ATTACHMENT 1

Scientific Report

Peat-derived Organic Humifulvate Concentrate (HFC): A New Multimineral Dietary Supplement

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Executive Summary

Humifulvate is a chemically identifiable source of a standardized peat-derived humic acid, fulvic acid and phenolic acid complex intended for oral consumption. Humifulvate is the base compound used in combination with minerals and trace elements as a dietary supplement. Since 1993, humifulvate has been an approved dietary supplement sold in numerous European countries as an OTC drug in the form of a liquid mineral concentrate called Humet®-R. A significant body of research has been carried out on the pharmcokinetics, toxicology, and efficacy of this product.

The uniqueness of Humifulvate is attributed to its specific properties, method of preparation, as well as its source, near Lake Balaton, Hungary. Humifulvste is solely derived from a fern peat deposit discovered over forty years ago near Lake Balaton, Hungary. The discovery was made after veterinary doctors observed that animals grazing on or near this ... deposit were exceptionally healthy, compared to other animals, especially off-spring. Studies found that the animals, consuming the grass growing on these peat deposits experienced accelerated growth and resisted diseases commonly seen in other regions of Hungary and nearby countries. This suggested that the animals had ingested something that had enhanced their immune function and promoted optimal growth and disease resistance. It was believed that the peat enhanced absorption of minerals found in the plants livestock was eating.

Extensive scientific research has established that this peat deposit contains significant quantities of two predominant humate compounds, humic acid and fulvic acid. Phenolic acid is a minor constituent. The chemical ratio and characterization of these humates has been isolated for their structural and chemical properties. Initially, the humic acid and fulvic acid compounds in this peat deposit were characterized as distinct entities; however, recent analyses, using infrared spectroscopy, has fingerprinted humifulvate as a distinct complex mixture of humic and fulvic acids. Therefore, this humate complex will be referred to by its predominant composition, namely, humifulvate. Since humifulvate is combined with minerals and trace elements to produce a liquid dietary supplement, we refer to this combination as humifulvate concentrate.

In a recent human trial, it was demonstrated that a standardized humifulvate concentrate (HFC) is able to rapidly chelate one of the most toxic metals known to mankind, cadmium. Normally, cadmium has a half-life in human tissue of 10-30 years. However, HFC was able to cause a significant increase in the urinary excretion of this toxic agent within weeks of administration. HFC has also been shown to favorable effect blood lead levels and increase the urinary excretion of this heavy metal. HFC has demonstrated the capacity to normalize iron levels and positively influence mineral and trace element levels in humans. The former property has been demonstrated in humans regardless of initial iron levels.

There is considerable animal and human evidence on the safety of HFC as a dietary supplement. A series of acute and cumulative toxicological studies and mutagenicity studies evaluating the safety of HFC concentrate have been conducted by a number of investigators in Hungary, including the National Institute for Food and Nutrition in Budapest, which functions similar to the U.S. FDA in regulating drugs and foods. In addition, a pre-clinical pharmaco-toxicological report by an independent M.D., Ph.D has documented the beneficial effects of HFC concentrate as well as its safety in laboratory animals.

The documentation of safety data in animals is considered adequate and applicable to humans as evident by the same mechanism of action that is thought to occur in both animals and humans. Most importantly, clinical documentation of both the short term and long term use and safety of HFC in humans is available. All animal and human studies we reviewed used the same HFC to affirm safety, including studies with humans, which monitored its effects for up to five years of use. Further, to date none of the countries that have approved Humet®-R, which contains humifulvate mineral

and trace elements, have reported a single adverse event associated with its consumption. Furthermore, an independent elemental analysis of Humet®-R has found that the concentrations of lead, cadmium, arsenic and aluminum, are significantly below the levels found in the daily diet, and comply with the State of California's Proposition 65 for lead and arsenic levels. The presence of carcinogenic polycyclic aromatic hydrocarbons (PAH) were found to be below detection levels in work carried out by the Hungarian equivalent of the U.S. FDA.

Humifulvate, when combined with minerals and non-toxic trace elements is safe for human consumption as a multimineral dietary supplement.

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INTRODUCTION

Humifulvate is a chemically identifiable source of a standardized peat-derived humic acid, fulvic acid and phenolic acid complex intended for oral consumption. Humifulvate is the base compound used in combination with minerals and trace elements to enhance mineral/trace element absorption. Since 1993, humifulvate has been an approved dietary supplement sold in numerous European countries as an OTC drug in the form of a liquid mineral concentrate called Humet®-R. A significant body of research has been carried out on the pharmcokinetics, toxicology, and efficacy of this product.

