter with which to filter tap water. Use of bottled water is not an appropriate alternative due to the lack of testing on bottled water quality and the leaching that occurs from packaging materials (evidence and discussion in Tip #4: Reduce or eliminate use of plastics).

Evidence: The EWG provides excellent information regarding the ubiquitous contamination of US water sources. While there is some regulation that monitors tap water contaminants, the EWG reports found 315 contaminants when they tested multiple water sources. Of these only half were regulated by laws – meaning that municipal water sources test for their presence and take action if the levels are above certain thresholds. For those unregulated contaminants, these testing procedures are not performed, meaning that they can be present in drinking water at any level. Unfortunately, even the regulations already in place do not guarantee safe levels, as the EWG found that 49 regulated contaminants were present at concentrations above the acceptable limits in certain locations (EWG 2009).

Resources: The best option appears to be filtering tap water. Many water filters use carbon technologies which vary in effectiveness based on the brand and type – which means that the sort of contaminants removed will also vary. The most comprehensive filter is a reverse osmosis system combined with a fine carbon filter. These systems can remove the inorganic contaminants in water, including metals such as arsenic and hexavalent chromium as well as chlorinated compounds. Also, look for product certifications such as the California certification and NSF certification, which require evaluation of the manufacturer's claims about toxin removal. (EWG, tap water)

Drinking bottled water isn't necessarily a safe alternative. The EWG found 38 contaminants when it tested 10 brands of bottled waters. Bottled water is held to the same contaminant standards as tap water, but unlike municipal sources of water they are not required to make public their water quality test results. The image of bottled water from pristine mountain springs is inaccurate at best and pure fabrication at worst – bottled water is often municipal water packaged and sold with less testing than required for tap water, resulting in higher levels of contamination. The FDA does not carefully regulate the contents of tap water, with the result that its pollutants may include: radioactive pollutants, synthetic chemicals including hexane and acetaldehyde, pharmaceutical residues, and disinfect byproducts (EWG 2008).

Tip #4: Reduce or eliminate use of plastics

Basic Idea: Plastics are ubiquitous. Our bottled water, softdrinks, juices, nutritional supplements, and food are packaged in plastics or stored in cans lined with epoxy resins. Most people view these containers as inert. Studies have demonstrated that even under normal storage conditions these containers can release toxins. Most chemicals leaching from plastics haven't been tested for their safety or extensively studied. Of those chemicals that have been studied many are endocrine disruptors which make them important to men's health. Since we consume plastics everyday – we are at risk for some known and likely many unknown health risks – therefore it is important to make choices that reduce exposure. Some of the major toxins from plastics include bisphenol A, phthalates, antimony, and brominated compounds and ways to reduce exposure to them will be discussed here.

A. Basic Safety tips for plastic use

Chemical leaching is increased from plastics that are heated or undergo a lot of use and demonstrate signs of wear. So avoid things like heating foods up in plastic containers, pouring hot beverages into plastic cups or travel mugs, and using water bottles that have scratches and other signs of abrasion.

B. Bisphenol A

Bisphenol A (BPA) is a common component in polycarbonate (hard) plastics and epoxy resins. BPA leaches from plastics in contact with food and beverages. Exposure via this route likely explains the fact that 92.6% of the US population has detectable levels of BPA in their urine (Calafat 2008 – NHANES study).

Evidence:

Water bottles: Patients may be able to reduce their exposure to BPA by eliminating the use of hard plastic drinking bottles and Tupperware. In an intervention study conducted by Harvard

School of Public Health, participants consumed all cold beverages out of BPA containers for one week and urine concentrations of BPA increased by two-thirds (Carwile 2009).

Food packaging: BPA is also found in the epoxy resin that lines tin cans and plastic food packaging. In another intervention study where families consuming canned and packaged foods on a daily basis were asked to consume fresh foods for three days, urinary levels of BPA were reduced by 76% (Rudel 2011).

Since 2008, polycarbonate water bottles have been quickly replaced with BPA-free water bottles. However, the contents this BPA-free plastic called "Tritan" remain an industry secret. From a precautionary and historical standpoint, it would be naïve to assume that this new polycarbonate plastic is safe – there may be other unidentified toxins leaching from it.

