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Commentary

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Evolving Concepts of Food Safety: The Need for Understanding Mechanisms of Food Toxicology for Public Policy

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Abstract

Nutrition and diet, which are fundamental to human development and health, in the context of food safety, can be major determinants in the prevention and contributor to both acute and chronic diseases. While the predominant and legitimate concern is to detect and eliminate microbial pathogens that can cause acute illnesses and deaths (estimated 3-5 thousand deaths in the United States and millions of various acute disorders), food components (nutrients, pollutants, additives, processing by-products, etc.) are major factors in chronic diseases (e.g., "metabolic diseases" of diabetes, cardiovascular diseases, cancers). They contribute to millions of long-term health problems and deaths, globally. The objective of this "Communication" is to integrate a shared underlying mechanism of toxicity between acute and chronic diseases. The traditional separation of the strategy to understand "causes" of acute and chronic diseases, while for some practical tactics is understandable (i.e. screening for food-associated pathogens), it fails to recognize that these microbial -associated toxins work by exactly the same molecular/biochemical and cellular mechanisms as the toxicants- causing chronic diseases. Since all chemicals work by mutagenic, cytotoxic or "epigenetic" alteration of gene expression at the transcriptional, translational or post-translational levels, understanding characteristics of all three of these toxicological mechanisms is important so that public policy- strategies for prevention of both these classes of food –related diseases can be made and that a solid foundation for the concept of "functional foods" be made. A moral imperative has to be given to the critical role that safe food can make during pregnancy in preventing long-term health effects later in life.

Keywords: Mutagenesis; Cytotoxicity; Epigenetic mechanisms; Food toxicology; Adult stem cells; Barker hypothesis; Metabolic diseases; Chronic diseases; Microbiome; Functional foods

"Certainly, looking for simple relation will not be sufficient, but delineating the exact mechanisms of cell cycle control and stem cell development in prostate cancer should be helpful in understanding these early preneoplastic lesions and their relations to diet" [1].

Introduction: Shared Mechanisms of Toxicity in Acute and Chronic Food Safety.

Traditionally, the concept of "Food Safety" usually encompassed the practical goal of understanding the science of understanding the causes of acute illnesses and diseases in animals, especially human beings, caused by biological microorganisms, in order to prevent these illnesses. From diarrhea, allergies, anaphylactic reaction, nausea, and even acute death, it has been the mission of all societies throughout history to practice safe food production, food handling, food storage, food packaging, food transportation and food preparation. In each of the global cultures, because all depend on safe foods to eat for healthy individuals' need for survival and reproduction, prescriptions for what, how and when to eat certain foods were determined by the sad results of trial and error. The list of hundreds of cultural prohibitions exists for what to do or what not to do to minimize illnesses and acute diseases. In most cases, these prohibitions were seen quickly associated with the eating of certain foods. For example, eating the poisonous blowfish in Japan, deemed a delicacy, has led to governmental regulations of the preparation of the sashimi from these fish by licensed chefs.

After the introduction of the germ theory of diseases, this relatively recent concept of "food safety" included the need to prevent microbiological agents/toxins, which was the result of contamination during the "farm/forest/sea to fork". This was to protect the young, immune-compromised, genetically predisposed and elderly from causing these acute health effects caused by various bacteria, fungi and viruses that could be associated by being on or in the foods. Today, with the globalization in the agri-business production, preparation of foods, their distribution, global environmental pollution, genetically modified foods and the diaspora of both people and foods, new pressures are put upon this traditional view of "food safety". New food safety issues, related to the safety of food supplements and food fraud, which, in most countries, are not regulated or tested, are clearly linked to potential human health problems. Even the historic diaspora of food stuffs, such as wheat from the Middle East, where it was grown and stored in dry climates, to places around the world to be grown and stored in wet climates, now is associated with mycotoxins.

Even more challenging to the understanding of food safety, there is the growing awareness of foods/ diets on the powerful and wide influence of the gut microbiome has on human health and diseases [2-7]. Without trying to define the vague terms of what constitutes the difference between "health" and "disease", this growing science of the gut microbiome brings, in my opinion, the new expanded concept of "food safety" to include the seamless connection of acute diseases to the chronic diseases [8,9].

This broader concept of "food safety" must include the fact that nutrition, diets (nutrients; vitamins/minerals; calories), as well as low level toxins and toxicants, medications, and supplemented synthetic chemicals, can alter chronic diseases via exactly the same underlying molecular mechanisms that affect acute diseases [10]. Even with the emphasis on the emerging concept of "Functional Foods", understanding the complex role of the interactions of many factors (genetic; gender; concentration of chemicals; dual roles of natural chemicals in foods; timing of ingestion; concentrations/ amounts of chemicals in and on foods; pollutants in/on foods; genetic modification of foods; food production ; food storage/preparation; person behavior; cultural practices; economic and political choices, etc.) must be integrated in this concept of "functional foods". In other words, after we can experience and witness acute illnesses associated with foods, we might not "see" the effect of the same natural/synthetic chemicals on the pathogenesis of the metabolic diseases of diabetes, cardiovascular diseases or cancer, which occur much later in life [11-13]. It is critically important to realize that there are only three mechanisms of toxicity, e.g., ***(a) mutagenesis; (b) cell death or cytotoxicity and (c) epigenetic toxicity or the alteration of gene express in a cell at the transcriptional, translational or posttranslational levels*** [14], any acute or chronic illness will have a part of its pathogenesis due to one or more of these mechanisms of toxicity. (More on the role of these mechanisms and human diseases later).