The uniqueness of Humifulvate is attributed to its specific properties, method of preparation, as well as its source, near Lake Balaton, Hungary. Extensive scientific research has established that this peat deposit contains significant quantities of two predominant humate compounds, humic acid and fulvic acid. Phenolic acid is a minor constituent. The chemical ratio and characterization of these humates has been isolated for their structural and chemical properties. Initially, the humic acid and fulvic acid compounds in this peat deposit were characterized as distinct entities; however, recent analyses, using infrared spectroscopy, has fingerprinted humifulvate as a distinct complex mixture of humic and fulvic acids. Therefore, this humate complex will be referred to by its predominant composition, namely, humifulvate.

Research conducted at several Hungarian universities and government institutions has established a scientific basis for the use of humifulvate as a mineral delivery agent. Humifulvate has received regulatory review and approval by the Hungarian National Institute of Pharmacy for its inclusion in a non-prescription, multimineral syrup. [1] The Department of Human Genetics of the Medical Research Institute of Budapest, Hungary has focused on its mutagenicity and safety. In addition, continuing research by government bodies, academic institutions, and independent laboratories have documented the safety of this humifulvate multimineral liquid concentrate (HFC). Studies over the last decade, on the properties of humifulvate, suggest that humifulvate has the ability to enhance mineral absorption without bypassing the body's homeostatic mechanisms for controlling toxicity. Further studies in humans and animals demonstrate that HFC reduces heavy metal burdens when taken over time.

The organic components, specifically humate compounds, found in some soils and peat deposits play a vital role in terrestrial and aquatic ecosystems [2]. Consequently, humates are used by farmers and agronomists to accelerate germination and improve rhizomic growth [1]. Humates are believed to stimulate oxygen transport, accelerate respiration, and promote efficient utilization of nutrients, by plants. [1] [3] [4]. Additionally, livestock fed food fortified with humates, have shown improvements in their reproductive cycle, resistance to disease, and growth rate [5]. These observations were what prompted researchers to study the specific properties of humate compounds and their possible benefits in improving the health and well being of humans.

At this point, less is known about the physiological properties of humic substances than their physical and chemical properties. Humic substances do not correspond to a unique chemical entity in structural and chemical terms and their characterization is difficult. However, Aiken and colleagues [6] have generally defined these humates as follows: "Humic substances are a category of naturally occurring, heterogenous organic substances of high molecular weight that can be isolated from the environment and operationally defined in terms of their solubilities." Several different humic substances have been identified:

Humin. The fraction of humic substances that is not soluble in water at any pH value.
Humic acid. The fraction of humic substances that is not soluble in water under acid conditions (below pH 2), but becomes soluble at a greater pH.
Fulvic acid. The fraction of humic substances that is soluble under all pH conditions.
Phenolic acid. Not defined based on solubility but identified as a component of humic substances

The two most important groups of potentially therapeutic humic substances are humic and fulvic acids [1]. The scientific literature has documented two potential therapeutic benefits of humic and fulvic acids: 1) their ability to positively influence mineral and trace element absorption, and 2) the capacity to bind heavy metals and decrease potential metal toxicity. These activities are attributed to the ability of HFC to chelate and facilitate the utilization of metal ions; therefore, supporting the normal transport, absorption and distribution of minerals and trace elements. Phenolic acids have also been widely studied for their potential therapeutic properties, specifically their metal chelating ability. However, their therapeutic role as part of humic substances remains underresearched.

Experiments in rats have shown that humates enhance the rate of cellular uptake of essential minerals, such as manganese, iron, zinc, and copper, thereby increasing their transport through intestinal membranes [7]. A plethora of research now suggests that humic substances are essential for the transport of nutrients to most members of the plant kingdom [2] [4] [6]. Specifically, humic substances have been shown to transport chelated minerals towards the plant roots, and stimulate the translocation of these ions into the plant [2]. These results in plants and animals are promising and provide a base for researchers in elucidating the role of humic substances in animal and human health. Recent research *in vivo* and in humans has substantiated the action of humic substances as mineral transporters.

The humic and fulvic acid constituents of HFC are negatively charged, thereby chelating positively charged ions and molecules. Thus, trace minerals are chelated to HFC in an organic bond resembling the transport proteins of the body, allowing effective absorption, distribution and elimination of these elements. These activities support the function of transport proteins, and prevent damage from toxic metal exposure. For example, transferrin, a protein that binds and distributes iron throughout the body, ensures that all tissues receive an appropriate amount of this mineral. However, when this transport protein is completely saturated, as might occur with chronic environmental exposure, transferrin cannot bind iron and the excess is deposited in the liver and other organs where it can have toxic effects. HFC acts like an ion exchanger,

releasing metal ions of low atomic mass while binding those that have a higher mass (e.g., Pb, Hg, and Cd) [1]. Therefore, HFC may serve as another transporter to distribute essential minerals and simultaneously reduce the deposition of toxic heavy metals in various tissues and organs.