C. Phthalates

Phthalates are found in highly flexible plastics and are not chemically bound to those plastics, thereby allowing them to leach into ambient air, dust, and food (Crinnion). They are also found in many personal care products, children's toys, in enteric coatings of pharmaceutical pills, and are used to soften polyvinyl chloride (PVC).

Evidence: . Families consuming canned and packaged foods on a daily basis were asked to consume fresh foods for three days, urinary levels of a phthalate metabolite from PVC was reduced by more than 50% (Rudel 2011).

D. Water bottles:

Release EDs into water including BPA and other alykl phenols including 4-nonylphenol, adipates, phthalates, and antimony (Sb) (Amiridou 2011;

Evidence: Studies have indicated that when water bottles are exposed to environmental stressors such as UV exposure and elevated temperatures, there is increased leaching of contaminants such as antimony into the water (Keresztes 2009; shoytk 2006). Leaching of antimony and brominated compounds has also been observed to increase in response to the type of water – for carbonated waters and those enriched with other substances there is an observed increase in leaching (Andra

2012). The level of leaching also varied in response to the type of plastic being used – where PET had the highest level of leaching for antimony and brominated compounds including BDE-209, a flame retardant used in the manufacturing of plastics, while PC, HDPE, and PS had significantly lower levels. (Andra 2012). Recent studies assessing the estrogenic activity of bottled water samples found that water packaged in PET demonstrated 3 times higher estrogenic activity than the same water packaged in glass (Wagner and Oehlmann 2011).

Tip #5: Modify fish consumption

The fish we consume often contain a number of pollutants, including lipid-soluble persistent organic pollutants such as PCBs, PCDDs (polychlorinated dibenzo-*p*-dioxins), PCDFs (polychlorinated dibenzofurans), PBDEs (polybrominated diphenyl ethers), OCs (organochlorine pesticides), and PFAs (perfluorinated acids). Fish may also have high levels of mercury, which is concentrated in their muscle. As previously discussed, PCBs may impact reproductive and prostate health. They are highly lipophilic and therefore are concentrated in fat tissues. Modifying fish consumption is an important way of reducing PCB exposure.

One way to reduce exposure to PCBs is to trim the fat from fish before or cooking or to cook the fish with broiling, baking or grilling in order to cook the fat off (EWG). These methods of cooking are preferable to frying or sautéing, where fats remain in the pan and are incorporated into any other foods you may be cooking with, such as vegetables.

Another way to reduce PCB epxosure is to alter your selection the fish you eat, avoiding those fish and marine mammals that are most high in PCBs. General guidelines about selecting fish low in PCBs are difficult to outline, as it is always difficult to know exactly what you are getting and from where.

If you are catching your own fish or obtaining fish from local fisheries, it is best to consult state or region-specific guidelines for fish consumption as these will vary depending on the local levels of water pollution. For example, the Hudson River, which has had a long history

of PCB contamination initiated by a power plant in the upper Hudson region, is a region where officials still consider the fish uneatable. The New York State Department of Health provides guidelines for fish consumption from lower regions of the Hudson based on proximity to the source of pollution and known PCB concentrations of fish.

Though specific choices will vary based on the source of fish and also the life stage of the person consuming it, there are some general recommendations that can be made when sufficient information is not available. The first general rule is to choose fish that are smaller and from cold-water areas. The second general rule is to avoid farmed salmon unless it comes from Canada. Most farmed salmon is extremely high in PCBs because the feed used for the fish is comprised of other seafoods rich in lipids and concentrated with PCBs. Canadian fisheries have rectified this problem by testing feed for PCB levels and choosing options with low or no PCB. When possible, choose wild and canned Alaskan salmon rather than the farmed variety.

The third way to lower PCB exposure is to evaluate the safety of your fish oil supplements. Depending on the source, PCB levels in fish oil can range from low to extremely high. A study evaluating the PCB levels of 17 n-3 PUFA supplements in Canada found that those derived from seal had the highest levels of PCBs and those from small, cold-water, fatty fish such as krill had the lowest (Bourdon, 2010). Selecting fish on the lower end of the food chain for both supplements and consumption reduces PCB exposure because PCBs bioaccumulate and dramatically increase as one moves up the food chain.