Chronic Diseases as a Component of Food Safety

A "systems" approach to "food safety" must include the **mechanisms** by which natural or synthetic chemical toxins/toxicants, in/on food, interact with the **pathogeneses** of acute and chronic diseases. In both cases of acute and chronic diseases a number of factors need to be considered: (a) Individual genetic differences, developmental state of exposures, gender status, together with interactions of other endogenous/exogenous chemicals; specific biology of cells being affected (organ-specific adult stem cells; their progenitor derivatives, and the terminally-differentiated offspring); (b) the mechanisms by which presence/absence of toxins/toxicants and nutrients interact to cause or prevent toxicities; and (c) how those mechanisms are involved in the pathogeneses of acute and chronic diseases. It is becoming clear that one of the shared underlying pathological mechanisms between these two categories of diseases is inflammation [15,16].

Moreover, one cannot ignore the biological and cultural evolutionary roles that interact in food safety in both the biological effect of toxins/ toxicants [17,18]. For example, threshold levels that are needed to trigger mutagenic, cytotoxic and epigenetic changes during the acute or chronic levels of exposure; and the differential effects of these food toxins/toxicants have on stem cells, progenitor and terminally differentiated cells [19]. To say this in another manner, all life depends on a food source for individual and species survival. The slow biological evolutionary selection of genes, needed to convert available food sources into energy, occurred during the inevitable change that occurs in the environment. As soon as pre-human life evolved into the human species, culture emerged with the ability to make tools, fire, domestication of animals, farming practices, etc. This unique attribute of ***Homo sapiens*** led to cultural evolution, whose consequences had to interact with the genes of each human ethnic group that left Africa to occupy deserts, jungles, and mountains, aquatic, artic and temperate areas of the globe, all of which provided unique food sources. In more recent times, that "cultural evolution", which occurs at laser-speed, causes real incompatibilities with our "biological evolution" of those genes needed to cope with new foods and food preparation [20]. ***In other words, we are in real collision between biological and cultural evolution that is contributing to the global "metabolic disease" crisis*** [17,18].

Nutrition and Diets in the Modulation of Carcinogenesis as Examples of an Expanded Concept of Food Safety

To make this conceptual expansion of "food Safety" to include both acute and chronic diseases, the example of food safety association with cancer will be illustrated. Although, admittedly, the understanding of human carcinogenesis is yet incomplete, new insights should help to understand the multiple ways that toxins/ toxicants, as well as dietary modulation of nutrients/vitamins/ minerals/calories, can either enhance or reduce risks to cancer. One of the most important observation is that food safety of the pregnant mother might be one of the most significant societal imperative we have, since this period of the embryo/fetal/neonatal human development is the most important for both the acute and chronic susceptibility to diseases later in life (The Barker hypothesis") [21]. The basic implication of this assumption is that "food safety" can only be assessed with the understanding of how food could be toxic or could have cancer prevention capacities. One must understand the mechanisms of toxicity caused by food and how the mechanism of toxicity interacts with any acute or chronic pathogenesis of any particular disease, such as carcinogenesis.

Food Safety is Dependent on Understanding Food Toxicology

Any public policy to assure any food product is "safe" depends on a series of validated tests to prevent any step from "farm to fork" that might contribute to either an acute or chronic disease. That, in itself, is a very daunting task, since a number of scientific and technological steps, with known limitations and basic assumptions has to be considered. Moreover, multiple scientific disciplines, from epidemiology to toxicology, must be considered, as well as the non-scientific decision- element of human values [22]. In other words, given the available scientific facts, which are, at best, incomplete, any decision to use or not use that information depends on values. In a pluralistic world and pluralist societies, this risk/benefit policy exercise becomes very complicated and complex because even the value component to food safety is never absolute or universally accepted. Since the concept of safety incorporates the reality that nothing can be absolutely "safe", a risk/benefit, analysis must be done with this in mind. Most importantly, the task of risk is complicated by the fact that at the individual level, genetic, gender, developmental state, life style behavior, can influence the risk on any individual, even with "precision or personalized" medical technology to characterize the individual [23-25] This, then, becomes even more perplexing when public policy to implement the scientific basis of the identified toxic mechanism of any food component to the population level, when knows that no population is universally

going to react to any food component the same manner. Implied in this, is the reality that facts, alone, are not the sole determinants of the decision, but the values of a pluralistic global society. However, as the late Dr. Van R. Potter stated, "While values cannot be derived from facts; neither should values defiantly ignore facts' [26].

To address the "factual" dimension of this human decision-making process, the fundamental underpinning of the science of how food can be toxic (toxicology) involves the three known mechanisms by which a cell or organism can be deviated from maintaining a homeostatic process to survive and reproduce for the healthy existence for itself and the species. If a cell is exposed to a physical, chemical or microbiological agent, four possible outcomes are possible: (a)the cell survives the encounter with no detectable change; (b) the cell's genetic information is irreversibly altered by a mutation (gene or chromosomal); (c) the cell dies by a number of cytotoxic mechanisms (e.g. apoptosis; necrosis, etc.); and (d) an alteration of gene expression occurred at the transcriptional, translational or posttranslational levels- (an "epigenetic" event) [27]. In all organisms, such as a bacterium, pig or human being, all three of these mechanisms exist.

Mutagenesis is an evolutionary adaptive mechanism needed for species survival in an ever-changing environment. In a metazoan, mutations in the germ line are also needed, if not too prevalent, for the survival of the species. On the other hand, mutations in the somatic cells of the metazoan could, depending on the gene, number of cells that are mutated, and of the cell type (adult organ-specific stem cell; progenitor or differentiated cells), could have serious health consequences [28,29]. Both germinal and somatic mutagenesis can lead to many hereditary and somatic diseases. Mutations can be the result of either "error in DNA repair" of DNA lesions caused by agents, such as UV light, that lead to skin cancer in the human skin-cancer prone, xeroderma pigmentosum [30-32]. In addition, mutations are also possible by "errors in DNA replication" especially in adult stem cells whenever they are forced to proliferate [33].