Lead and cadmium are toxic metals that we are exposed to daily. These heavy metals have almost become pervasive and are found in food, water, cigarette smoke, pesticides, near all roadways, and are found in various industrial applications. Their cumulative effect can reach dangerous levels harmful to health. The increasing prevalence of lead and cadmium as environmental contaminants has led to subclinical exposures, which often result in subtle, yet significant, adverse health effects such as fatigue, dizziness, weight loss, hypertension, and kidney function defects [8] [9]. In addition, cadmium and lead are both believed to be carcinogens [8]. A potential preventive measure for these consequences has been emplified in several human clinical trials. In all trials, a significant amount of individuals, that had been exposed to the toxic metals, cadmium and/or lead, benefited from treatment with the humifulvate complex combined with essential minerals and trace elements [10] [11]

Although preliminary data exists on the mechanisms of action of HFC and its humic and fulvic acid constituents, a complete understanding of their activity in humans is still in its infancy. Yet, there has already been a considerable body of research that has documented the potential benefits and safety of HFC, its ion exchange capacity, and ability to influence the absorption, transport, distribution and elimination of metal ions. The following review documents the structural and chemical properties of HFC, discusses its potential health benefits, and provides pertinent toxicological data related to its use in animals and humans.

HUMIFULVATE

Origin and Description of Humifulvate in Peat

A unique source of Hungarian humic acid and fulvic acid (humifulvate) has been chemically identified from geologically young Hungarian peat, estimated 3,000-10,000 years old [1]. The richest source of humifulvate is found in a layer between 5,000 to 10,000 years old. This may be due to the botanical composition of the deposit growing at the time, necessary to form this composition. This unique form of humifulvate rich material is derived from a specific type of peat contained within a square mile found only at a site near the northern embankment of Lake Balaton in Hungary.

Also known as highly organic soil, peat is the organic deposit, or accumulation of plants and vegetable matter that have humified over a period of thousands of years. Peat is formed during a process known as humification, which involves the degradation of plant material. Due to differences in the source and nature of the surrounding aqueous environment, peat varies in its botanical origin, extent of humification, and present flora [6]. However, its increasing degradation leads to the progressive evolution of humin first, then humic acids, and finally fulvic acids [2]. As a result, humic substances are an integral, characteristic, and substantial constituent of peat. Peat is a storehouse of nutrients. Its use has been

exploited to produce high quality vegetable crops [6]. Peats are also known to contain more phenolic compounds than humified organic soils [6] which arise during the humification of plant matter.

Humic and fulvic acids are complex organic molecules, which comprise some 60-80% of peat and soil organic matter [12]. The bacterial and chemical degradation of lignins (substances deposited in cell membranes that help give the plant support and rigidity) and other structural carbohydrates in plants are responsible for forming the intermediate products of humic and fulvic acids. [12] [13] These intermediate products are then polymerized in the presence of polyphenols, which are leached by rain, from the leaves and other plant components. Polyphenols are plant metabolites that are essential to plant physiology. They contribute to pigmentation, growth and reproduction, and resistance to pathogens and predators [14]. Polyphenols can be oxidized to quinones (a smaller phenolic compound) either spontaneously in the presence of molecular oxygen or enzymatically mediated by a wide variety of microorganisms.

Since polyphenols from plant degradation are involved in the formation of humic substances, phenolic acids would then contribute an important part to the structure of these molecules. The phenolic acids that are thought to contribute to the structure and activity of humic substances include; protocatechic acid, vanillic acid, vanillin, resorcinol, ferulic acid, and benzoic acid. These phenolic acids have at least one carboxyl group (-COOH) and one phenolic hydroxyl (-OH) group [15]. These are the functional groups that are thought to possess the mineral chelating capacity of humic and fulvic acids. The phenolic compounds, quinones, and proteins condensate by the action of soil microorganisms on soil carbohydrates This helps to form the structure and composition of humic and fulvic acids and thus humic substances [6] [12]. Figure 1 below includes a schematic of the mechanisms for the formation of soil or peat humic substances

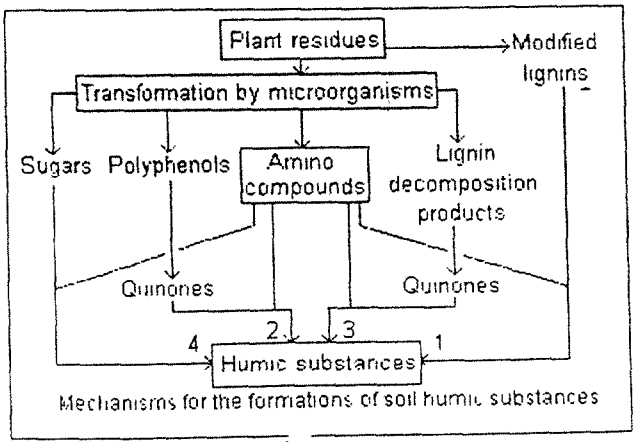


Figure 1 Proposed formation of soil and peat humic substances [6]

Structural and Chemical Properties

Humic substances represent an extremely heterogeneous mixture of molecules, which, in any given soil or sediment, may range in molecular weight from as low as several hundred to over 300,000 daltons [6]. The nature of their building blocks and their structural arrangement continues to be investigated. Due to the complex nature of these biopolymers, determination of molecular mass, elemental composition and chemical moieties has been difficult [6] [16] [17]. Although research does indicate that each and every molecule in a given humic acid or fulvic acid fraction is most likely to have a different structure, the samples in one specific environment contain functional groups of similar types and in a similar number [1]. Elemental composition and functional group analyses have shown that peat humic acids tend to be similar to those from mineral soils [6].