Tip #6: Investigate other possible sources of toxins

The important thing to remember about toxin identification is that many toxins may not be easy to identify. Here are some important questions to ask when considering toxins that may be specific to an individual's history:

1) Obtain a geographical history from the person by finding out where they were born and where they have lived. Then do the necessary detective work to obtain information about

those areas. Are there any known cancer clusters in those areas? Are there any notable superfund sites or chemical accidents? Are there any major sources of pollution – power plants, air pollution, pesticide usage, refineries, contaminated drinking water?

2) Obtain an occupational history from the person. What exposures have occurred via work, hobbies, or other activities? Was there exposure to any known chemicals or fumes? Possible considerations include pesticides, construction materials, refining byproducts, and radioactive materials. Hobbies such as gardening, metal sculpture, woodworking, and other arts may implicate specific exposures.

Aging and cancer

Genomic instability

Exposure to toxins can impact cellular health through many mechanisms. Current research is asking how aging and cancer – perennial concerns – may be related to toxic exposure.

There are many ways to answer this question and many lenses through which to view it – the focus here will be on *genomic instability*, which is the large-scale damage to chromosomes that results from repeated damage to DNA, inhibition of DNA repair pathways, and disruption of mitotic DNA replication. This discussion will review some of the toxins that promote the progression of genomic instability, its involvement in cancer and aging, and clinical strategies to mitigate it.

During cellular proliferation, during reproduction, and during life – faithful replication of DNA is integral in maintaining not only health but also functionality. Every cellular activity requires faithful replication of DNA: the formation of proteins and enzymes, the regulation of signaling pathways, and the progression through the cell cycle all require the genetic information encoded in the DNA. Faithful replication of the genome is integral and there are a multitude of redundancies in place to ensure that damaged DNA is repaired, that cell cycle progression waits for DNA to be repaired, and during the delicate process when replicated strands of DNA are

separated from each other during anaphase, that their segregation is exact, with no lagging strand, and no broken fragments. Despite this system of redundant checks, genetic instability can and does occur. Genetic instability happens through the occurrence of events that alter DNA structure and base patterns – these events lead to inappropriate DNA expression, mutations that alter protein structures, and changes in the genetic landscape that fundamentally alter the way the cell functions. These events include point mutations, amplifications, deletions, chromosome fragmentations, and aneuploidy (inappropriate chromosome number in a cell).

Environmental toxins can interact at every level of the various processes that maintain genomic stability, both by injuring the DNA and by interfering with its repair. Mutagens are toxins that directly damage DNA. Some mechanisms of damage include: inducing oxidative damage (chromium and cobalt), the formation of bulky DNA adducts (benzo-a-pyrene), cross-links in the DNA (UV radiation), and double strand breaks (ionizing radiation). The cell's natural defense systems are capable of dealing with each of these types of damage, and under normal conditions in the absence of environmental toxins these types of damage are often successfully repaired. But when the extent of damage exceeds the endogenous levels of DNA damage response, DNA damage may go unrepaired, leading to mutations in the DNA, strand breaks, and inappropriate fusion events. Moreover, if other toxins are present, mechanisms of DNA damage sensing and repair may be inhibited, further exacerbating the level of damage. For example, arsenic can inhibit base excision repair and nucleotide excision repair – it can also block p53 (a safeguard against the proliferation of damaged cells) and thereby promote cell cycle progression even when DNA damage has occurred. Therefore arsenic alone can increase the rate of spontaneous mutagenesis by inhibiting repair. In combination with a mutagen like UV radiation, it can lead to rapid progression of skin cancer (Rossman 2004; Burns 2004).

Other toxins may work through epigenetic routes to promote genomic instability. These toxins alter the likelihood of certain genes being transcribed into proteins. Control of gene

transcription is not a one-to-one correlate as previously thought: all genes are not transcribed equally. Research in this area indicates that epigenetic programs that determine whether genes are transcribed is of equal or greater importance in maintaining health than individual genetic predispositions. Therefore, the study of epigenetics has recently exploded and with the development of new technologies, we will likely spend at least the next decade exploring this system and its impact on our health.