The reason that mutagenesis is brought up in the context of food safety is because it has been a primary assumption that agents, affecting food safety that are associated with human diseases, are mutagens. One recent example came from the use of a bacterial mutation assay to test chemicals that were associated with the carcinogenic process, namely, the "Ames Assay" [34]. This novel, inexpensive, and easy to use bacterial system was used to screen for chemicals that might "cause" cancer in human beings ("Carcinogens as mutagens"). For multiple reasons, this approach was shown to be a faulty assumption for testing mutations that might occur in human cells [35,36]. In addition, in vitro assays, designed to detect mutations in animal and human cells have also proven to be less than accurate in predicting the mutagenicity of chemicals [19].

In brief, carcinogenesis in human beings is a multi-step, multi-mechanism process [37,38], involving (a) the "initiation" or irreversible change in a single cell of the human being; (b) the clonal expansion or "promotion" of that single cell into a pre-malignant lesion , such as a papilloma, enzyme altered enzyme of the liver, nodule in the breast or polyp of the colon; and (c) the conversion of that initiated and promoted cell into an invasive and metastatic cancer cell or the "progression" step [39,40]. In other words, while mutations do occur in most human cancers (the exception being teratomas, it is the promotion phase that appears to take decades in adult human cancers and is the rate limiting phase of carcinogenesis [41]. The most contested view of the role of chemicals, natural or synthetic, in the human mutagenic process is that these chemicals are not mutagenic to the human genomic DNA, but could mutate mitochondrial DNA [42].

That, then, brings one to the promotion phase of human carcinogenesis. While one out of three human beings will get cancers before we die, 2 of three of us will die before we are diagnosed with cancers, even though all of us has somatic mutant or "initiated" cells in most of our tissues. The question is "Why"? One answer depends on the mechanism of tumor promotion. It seems that many of the environmental or food- associated chemicals, such as DDT, polybrominated biphenyls, bisphenol A, arsenic, estrogenic disruptors, TCDD, methyl mercury, etc., are "epigenetic" , not mutagenic" toxicants. Even aflatoxin, vomatin, T-2 toxin, and poly aromatic hydrocarbons, while capable of inducing oxidative stress and free radicals, are able to act to induce cell intracellular signaling and altering gene expression [27]. Endogenous chemicals, such as hormones, growth factors and cytokines, are also tumor promoters [43-47].

One of the validated mechanisms that tumor promoters, acting at non-cytotoxic and non-mutagenic levels, seem to be able to inhibit a most fundamental metazoan mechanisms needed for homeostatic regulation of cell proliferation, cell differentiation and apoptosis, namely cell-cell communication by either (a) secreted cell-cell communication between adult stem cells and differentiated cells ; or (b) gap junctional intercellular communication (GJIC) between somatic initiated, progenitor and differentiated cells [48]. All of those known animal tumor promoters, including the endogenous, or exogenous chemicals, including those associated with foods (pesticides, herbicides, bacterial/fungal toxins, arsenic, methyl mercury, PAH's, etc.), work by inducing intracellular signaling, including the induction of free radicals and oxidative stress, can inhibit cell-cell communication, reversible at threshold levels, in a species, gender and developmental stage manner [49-51]. By inhibition of cell-cell communication, cell growth can be disrupted leading to hyperplasia; cell differentiation can be blocked; and apoptosis can be blocked [52]. Any natural or synthetic chemical on or in foods that pregnant women, to which she might be exposed, could, depending on many circumstances, alter the proliferation of organ-specific stem cells (too many); the premature differentiation of those adult organ-specific stem cells (too few at birth); or blockage or induction of apoptosis leading to birth defects, such as cleft palate or spinal bifida [53]. Even the induction of the autism spectrum of children might be related to the interference of the epigenetic regulation of cell communication during early brain development [29].

The epigenetic mechanism of cell -cell communication during carcinogenesis must occur in the presence of a threshold concentration and in the absence of "epigenetic-acting" "anti-promoters" [50,51] . This is very relevant to food safety as it relates to nutrition and diets. Many natural and synthetic chemicals have been shown epidemiologically or experimentally to be cancer chemopreventive agents, such as retinoids, caretinoids, green tea components, resveratrol, CAPE, and beta-sitosterol [54-60]. These chemicals have been shown to either prevent the inhibition of cell to cell communication by known tumor promoters, or enhancing

gap junctional intercellular communication. Even BHT, a food preservative, has been shown to prevent the tumor promotion phase of carcinogenesis but can be a tumor promoter [61,62].

Lastly, a couple important observations must be kept in mind when considering whether a natural or synthetic chemical can be a tumor promoter or an anti-tumor promoter. It has been shown that chemicals capable of being an anti-oxidant can, under other circumstances, including concentrations, can become pro-oxidants [63,64]. One classic example of an epigenetic chemical, although not a food safety- related chemical, thalidomide, is a well -known human teratogen, a sedative, a therapeutic drug for leprosy, and an anti-cancer agent [65-68]. Linking these diverse observations to the mechanism of action of epigenetic compounds that could have pathologies and beneficial consequences, it gave been shown that thalidomide can modulate gap junction function [69]. This observation creates the major challenge to food regulators, namely, the detection of a given chemical in or on food, by itself, does not afford the regulator the clear choice of putting a red flag or a green flag on that chemical to be "safe" or "toxic". It must be analyzed in the context of the many factors related to how epigenetic chemicals work. One must note that these epigenetic food-related chemicals work by different biochemical, signally pathways on different cell types, namely, organ-specific adult stem cells, progenitor cells and terminally differentiated cells [70]. Therefore, while one epigenetic agent (endogenous or exogenous) might inhibit GJIC to be potentially toxic, there will never be "silver bullet" to counter act as a universal dietary chemo-preventive agent. In the same manner, while various oncogenes, which code for via different intracellular signaling pathways, there will not be one anti-oncogene inhibitor strategy to block all oncogenes.