Humifulvate contains oxygen, nitrogen, and sulfur containing functional groups that make it very well suited as a metal complexing ligand [6] [17]. At several sites and with varying strengths, metals are bound to the polypeptides and phenolic acids connected to the polynuclear heteroaromatic nucleus of humifulvate. The properties of humifulvate include its ability to bind heavy metals as an ion exchange agent [13] while acting as a carrier molecule of minerals and trace elements that are essential to human and animal health. This capacity has been attributed mainly to the presence of hydrogen ions in the aromatic and aliphatic carboxyl (-COOH) and phenolic hydroxyl (-OH) groups of the humic substance [6] [12]. When metal ions and humic substances interact through ion exchange, protons on carboxylic acid and phenolic hydroxyl groups of the humifulvate molecule are replaced by metal ions [13]. Evidence for this mechanism is supported by the fact that when these functional groups are masked by methylation (i.e the addition of a methyl group) and acetylation (i.e. the addition of an acetyl group) the extent of activity and metal binding is drastically reduced [1] [2].

The percentage of the humin, which occurs in the various humic fractions, varies considerably from one soil type to another. The humus of forest soils is characterized by a high content of fulvic acids while the humus of peat and grassland soils is high in humic acids. The humic acid / fulvic acid ratio usually, but not always, decreases with increasing depth.

Practically all the cation exchange capacity of highly organic soils (peats), as well as the humus layers of forest soils, is due to organic matter. In these cases, the greater the degree of humification the higher is the cation exchange capacity. The contribution from humic and fulvic acids is due largely to the ionization of carboxylic (COOH) groups, although some contribution from phenolic hydroxyl (OH) and amine (NH) groups is expected. The maximum amount of any given mineral ion that can be bound is approximately equal to the content of acidic functional groups, primarily carboxylic acid groups (COOH). Bonding mechanisms for the retention of organic compounds by humic substances in soil include ion exchange, hydrogen bonding, van der Waals forces (physical adsorption), and coordination through attached metal ion (ligand exchange) [6].

Biological Role

Although data on the exact biochemical properties of HFC is limited, it is evident that humic substances can affect several biological processes [7]. Research in animals and humans have demonstrated several potential therapeutic applications for HFC. HFC has shown the ability to support the normal transport, absorption, and distribution of essential nutrients and minerals in the body.

Humifulvate has the ability to transfer metals to and from metallo-proteins (also called metallothionein (MT)) *in vivo*. [18]. These proteins play a role in metal storage and sequester excess metal ions, preventing toxicity. Metalloprotein concentrations are highest in the liver where metals accumulate in the MT portions of this organ. MT is found in many other human tissues, including small amounts in the blood plasma, which suggest that MT plays a role in the transport of metals as well. [9]

When the free metal binding capacity of humifulvate is saturated, or contains a high concentration of a metal humate (attachment of metal to humifulvic acid), then humifulvate will transfer this metal to the protein type molecules that are able to bind and utilize it. On the other hand, if the free metal binding capacity is high then humifulvate will form complexes with metals that are free or attached to metalloproteins, aiding in the excretion of these metals (i.e. in the case of toxic heavy metals like cadmium). It is also safe to assume that humifulvate may act somewhat like metalloproteins due to their chelating activity and ion exchange capacity. When metals are a part of a metalloprotein, they can modulate biochemical reactions [19]. For example, these reactions can manifest themselves as changes in immune function, such as the release of molecules involved in intercellular communications. This gives further evidence of the supporting role of humifulvate, by assisting in the transfer of metals to metalloproteins. In addition, if humufulvate is considered to act like a metalloprotein, this could suggest that it directly modulates biochemical reactions in the human body.

Safety and Toxicology

Humic substances have existed in nature well before human existence and their importance in the environment is well documented. Due to their ubiquitous nature, research continues today to determine if humic substances pose a threat to human health. Some researchers in China have attempted to link humic substances in well water with two different endemic diseases, Blackfoots disease and Keshan Beck disease. However, it appears that many factors are involved in the formation of both conditions, and the etiology of both diseases as related to humic substances remains equivocal. Those endemic conditions are assoicated only with well water humic substances, which are ingested in extremely high amounts and are also contaminated with high levels of arsenic and other toxic compounds, and not peat derived humic substances It is imperative to distinguish the standardized HFC derived from Hungarian peat from these humic substances. Due to their different origins and based on independent laboratory analysis of HFC documenting the absence of arsenic and other toxic compounds, one concludes that the Hungarian peat source of HFC is quite different than the humic substances found in well water in China.