Epigenetic regulation of DNA expression occurs at multiple levels and is a highly physical and structural event. Visualizing DNA structure is important in understanding epigenetic events, because DNA structure is not static in the cell: it moves between being fully unwound, to slightly wound, to highly compacted. Just as a rubber band continues to fold over on itself the more you twist it, so too does DNA. DNA is often found it its most twisted form, which is considered a chromosome. When the DNA in a single human cell is fully unwound and stretched out it is two meters long - so keeping DNA compact *and* making routinely important genes easily accessible is critical, and that's what epigenetic modifications do. These modifications are used on DNA at all the levels of compaction, influencing the degree to which it continues to compact or unwind, and in turn the extent to which DNA transcriptional machinery can access certain genes.

From a visual perspective epigenetic modifications occur at these different levels of scale. At the smallest scale, strands of DNA that are fully open and available for transcription can undergo the addition of methyl groups. When methyl groups are added at the promoter of a gene, their presence physically blocks the DNA reading machinery from binding to the DNA and therefore prevents the gene from being expressed into RNA and protein. Many toxins are known to cause this form of hypermethylation at the promoters of tumor suppressor genes and other important regulatory genes. Zooming out a bit, the DNA is wrapped around histone proteins that serve an integral purpose in keeping the DNA organized as it is folded into higher order structures – much like the reels around film, DNA spools and unspools around histone proteins, where each protein manages about 166 base pairs of DNA, thus there are millions of histone proteins in a

single chromosome. When visualized at this scale DNA looks like beads on a string, where DNA is the string and histone proteins are the beads. Another avenue through which epigenetic regulation occurs is through post-translational modification of these histone proteins that influence how tightly wound the DNA is, as well as how attracted the histone proteins are to each other. Toxins like nickel can interact with epigenetic enzymes and alter the abundance of histone marks, leading to systemic repression and overexpression of certain genes.

The epigenetic activity of many known toxins are now being evaluated and explored through gene expression analysis, where cells exposed to a toxin demonstrate markedly different gene expression profiles, indicating that epigenetic changes are likely occurring. Cancers are known to have altered gene expression profiles and while these changes occur globally throughout the genome, there are certain genes that have garnered special attention. Some changes in gene expression confer special abilities to cells, allowing them to proliferate uncontrollably. Repression of tumor suppressor genes allows cells to evade normal pathways and checkpoints that would ordinarily halt their proliferation, while overexpression in oncogenes enhances their ability to grow, proliferate, and obtain nourishment.

While DNA mutations are critical in the formation of cancers, so too is the ability to proliferate uncontrollably. Telomeres usually prevent this uncontrolled replication. Functionally, telomeres serve to protect the ends of chromosomes so that DNA repair machinery doesn't treat the end of a chromosome as a strand break and fuse it to another chromosome, which would increase genomic instability. As cells divide, telomeres naturally shorten due to their method of replication. Based on the length of the telomeres, one can determine approximately how many more divisions the cell will live before it goes into senescence where it stops dividing – while senescence may be associated with old age, here it is truly a sign of health. Short telomeres trigger cellular senescence, and cells that are senescent cannot be cancerous. Therefore all cancers must bypass this senescence barrier in order to gain the ability to proliferate uncontrollably and beyond the length of their telomeres. This bypass event occurs when a

mutation in the DNA activates the telomerase gene, which is capable of maintaining telomere length during cellular division. When a cancer cell has telomerase activated, all of its mutations and its new genetic landscape are propelled forward in a course of aggressive proliferation.

Importance of Micronutrients

Integral to maintaining genomic stability is the functionality of the enzymes and proteins involved in DNA repair, epigenetic regulation, and cell cycle progression. At various stages in these processes, biochemical reactions rely on nutritional inputs in the form of specific nutrients. In 2006, Bruce N. Ames presented the triage theory, in which he suggests that humans have adapted to live through periods of food scarcity by allocating scarce micronutrients to areas which need them for short-term survival (Ames, 2006). The essential micronutrients that are critical for enzyme function, cellular maintenance, and DNA repair may be prioritized to support short-term function over long-term longevity. At a cellular level, this translates to favoring enzymes involved in ATP production over those enzymes involved in DNA repair. Recently, research in this area has demonstrated that micronutrient deficiencies can produce DNA damage that is of the same magnitude as exposures to toxins (Branda 1993).