The other observation is the concept of "green chemistry". As this concept can be related to "food Safety", it has to be stated loud and clear that chemicals are chemicals, whether they are found in nature or synthetized by human beings. To assume that one can find structural alternatives to known synthetic chemicals, while true, one cannot automatically conclude that just because a chemical, found in natural sources, will be "safer" than ones found in a chemical synthesis laboratory is false. If any chemical has a biological effect on health, depending on the circumstances of its use, there could be either beneficial or detrimental effects. One can only speculate that, with only limited knowledge of how a natural chemical might work, e.g., in utero stage of development, on each gender, concentrations, presence of absence of other chemicals with synergistic, additive or antagonistic effects, protracted or episodic use, harmful- effects might follow. Uncontrolled supplementation of dietary chemicals, such as folic acid, might be related to detrimental consequences, even though controlled supplementation can be beneficial when it is shown that deficiencies exist [71,27]. This gets to the point that the human body at any given moment in time, might be sufficient or deficient for vital minerals, vitamins and other nutrients. Therefore, taking these nutrient supplements might either non-effective, effective or even toxic.

The role of Epigenetics in Food Safety

Obviously, many of the traditional approaches, such as detection of microorganisms found in food products, the toxins they produce, antibiotics, antibodies, techniques of handling, sterilization, storage, packaging, transportation, and preparation of foods, are needed, as well as the upgrading of the technology, such as state of the art genomic technology, to perform these functions as they relate to the prevention of acute diseases, the concept of epigenetics in food safety and food toxicology is relatively new [72-76]. Since epigenetics or the regulation of gene expression is critical for the development of the multi-cellular organisms after the evolutionary appearance of the stem cell, it is especially critical that careful regulation of a subset of the total genome be expressed/repressed in each type of the 200+ types of differentiated cells in our organs [78], each containing an organ-specific adult stem cell, their progenitor and differentiated derivatives [28,77,78]. From the single toti-potent fertilized zygote to the geriatric adult with over 10^{12}-10^{16} cells, a delicate coordinated homeostatic regulation of extra-, intra- and gap junctional inter-cellular communication must occur in each cell type that shares the total genome in each cell type but only requires a restricted set of gene expression [77,79]. A very descriptive analysis of this epigenetic process was given by C. L. Markert [80].

". . . The first embryonic cells, blastomeres, of mice and other mammals are all totipotent. During cleavage and early morphogenesis these cells come to occupy different positions in the three-dimensional embryo. Some cells are on the outside, some inside. The different environments of these cells cause the cells to express different patterns of metabolism in accordance with their own developing programs of gene function. These patterns of metabolism create new chemical environments for nearby cells and these changed environments induce yet new programs of gene function in responding cells. Thus a progressive series of reciprocal interactions is established between the cellular environment and genome of each cell. These interactions drive the cell along a specific path of differentiation until a stable equilibrium is reached in the adult. Thereafter little change occurs in the specialized cells and they become remarkably refractory to changes in the environment. They seem stably locked into the terminal patterns of gene function characteristic of adult cells. The genome seems no longer responsible to the signals that were effective earlier in development."

There exists a series of molecular mechanisms, such as methylation/ acetylation of nucleic acids/ histone proteins at the transcriptional level, spliced messages can occur at the translational level, while posttranslational modification of proteins or micro-RNA regulation of protein can occur at the posttranslational level. However, one has to remember that these molecular changes are "down-stream effects" caused by earlier biochemical events (triggering of intracellular signaling by endogenous and exogenous extra-cellular factors that interact with the cell via either receptor or non-receptor interactions). Epigenetic changes occur during normal development as cells are required to proliferate, other cells are induced to differentiate and still others are induced to apoptose. Therefore, to distinguish normal and abnormal epigenetic changes at the whole organism level is a major challenge. The homeostatic regulation of fetal, neonate, adolescent and mature development required this delicate modulation of extra-, intra- and gap junctional inter-cellular communication between homologous and heterologous cells, and between tissues in other organs. These natural epigenetic changes occur during the diurnal cycle, eating cycles, exercise, stress, growth and wound healing.

To put the challenge of how chemicals, in and on foods, might induce human diseases via epigenetic mechanisms, any food item, by itself, is a mixture of thousands of endogenous and exogenous chemicals. If it has been produced in contaminated water and soil, such as containing radioisotopes, arsenic, mercury, DDT, etc.), those agents,

especially their concentration, must be added to the list. Later, if the food has been infected by bacteria or fungi, the toxins produced by these chemicals must be accounted. Depending on the storage and packaging conditions, additional chemicals and the status of the aforementioned chemicals will have been altered. When the foods are ingested (daylight or nighttime hours), the genetic background, gender, developmental state and other interacting conditions of the individual (alcohol; drug status; smoke from cigarettes or stoves, etc..) will affect the metabolism and interaction of the metabolites. Remember, human beings evolved as needing a circadian rhythm. Human beings evolved not as nocturnal animals. With a major global shift in people working and eating at night, where they do not produce the brain hormone, melatonin, an antioxidant, it has been argued that susceptibility to various diseases, such as breast cancers in women, who work at night, might be the result of this phenomenon [81].

Moreover, the status of the gut microbiome now becomes a major modulator of these food-associated chemicals and vice versa. Again, during the course of human evolution, the symbiotic relationship of these microbes for human health was the result in their assistance in the digestion of foods, if not some detoxification of some food byproducts. However, with populations, that have established a stable homeostatic symbiosis with beneficial microbiome, now eating foods that challenge the established microbiome, are creating new gut microenvironments for new microbiome populations that might not be compatible for normal tissue health [82-85]. The consequences of these new microbiome secreted toxins might have all kinds of health effects along the gut/brain axis [86]. With the trillions of various populations of microbes in the gut, confronting all kinds of foods, grown and processed differently, together with medications, pollutants and supplements, it seems that the chemical sequelae of the signaling from this metabolism on the physiological responses of all the organs affected would be unpredictable. This complex chemical mixture is now at the next level of interaction to either enhanced potential toxicity or reduced toxicity by the co-digestion of components of high anti-oxidant containing foods. The net effect of these interactions now must face the inherent barriers from the stomach, intestine to the liver and beyond, via the systemic systems.