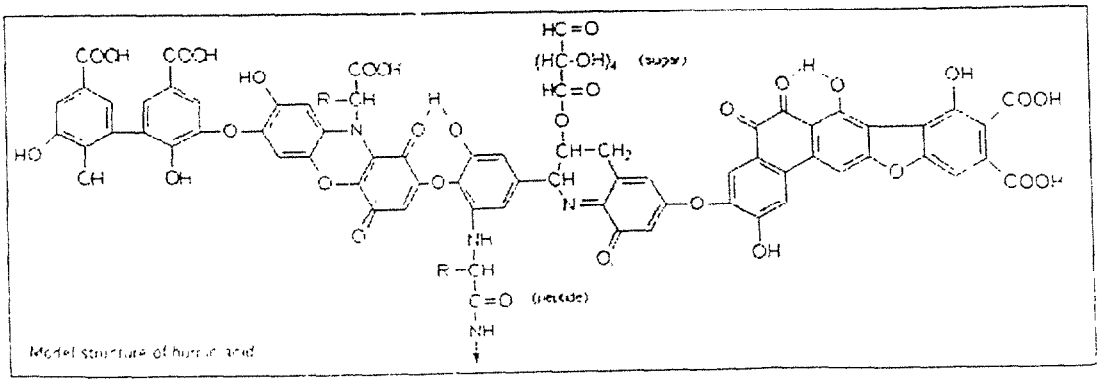
The consensus at this point is that humic substances are an essential part of geochemical interactions whose benefits outweigh any questions related to its ingestion. However, because humic substances are being considered for use as a supplement in the diet, an acceptable threshold for this dietary supplement had to be determined Acute toxicity testing in rats demonstrated that the lethal dose of the standardized HFC is extremely high, at more than 10 gm/kg body weight of the animals used in the study. This suggests that the typical dose administered to humans in a dietary supplement would produce no harm Cumulative and sub acute toxicity and mutagenicity studies have also documented the safety of HFC. Furthermore, a review of human clinical studies indicate a lack of significant side effects from the ingestion of HFC. The amounts of humic substances in HFC are extremely low and have been documented as safe from animal and human studies. The following review will provide a more detailed discussion of the safety of each compound and ingredient found in HFC in its appropriate section

HUMIC ACID

Structural and Chemical Properties

One could assume that due to the ubiquitous nature and structural and chemical properties of humic substances that the environment is dependent upon them. It is known that the specific functional groups of humic acids are responsible for chelating various compounds in the environment, thereby improving nutrient utilization and preventing metal toxicities in waters and soils, and thus possibly in plants, animals, and humans as well.

Humic acids from peats show significant levels of phenolic carbons (C_{ar}) and methoxyl carbons (-OCH_3), associated with the presence of lignin-like materials [2]. Lignin, being the starting material of humic and fulvic acid, and various phenolic compounds such as vanillin, vanillic acid, resorcinol, ferulic acid, protochatechuic acid, and benzoic acid are the degradation products of these lignins [15] [20]. It is apparent that humic substances consist of a heterogeneous mixture of compounds for which no single structural formula will suffice. However, humic acids are thought to be complex aromatic macromolecules with amino acids, amino sugars, peptides, and aliphatic compounds involved in linkages between the aromatic groups. The hypothetical structure for humic acid is shown in figure 2 below. It contains free and bound phenolic OH groups, quinone structures, nitrogen and oxygen as bridge units and carboxylic acid (COOH) groups variously placed on aromatic rings.



Model structure of humic acid

Due to the variable molecular composition of humic acids, a wide range of dissociation constants (binding stability) exists for the metals that are chelated by humic acids. [1]. In addition, different metals are bound to humic acids with varying strength meaning that a particular chelate-bond cation will modify the binding stability of the other metal linkages. This peculiar metal binding capacity of humic acids is exemplified by the fact that when some alkali metals, such as K and Na, are bound by previously empty functional groups, then the chelated bonds of Fe and Al may rupture easier than if the humic acid molecule contains an alkali earth metal, such as Ca [1]. This is why vegetation suffers from micro-element deficiency in the presence of Ca-humate, a characteristic constituent of basic soils, although the needed elements abound in the humus. This peculiar metal binding capacity also protects plants by the ability of water-soluble fractions of humic substances (humic and fulvic acids) to form precipitates with a number of metal ions (Ca, Cd, Hg, Pb, Ba), forming insoluble complexes. The complexes formed are not available to plants and the concentration of toxic cations in the soil solution is reduced [2]. This may also partly explain the role of humic substances in alleviating heavy metal toxicity in humans.