There are many vitamins that are integral to genome stability. Magnesium, niacin, folate, vitmin B6, and B12 are all necessary for effective DNA repair. Vitamin C, vitamin E and antioxidants prevent damage to DNA and lipid peroxidation. Niacin is necessary to maintain telomere length; zinc for proper function of p53; zinc and manganese for superoxide dismutase; calcium for the regulation of chromosome segregation during mitosis; selenium is involved in methionine metabolism while deficiency in it can lead to telomere shortening (Fenech 2010).

Deficiencies in each of these micronutrients results in observable changes in the levels of DNA damage: therefore maintenance of the appropriate vitamin levels is a critical tool for maintaining lifelong health. However, caution must be used because at inappropriately high concentrations, many of these micronutrients can also have the reverse effect – they demonstrate

U shaped toxicity curves. Where extremely low doses elevate the level of DNA damage, moderate doses decrease the level of damage and high doses promote it. In some cases, as with metals such as iron and copper, elevated doses may increase the concentration of ions beyond the available protein binding sites, leaving them free to participate in oxidative chemistry where they generate hydroxyl radicals that go on to produce damage to lipid membranes and DNA.

Case Study: Folic Acid

Folate deficiency has been associated with increased risk of colon, esophageal and cervical cancer (Blount 1997). Research has indicated that folate deficiency produces equivalent levels of damage on the order of exposure to x-rays and gamma rays (Fenech 2010; Branda 1993). A study conducted in 1993 showed that folic acid deficiency induced approximately the same amount of strand breaks as 26 cGy of gamma radiation (Branda, 1993). Folate deficiency is highly problematic because it directly interferes with the foundational responses to endogenous DNA damage by 1) impairing the DNA repair through decreased thymine production and promotion of uracil misincorporation, and with that 2) increasing the level of DNA repair that is required. These processes increase the level of stress in the system to a point that significantly increases the likelihood of double strand breaks, especially in vulnerable regions like telomeres (Fenech, 2010). Folate is required for purine and pyrimidine synthesis, which are building blocks of DNA, but largely its impact on DNA production occurs with respect to thymine (Branda, 1993), whose incorporation into DNA depends upon the dUMP/dTTP ratio (Fenech, 2010). When folate is low, uracil fails to be methylated and therefore is not converted to thymine, and as a result, the ratio of dUMP/dTTP increases and uracil is erroneously incorporated into the DNA instead of thymine (Fenech, 2010). Uracil incorporation into the DNA can result in DNA double strand breaks. Folate further impacts genome stability by promoting DNA hypomethylation, which can also increase uracil levels in DNA because unmethylated cytosine spontaneously deaminates to uracil and in this case leads to a mutation in the DNA (Kronenberg et. al., 2008).

Hypomethylation at the centromeric sequences can promote DNA unwinding and interfere with centromere function, resulting in higher rates of aneuploidy (Fenech, 2010).

Taken collectively, folate deficiency impacts genome stability through physical alteration of the chromosome structure as well alterations in sequence and methylation markers.

Conclusion

Maintaining cellular health and DNA integrity are integral to cancer prevention, reproductive health, and overall well-being. Protecting DNA and repairing damaged DNA are necessary steps in any comprehensive health plan, requiring careful thought by both the practitioner and patient involved. In short, we must avoid those substances that are toxic to cellular health and DNA integrity and remember to include the necessary vitamins and micronutrients that repair damage when it does occur. Avoiding toxins may be a daunting task, but there are many easily implementable ways to reduce exposure to many toxins.

DNA integrity and cellular health are important for men's health concerns as they may impact the progression of prostate cancer as well as male fertility. Preconception health for men requires more attention as there are many toxins that may impact the quality of sperm and reduction of these exposures may improve fertility in many men.

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