To put the ability to determine if any chemical can contribute to any disease, especially chronic diseases; one needs to look at the success of epidemiologists in correlating smoking with lung cancer, alcohol with liver cancer, and exposure to arsenic or sunlight with skin cancer. In each of these epidemiological studies, regular and sustained exposure to heavy smoking or drinking, and every day to intense sun exposure was found to be correlated with specific pathologies. However, the observation that the amounts of exposures to these agents was important only demonstrates that other factors, such as threshold levels, genetic/gender, development state and exposures to other anti-toxic agents, could affect the health effects even in individuals exposed to the same toxic agent at the same or higher levels. In the case of food safety, individuals exposed to the same microbial-contaminated foods can share the same risks to acute food poisoning, yet not suffer the same health consequence. Eating is an everyday event during one's lifetime, as can be smoking or exposure to ultraviolet light from the sun. Therefore, chronic diseases, such as Type 2 diabetes, atherosclerosis, and cancers could be related to sustained exposures to epigenetic toxicants associated with the food. Since no two people, even the same development state, gender or even identical twins, will eat the same foods for a long period of time, it should be no surprise that the foods of one individual might contribute to a disease but not to another individual. Only large database of a similar population might detect a pattern where a certain food that is eaten very regularly might be associated with an increase or decrease of a chronic disease.

Food Safety's Role in Human Carcinogenesis: Dietary Modulation of the Multi-Stage, Multi-Mechanisms of Human Carcinogenesis: Effects On Initiated Stem Cells and Cell-Cell Communication

Using human cancer as only one chronic disease that must be included in public policies concerning "food safety", given (a) the "initiation"/"promotion"/"progression" model of carcinogenesis (and possibly atherosclerosis), and (b) the fact that various food-associated toxins (aflatoxin) and toxicants (alcohol; polycyclic hydrocarbons in grilled red meat) have been shown to be associated with various cancers, it must be determined at which stage foods might have its greatest impact on either the enhancement or inhibition of cancer [87-89]. Since the "initiation" of a single organ-specific adult stem cell can occur by either an "error of DNA repair" or by an "error of DNA replication" that causes a mutation in the gene(s) that controls a stem cell's ability to divide asymmetrically. These initiated stem cells can only divide symmetrically to form a clone of partially differentiated, premalignant cells [48]. Because this initiation event can occur anytime, a stem cell proliferates (either with or without a DNA lesion) during the development and lifetime of the human being, to reduce this initiation event to zero level by any nutritional/dietary intervention policy would be impossible. Of course, food-associated nutritional content and dietary practices could modulate initiation to some extent, especially during in utero development [11,17,53,90]. This could happen by food-associated natural minerals/vitamins/and other nutrients/toxins/toxicants preventing the proliferation of organ specific adult stem cells or by stimulating the proliferation of these stem cells [91-93]. Since these stem cells are the "target" cells for the initiation step, increasing or decreasing their numbers during in utero development would increase or decrease, respectively, the risk for cancer later in life (The Barker hypothesis) [11, 17. 21, 42, 75, 77].

There have been several human examples of both restricted caloric intake and specific dietary intake on human health [94]. During the Second World War, it was noted that prenatal exposure to under nutritious diets correlated with adult diseases later in life [95]. Moreover, the example of breast cancers in the Japanese survivors of the atomic bombs might illustrate this possibility [96-98]. The relatively low frequency of breast cancers in these women was seen only because the background frequency of the non-irradiated Japanese women at that time was extremely low. The question then was, "What caused the low frequency of breast cancer in Japan at that time?" The answer could be that the Japanese diet at that period in history was: (a) low caloric; (b) very uniform through Japan; and (c) the diet featured some rice; vegetables, soy products, raw fish; green tea and no smoking and little alcohol or fried/grilled red meat. While caloric restriction can reduce risk of many chronic diseases, two natural chemicals in soy products, Bowman Birks inhibitor and genistein have been shown experimentally to act to reduce cancer risks, as well as increase risks [94,99-101]. More directly, genistein has been shown to induce differentiation of human adult breast stem cells [102]. At that time in Japanese history, the young women were born of small stature. At the time of puberty, breast sizes were small.

Consequently, one could speculate that during pregnancy, the female fetus's breast stem cells could have been reduced in number by premature differentiation or apoptosis. At the time of puberty, these young Japanese women had few adult breast stem cells (which express the estrogen receptor to form breast tissue or have many breast stem cells to be targets for the initiation event [103]).

Because breast cancers seem to favor metastasis to the bone, it might be speculated that the bone stem cells and their niches might attract these breast cancer stem cells [104]. If during in utero development, these bone stem cells behaved the same manner as the breast stem cells, i.e., they prematurely terminally differentiated, then, not only would these female offspring be short in stature, but, because they lived longer because of few breast cancers, they suffered osteoporosis because they had few bone stem cells to make more bone as they aged.

An opposite example might be illustrate when one observes an increase of blood cancers in children born of over - weight mothers [105]. In brief, nutrition/diets could increase or decrease the initiation of organ-specific stem cells, especially during in utero development. Therefore, "food safety" must be considered during in utero development because food related chemicals in the diet could affect both the production of the "initiation" of stem cells by forcing "errors of DNA replication" and by increasing or decreasing the number of organ-specific adult stem cells, thus increasing or decreasing the risk of initiation.