Conformational changes also occur when metals bind to different sites of the humic acid. This can affect the binding stability through competition with or enhancement of various metals. [13] [21]. The fact that different metals have different affinities for different binding sites of the humic acid will also affect binding stability. The pH, ionic strength, molecular weight, and functional group content are all factors influencing the quantity of metal ions bound by humic substances [6]. But most importantly, due to the heterogenous molecular composition of humic acids, a given metal may bind very strongly, while another humic acid may affix or release the same metal much easier [1].

In regards to the metal ion exchange processes, it is thought that the humic acids with smaller molecular mass bind metals 2 to 6 times better than larger molecules from the same sample. Additionally, bivalent ions (e.g. Zn^{+2}, Cu^{+2}, Ca^{+2}, Mn^{+2}, Cd^{+2}) are more likely to become bound as compared to trivalent ions (e.g. Cr^{+3}, Al^{+3}). The bivalent ions that have been characterized based on their ability for cation exchange are in the following order: Pb≥Cd≥Cu>Ni>Fe≥Co≥Mn≥Zn≥VO [1].

Biological Role

Although the physical and chemical properties of humic acids and their biological role in biota (bacteria, fungi, viruses) and plants have been well documented, it is not yet clear exactly how humic acids affect mammalian cells. Research indicates that humic acid is absorbed *in vivo*, and can act as an active agent modifying biochemical reactions. Its effects on cell metabolism, enzymes, free radicals, and minerals have been documented in the literature. Furthermore, due to the ability of humic acids to form bonds with metal ions, they are also responsible for forming complexes with amino acids, peptides, carbohydrates, and steroids. These physiochemical properties of humic acids may also be responsible for some of the effects occurring in tissues; including the elimination of heavy metals, desmutagenic effects (extracellular interception of mutagens), and antioxidant and antiocoagulant activity.

Absorption and Bioavailability

It is thought that humic acids can be absorbed in the body through intestinal tissue as well as influence various metabolic processes. ^{14}C labelled humic acids were administered ad libitum to rats in their drinking water at a concentration of 1% and measured in the CO_2 of the air respired, feces, urine, and internal organs [7]. It appeared that some of the humic acids were absorbed and the liver seems to be one of the major organs in which humic acids are absorbed and/or broken down. The data indicate that during a period of 33 hours, approximately 10% of the administered humic acids had been completely metabolized into CO_2. Humic acids are known to contain hydrolysable peptide groups; however, whether the breakdown of the rest of the molecule (i.e. aromatic moiety) is complete is not known. The breakdown of humic acids will also take place in the gut, as several microorganisms are known to utilize them as their source of organic carbon [2]. Other research reports that after a single oral dose of humic substances to rats, the bioavailability was determined to be <0.1% of the administered dose, indicating that only a small fraction of the humic substances was absorbed through the intestine [22]. Therefore, further data are needed to clarify the absorptive process of humic substances.

Metabolic Effects

Visser [7] found that humic acids seem to accelerate cell metabolism as observed on the rate of breakdown of glucose, the amino acid leucine, and the nucleic acid base uridine by rat liver. The data indicate that the humic acids appear to retard the rate of incorporation of these organic molecules into the liver, but once they are absorbed, humic acids seem to accelerate their metabolism. Other data reported by Visser indicate that humic acids have the ability to increase levels of free amino acids in the serum. Humic substances are also thought to interact directly with enzymes activating and inhibiting their actions, by binding to the active sites on enzymes. This is exemplified by the fact that when the humic acid functional groups are methylated, total inactivation of the humic acid is observed [2] and activation or inhibition of the enzyme does not occur. Besides directly affecting enzyme function, the cation exchange properties of humic substances allow themselves to fixate bivalent cations, and indirectly influence enzyme function. Many mineral cations are often used as cofactors for enzymatic function, thus helping enzymes to facilitate biochemical reactions necessary for metabolic function, nutrient utilization, and growth.

Other data support the indirect influence of humic acids in enhancing the ability of the body to utilize nutrients. Humic acids are known to bind inorganic ions and thus facilitate the transport of these minerals through the intestinal membrane of rats [7] The amount of transfer across the intestinal membrane was found to increase in the order of alkaline metals (Na, K), earth alkaline metals (Mg and Ca), and heavier metals (Mn, Fe, Zn) by 1-16%, 50%, and 80%, respectively. Elements such as Mn, Fe, Cd, and Zn are known to be able to participate actively in ligand formation with organic compounds and therefore, the ability of humic acids to act as ligand formers, may explain their facilitatory action of tranporting inorganic ions through biological membranes

It has also been proposed that humic acids promote the restoration of energy levels by stimulating increased oxygen uptake resulting in the generation of energy rich molecules necessary for metabolic processes. Research suggests that humic acids can stimulate respiration and increase the efficiency of oxidative phosphorylation in rat liver mitochondria [3]. Cellular respiration, occurring only in the presence of oxygen, results in the breakdown of nutrient molecules to generate ATP. Cells, such as in the liver and muscle, use this ATP for energy to fuel various processes like stimulating the uptake of nutrients [23]. This in turn would provide more energy for the individual to maintain normal physiological processes as well as compensate for the extra energy demands brought on by illness or other stressors such as exercise.

Compared with non-treated mitochondria, 40-400 mg/L of humic acid solution resulted in an increased efficiency of oxidative phosphorylation indicated by a high P/O ratio (number of moles of ADP phosphorylated to ATP/atoms of reduced oxygen). However, the lower molecular weight humic acids at the higher concentration level were effective uncouplers of oxidative phosphorylation. The uncoupling of oxidative phosphorylation results in the electron transfer to oxygen but no ATP synthesis is coupled to this respiration [23]. This uncoupling effect is similar to what happens in most mammals, including humans in which an uncoupling protein, thermogenin, in brown adpose tissue is used to help generate heat with the electon transfer to oxygen. This energy of oxidation is not conserved as ATP but is dissipated as heat, which is necessary for the newborn infant to maintain warmth as well as for the hibernating animals during long periods without food

Upon longer contact with the smaller molecular weight fraction of humic acid, at low concentrations, the most efficient oxidative phosphorylation was induced [3]. The researchers state that, due to the smaller molecular weight of humic acid, penetration of the mitochondrial membrane would be easier thus promoting more effective induction even at lower concentrations. The ability of humic acid to induce cellular respiration and thus generation of ATP for energy should be studied further *in vivo* to substantiate the effects seen in this study. If future data support the current study, then it is possible to conclude that humic substances, specifically humic acid, could effectively induce energy production thus enhancing the ability of the body to utilize nutrients.

Elimination of Heavy Metals

It appears that the cation exchange capacity and ligand formation ability of humic substances may partially explain why humic acids can bind and release ions of lower atomic mass while binding heavier ions with a higher atomic mass. As previously mentioned, many other factors will affect binding stability; however, it is known that lead and cadmium are among those bivalent ions that are most likely to be bound to the humic acid molecule. This is of great significance since both metals are considered toxic when accumulated in biological systems.

Cadmium(Cd), lead(Pb), and mercury(Hg) are among the most toxic and ubiquitous environmental metallic contaminants to which the population is exposed. Shubert [24] has reported concentrations in the human body as follows: Cd~ 50 mg, Pb ~ 120 mg, and Hg~ 13 mg, which are relatively high Cadmium is

one of the most studied heavy metals. It is known to be toxic to every body system whether ingested or inhaled and tends to accumulate in body tissues with a very slow elimination rate (half-life ~ 20-30 years). Consequently, there is concern about the increase in environmental cadmium that has occurred as a result of its increasing industrial use. Cadmium is a known central nervous system neurotoxin and carcinogen. Daily intakes of 25 to 60 mcg of cadmium for a 70 kg individual have been estimated for typical diets in Europe and the United States [9].

There is concern that long term exposures to cadmium can result in renal damage due to the fact that this organ is the most sensitive to cadmium [9]. Cadmium toxicity is manifested by a variety of syndromes including; kidney dysfunction [9, 10], hypertension, hepatic injury, and lung damage after inhalation exposure [9]. Cadmium induced calciuria, radiologic signs of osteomalacia, and abnormal effects on bone remodeling have been reported in occupationally exposed workers [9] [25]. Furthermore, cadmium has a powerful affinity to bind metallothionein (a transport protein for minerals) and displaces the ability of Cu and Zn to bind and be utilized effectively in the body [19].

In an attempt to more clearly describe the role and mechanism of humic acid in alleviating heavy metal toxicity, several *in vivo* studies were conducted. These studies have produced conflicting results. In one study to determine the effect of humic acid on the absorption of cadmium in rat intestine, researchers found that an increased distribution of cadmium to the metallothionein fraction (transport protein) may contribute to a lower absorption of cadmium in the intestine [18]. Because the speciation of Cd in the incubation solution was not markedly affected by the presence of the humic acid, this provides some evidence that the complexation of Cd to the humic acid is not happening in the intestinal lumen. However, it is imperative to note that the Cd-Cl complex used for the solution, although widely accepted in the literature, may have affected the outcome. The investigators speculate that humic acid exposure may be responsible for influencing the metabolism of Cd inside the cells (as reflected by the increased distribution of Cd to MT) instead of affecting the absorptive process (uptake into the intestinal cells) [18].

Another study was designed to determine if the formation of cadmium complexes with humic substances would occur in the intestinal lumen. The observed effects on intestinal absorption and tissue accumulation of this complex were also studied. [22]. In contrast to the decreased absorption of Cd in the presence of humic substances in the intestines of rats in the previous study, [18] the fractional absorption of Cd in mice was not affected by humic substances [22]. However, the organ distribution of Cd was affected after absorption as indicated by the decreased fractional retention in the kidneys at the highest humic acid exposure level.