After conception, food safety could also affect the ability of stem cells to differentiation or apoptose normally. Either by inhibiting the stem cells from forming or having functional gap junctional intercellular communication required for growth control differentiation and apoptosis, the numbers of stem cells in various organs, such as the brain, could alter the function of that organ later in life by epigenetic mechanisms. One example might be autism spectrum disorder. It has been speculated that various environmental/food related chemicals might be responsible for autism [106]. The chemicals associated with autism in this study have been shown to modulate GJIC, not to mutate cells, in a dose-dependent, threshold fashion [107,108].

After birth, "food safety" issues must still be considered because chemicals, in and on foods, could act as "promoters" or anti-promoters for any initiated cell. Because of the requirement of eating regularly, both the elements of chronic, regular exposure at threshold levels and in the absence of anti-promoting, antioxidants, could increase the probability of converting these benign initiated stem cell- lesions to an invasive and metastatic cancer cell (progression phase). While there is no doubt that nutrition & diet play important roles in the cultural patterns of cancer, the late appearance of cancer after exposures to unhealthy foods seems to escape the traditional view of "food safety". Therefore, while there still remains an important objective to reduce the risk to acute diseases caused by "unsafe foods", the global rise of "metabolic diseases" in the 7 billion people on earth because we are living longer should be a stronger component of "food safety".

Global Crisis caused by the Collision of Biological and Cultural Evolution of Food

When one considers the global rise of "metabolic diseases", potentially associated with the availability of more calories and the observation that there seems to be more overweight people than underweight persons, the human suffering and drain on limited health care resources to take care of diabetes, cancer and cardiovascular diseases over the life time of these affect individuals should command our attention to this modern dilemma. So, while issues of implementing short time inexpensive tests to determine if food stuffs are "safe" against acute diseases is still an important objective, the philosophical and scientific examination of what has brought about this crisis in chronic diseases is, itself, equally important.

In short, since all living organisms need food for the energy needed for individual and species survival, the biological evolution of all those genes, needed for the acquisition and metabolism of minerals, vitamins, nutrients to generate that energy in specific environment, had to occur. Since no environment is going to remain unchanged or stable forever, there was a need for the delicate balance of genetics and environmental interaction. Any organism that acquired perfect mechanisms to prevent DNA damage and mutations would not survive. On the other hand, organisms that allowed too much DNA damage and mutation induction would also be at risk for non-adaptive survival strategy in an ever-changing environment. Consequently, during the environmental change in environmental temperatures, radiation levels, and gases in the air and water, a slow, but selective biological evolution, occurred from the anaerobic single organisms to phytoplankton to simple multi-cellular organisms to the pre-culture, non-human organism [109-111].

Along that very slow biological evolution, which involved the appearance of high levels of oxygen, molecules, such as oxygen-dependent collagen molecules, appeared to help the transition from single cell organs living in "quorun sensing", communicating loose populations to adhering societies of cells that now could communicate via a new family of genes, the "connexins" [28,112,113]. The genes coded for proteins that self-organized in cells to form gap junction channels, through which ions and small molecular weight molecules could freely go from contiguous cell to cell [114]. The specific connexin gene of this 20 gene family that was expressed, due to the channel size and protein composition associated with its regulation potential (i.e., being phosphorylated or not), provided means for the differentiation of different cell types, such as connexins 26 and 32 needed for hepatocytes, while connexin43 was need for skin fibroblasts [115].

While the exact timing of the new genes not found in single cell organisms, such as the bacterium, is unclear at this time, the evolutionary symbiotic fusion of the mitochondria, need for the utilization of oxygen for energy, and an early pre-multi-cellular organism, allowed for several new phenotypes to appear. Regulation of subsets of genes of the total genome or epigenetic mechanisms to regulate transcription, translation and post translation of proteins in multicellular organisms was needed. Growth control to regulate cell proliferation in this society of adhering cells. The appearance of both germinal and somatic stem cells that were needed for both maintenance of genes needed for the survival of the species and development and health of the individuals to live long enough to reproduce, and, in the case of human beings, long enough to raise their offspring to reproduce. These unique stem cells had the ability to divide either symmetrically to produce self-renewing daughters or asymmetrically to produce one daughter cell needed for maintaining unlimited self-renewal and one daughter that produced a finite surviving progenitor and differentiated daughter. The stimulus to

make that division choice of symmetrical or asymmetrical cell division seems to be external to the genome. In other words, either by a secreted hormone, cytokine or growth factor or by an exogenous environmental, including a food associated factor, could trigger the specific type of cell division.

These stem cells also seem to be characterized by the absence of functional gap junctions, which are required for cell growth regulation, cell differentiation and for apoptosis [116]. Stem cells (embryonic and organ-specific adult), which are undifferentiated, can be growth regulated by both niche extra-cellular matrices and secreted factors [117- 119]. On the other hand, since cancer cells are characterized by the lack of growth control, not being able to terminally differentiate or apoptose, lack either the expression of connexin genes or the function of gap junctions [48]. The demonstration that cancer stem cells, which express normal stem cell genes, such as Oct-4, and lack expression of connexin genes or function of gap junctions, is strong evidence that these cancer cells derived from normal adult organ-specific stem cells [20,120-124]. In addition, the fact that epigenetic, non-mutagenic chemicals that are tumor promoters (i.e., phorbol esters or anti-tumor promoters (beta sitosterol) can modulate gap junction function [60,125].

To bring this back to biological evolution, the time came when the pre-human primate obtained several phenotypes that allowed abstract thought; the translation those thoughts into language; the ability to make things; and finally, the ability to value the use or non-use of those technologies. The ability to make fire for warmth and food preparation; to make tools for hunting; to domesticate animals for food; and to perform agricultural production of food is that which helped to form "cultures". Because this creative process continued to change, it was the creation of "cultural evolution". Whereas, mutation of genes, that were adaptive for individual and species survival, took millions of years to occur, the diaspora of the cultural human beings from Africa to all parts of the earth, occurred because of the specific physical and food-associated regions, in which these humans found themselves (e.g., frigid Alaska; tropical jungles of the Amazon; arid desserts of the Middle East, isolated island in various oceans, such as Japan or New Zealand). The types of foods available in each region helped to select over thousands of years those genes in both the hosts and their gut microbiome to cope with the foods that were available.

However, within the most recent history, cultural evolution has made a dramatic effect on human health, in large part because of food production, agricultural practices, food transportation, storage, packaging and food chemistry. One moment in this transition from local survival, based on traditional agricultural practices for food production, changed when more humans left the farms to urban centers [126]. In addition, the diaspora of ethnic groups of people from the land that selected genes to cope with the foods that were available (i.e., raw fish for sushi in Japan) to new cultures with different foods helped to create some of those factors contribution to chronic diseases associated with food safety.

This complex interaction of the relatively stable genetic backgrounds of various ethnic groups with the rapidly changing cultural changes due to the diaspora of both foods and people, including economic/political forces, is seen in the change in frequencies of human colon cancer in various countries.

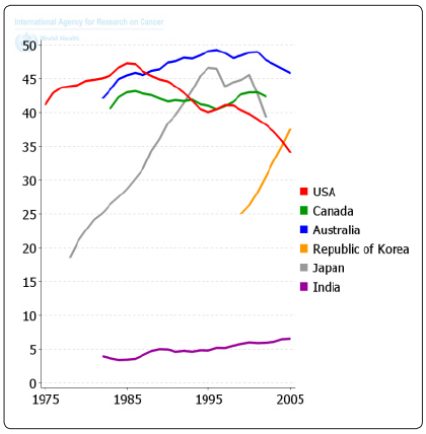

Figure 1: This figure illustrates the close correlation between the consumption of red meat on the frequency of colon cancer in different countries over the 30 years. This clearly demonstrates, in the case of the Japanese and Korean incidences on colon cancer that the "genetic background" of these two groups of people have little to do with the dramatic change in colon cancer. More likely, the changing diets in those countries are the driving force for the incidences of colon cancer. From: http://www.ncbi.nlm.nih.gov/pubmed/22212999 [127].

The frequencies of colon cancers in counties that produce red meet animals have been very high. Yet over the last 75 years, countries, such as Japan, South Korea and India, with their economic circumstances having increased, the colon cancers in these countries has increased as they now are consuming more red meat. This demonstrates the powerful role that nutrition and diets play in the modulation of cancer frequencies.

To make this observation even more related to "food safety" is the observation that colon cancers arising from the ascending colon seem to be less treatable than those colon tumors arising from the descending colon [128]. Possibly, not surprising, is the observation that the microbiome in those two regions of the colon are different [129-133]. Therein, illustrates this complex interaction of biological and cultural evolution, together with nutrients and diets, economics and politics.

Overview of the Interaction of Foods and Human Health and Diseases

Life depends on energy, in order to fend off entropy, in order to survive as an individual and to be able to reproduce to maintain the species survival. Evolutionary forces, which selected several distinct genes, in an oxygenated environment, led to those distinct biological features that led to the emergence of the Homo sapiens. These features included the creation of, (a) oxygen-dependent cell adhesion molecule, collagen; (b) low oxygenated microenvironment, the niche; (c) the formation of the "stem cell, which could divide either symmetrically or asymmetrically, for self-renewal of "stemness" or to terminally differentiate; or (d) to senesce. This *Homo sapiens* or primitive human being, survived or not, depending on the foods in the microenvironment found in him- or her-self and the inherited genes.

Most importantly, because these early creatures were "human", namely, because he/she had abstract thinking ability to create

symbols; could transmit those abstractions through language; covert those abstracts into things (technology); and to make choices as to whether to use or not to use those technologies, these early humans created culture.

Because the technologies that were created, e.g., fire; tools, agriculture, domestication of animals, etc., altered the nutrition, diets and health very fast. In fact, what was created in each area of the globe where these humans migrated was a rapid collision of the very slow **biological evolution of genes** needed for the local conversion of unique foods and the **rapid cultural evolution** that allowed for finding food, food production; food preservation and food production. With the current state of population explosion; environment pollution; ecological changes, diaspora of peoples from their ancient ancestral origins and traditional diets and the diaspora of foods and diets to all parts of the globe, new food production, distribution/preparation, biological changes in our genes cannot keep up with the laser speed cultural evolution affecting our nutritional and dietary, and life style behaviors.

The situation boils down to this; namely each human being is nothing more than a unique organized collection of chemicals that changes during development and that interacts with not only unique foods that are nothing more than a collection of chemicals. All foods are composed of both antioxidants and pro-oxidants. Additional chemicals can be exogenously added to these natural foods, such as coloring; stabilizers; pesticides, herbicides, preparation-induced chemicals. Today, foods can be genetically modified to be pest or draught-resistant. Humans, that eat the foods, take medications, antibiotics, eat at different times a day; and eat too much or too little. The foods eaten are often supplemented by various other food components (alcohol; sugar-laced drinks; caffeine-enhanced drinks).

As we now see, many seem to believe that by isolating presumptive safe or nutrient components out of food (omega 3 fatty acid; retinoids, caffeine, green tea, caffeic acid, etc.), and using them as "supplements", that these molecules will act in the human body the same way they would if ingested in the whole food product. In the rapidly changing dietary habits, the microbiome, which exists in the body, can be altered in such ways as to influence the genetic symbiotic relationship with their needed digestive characteristics that can influence the gut-brain axis, In addition, with more human beings working and eating at night, when via our evolutionary origin, humans did not hunt or eat at night [134]. This imbalance of melatonin production in the brain, an antioxidant, caused by its non-production when not sleeping, might have a major impact on our health due to the disruption of the microbiome and food digestion [135].

This, then, creates a major problem of determining, especially on either the individual or population levels, what "foods" are healthy and safe. There does not seem to be a qualitative/quantitative red or green flag to put on any food for any individual.

What happens when one might eat a natural product (marijuana) mixed with a processed item (a brownie)?

The reports of the positive medical effects of marijuana have to be viewed with the physiological effects of smoking marijuana, such as suppression of nausea and to stimulate appetite; to alleviate eye pressure; to stop convulsions and to decrease muscle spasms [136]. While the biological and mechanistic basis for the chemicals found in marijuana are not known, the reported effects of several chemicals in marijuana, such as the tetrahydrocannabinol or THC, on the immune system might provide a clue to the manner by which the many organ-system effects have been noted [137]. Given many of the organs of the body have their own intrinsic immune cells (liver, brain, skin, gut, etc.) and that they also respond to immune secreted factors from other organs, the wide spread marijuana effects might all be related, in part, to these chemicals trigger intrinsic immune responses to give rise to the many physiological effects seen after exposure.

In the same manner by which the short-term physiological responses to smoking cigarettes give rise to perceived pleasurable effects, the long-term consequences can be harmful, leading to lung dysfunctions. The chemicals of cigarette smoke have been shown to modulate gap junctional intercellular communication [138]. In the case of marijuana or the many chemicals in this plant, such as THC, have been shown to potentially slow the process in which mental decline can occur in many HIV patients [139,140]. The speculation is that cognitive function of HIV patients could be the result of chronic inflammation that occurs in the brain via microglial [141]. Recently, it has been reported that compounds in marijuana seem to act as anti-inflammatory agents [142]. Since, the cannabinols have been shown to modulate gap junctional intercellular communication, and since the gap junction coupled cell networks are target cells of inflammation, which, in turn, can spread to different organs and lead to system chronic inflammation, an integrated hypothesis suggests that this ubiquitous and fundamental biological process that helps to maintain homeostasis might be a target for further experimental [46,143].

Given the widespread role of inflammation in many chronic diseases, to prevent inflammatory by a natural chemical, such as THC, if given in the right protocol in a food product, rather than smoking a "joint", could be an effective preventive/therapeutic strategy to affect multiple brain dysfunctions. Given the many natural anti-inflammatory chemicals found in fruits and vegetables, many of which are known to modulate gap junctional intercellular communication, some mixture might be found that might be effective in treating several brain dysfunctions [51]. Care must always be practiced since these chemicals, under different circumstances, can be converted from an anti-inflammatory agent to a pro-inflammatory agent. Indeed, these chemicals could have very different effects at different stages of development and in different genders.

Conclusions

Again, viewing food safety as primarily associated with acute diseases still is an important way of helping public policy to protect human beings; the case has been made to integrate mechanisms of toxicities, shared in the pathogenesis of both acute and chronic diseases, in order to have a comprehensive approach to a modern day -food safety concept. Given a "One Health-One Planet" framework, where the "health" of the global ecosystem affects the health of animals and human beings, because of the universal requirement of safe foods for energy to sustain individual's and species' survival, understanding that all life forms share the same mechanisms by which physical, chemical and biological agents act toxicologically can provide a new perspective to shape public policies for food safety. However, since our environment will constantly change, especially now that human culture can impact those environmental conditions affecting food safety at the ecological animal and human levels, the effort

to define "sustainability", for "food safety" must, itself, take on a new meaning. In our attempt to prevent some bacterial contaminant in foods that might be associated with gastric cancers, the drugs being used might affect the microbiome of the colon, which in turn, could affect colon cancers in various regions of the colon to alter the resistance of the colon cancers to cancer treatments.

Therefore, while the aim of public health policies is to reduce risks to both acute and chronic diseases associated with foods, we will never eliminate death. What we must aim for is reducing, as best we can, unnecessary suffering from either food associated diseases early in each person's life. To date, many of the mechanistic contributions of endogenous and exogenous agents, in or on foods, appear to act as "epigenetic" toxicants. These epigenetic agents can alter, inappropriately, depending on many circumstances, to alter the number of organ-specific stem cells, especially during fetal, neonatal development. In addition, these agents can alter the expression of genes to modulate cell proliferation, differentiation and cytotoxicity of stem cells. Moreover to add to this complex manner by which food components act epigenetically to be "toxic", they can also, depending on circumstances, act to prevent certain mechanisms of toxicity (chemo preventive agents}. Designing practical assays to detect these epigenetic chemicals, without the need to use animals (which might not be reflective of the in vivo human condition; which would cost too much money; would take too long, etc.), new strategies that would be more relevant to the human situation must be developed [144]. Even the use of primary or abnormal human cells, grown in 2-dimensions, will not suffice [145]. The use of 3-dimensional human or 3-dimensional human organoids from organ-specific human stem cells from both genders will probably come as close to the human situation as one could get to a predictive model. Several examples, while still imperfect because they lack interactive factors such as stromal and immune factors, have provided potential toxicological and chemopreventive evidence that human breast cancers could be influenced by chemicals, shown experimentally and epidemiologically, to influence breast cancer risks [135,146,138].

Finally, the naïve goal to have food safety regulators put a "green" flag or "red" flag on a specific food component only reflects the ignorance of the role of mechanisms by which food components act epigenetically. Only by viewing "food safety" in a larger "Big Picture", can our policies start making a better impact, globally, than is currently practiced in our current narrow view of food safety. Making the decision to destroy more ecosystems to plant more corn to feed more cattle, pigs and chickens and to produce more alcohol to fuel the tractors and cars, as well as to produce more fructose for processed foods, while providing short term economic gain for a few, contributes to more global health problems for all.

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