The difference in results in the two former studies is explained by the authors. In the first study the intestinal lumen was carefully rinsed and competing complexing agents normally present were removed. However, in the latter study this was not the case and therefore, a dissociation of the Cd humic complex could occur in the presence of other binding ligands. Furthermore, the heterogeneous nature of humic substances and varying functional group capacity may also be responsible for different results. Further

studies are warranted to clarify the role that humic substances play in the speciation of Cd in the intestines and their role in the distribution of this metal in the body.

Desmutagenic Effects

Due to the chelating properties of humic acids, emphasis has been placed on the possible role of these substances in preventing mutagenesis. Many medicines, chemicals, and physical agents such as ionizing radiation and ultraviolent light have the ability to act as mutagens and cause genetic mutations. Some natural plant derived materials (i.e. humic acids, glycyrrhiza glabra extract, glutathione, and bioflavinoids) have been classified as desmutagens or antimutagens based on their ability to react with or bind to formed mutagens, or breakdown a mutagen or promutagen, thereby providing a means of defense against mutagenesis [26].

Research suggests that humic acids may display a desmutagenic as opposed to an antimutagenic role *in vitro*. The desmutagenic activity of humic acids is characterized by their ability to adsorb mutagens rather than decompose them [27]. By adsorption or through the formation of humic acid-mutagen complexes, humic acids may act extracellularly by preventing the formation of genotoxic compounds that would affect the DNA of the cell, [28] rather than directly protecting the DNA from damage at the intracellular level. Thus, the mechanism of action of humic acid is not by inhibition of metabolic activation of the mutagen, but the humic acid is instead binding to and inactivating the mutagen. Sato et al. [27] found that the ability of humic acids to adsorb mutagens increases with the molecular weight of humic acid.

Cardioprotective Effects

The leading health care problem and cause of death in the United States is cardiovascular disease (CVD), with 733,834 deaths in 1996. In 1994, 22.3 million Americans were reported to be suffering from art disease [29]. Risk factors associated with the development of CVD include obesity, high blood pressure, diabetes, smoking, and decreased antioxidant protection. Among the repercussions of this disease are ischemic events (decreased oxygen in the heart tissue) induced by atherosclerosis or narrowing of the blood vessels which lead to heart attacks. During an ischemic insult, alleviating arrythmias (loss of rhythm of the heartbeat) upon reperfusion (return of blood flow to the heart muscle that had become ischemic) is important in protecting the cardiac muscle. Additional damage upon reperfusion includes; increased free radical production, histological damage to the heart tissue, and increased blood-clotting activity, which can further exacerbate the narrowing of blood vessels and ischemia. It is thought that humic acid may work in alleviating this damage upon reperfusion by scavenging free radicals and decreasing blood-clotting activity. Studies have documented the potential cardioprotective role of humic acid [30] [31].

The myocardial reperfusion rates were studied in rats given 30 mg/kg body weight of humic acid and 10 mg/kg body weight of HPC with microelements for two weeks. In response to the ischemic insult, coronary blood flow, aortic blood flow, and left ventricular and diastolic pressure were improved in those hearts of the rats fed the humic acid and HPC [30]. The results indicate that humic acid and the

microelement complex containing humifulvate may have some beneficial effect on myocardial perfusion after ischemia, slightly improving myocardial function. Furthermore, no effects were seen when using the humic acid or HPC in nonischemic myocardium. The fact that supplementation with humic acids alleviate reperfusion injury is promising, but more data are needed to substantiate the mechanism by which the humic acid is beneficial as a cardioprotective agent.

It has been proposed that humic acid could play a protective role during myocardial reperfusion by exhibiting antioxidant activity. Humic acid may have the ability, as an antioxidant, to limit the potential formation of oxyradicals produced during tissue injury that occurs with ischemia and reperfusion. Neutrophils are thought to be the causative agent because the accumulation of these immune cells at the damaged tissue site produces significant oxidative stress to the surviving tissue cells. This leads to irreversible injury of these cells as result of the massive generation of superoxide anion and hydrogen peroxide. This damage has been successfully prevented with inhibitors of oxyradical generation and with antioxidants that destroy the radicals after generation (e.g. superoxide dismutase). Thus, in future research it will be important to measure the antioxidant capacity of humic acid after reperfusion injury to elucidate its role as a cardioprotective agent.

Another potential role for humic acids as cardioprotective agents has been exhibited in an *in vivo* study examining the anticoagulant effects of these humates. Klocking [31] found that 5 or 10 mg/kg body weight of sodium or ammonium humate, isolated from peat, contributed to the release of tissue type plasminogen activator in rats and rabbits, which plays an important role in dissolving thrombi (blood clots). Although a less potent anticoagulant (anti-blood clotting) effect was seen with the natural humic acids as compared to the synthetic, the use of the natural substances may still be effective in the treatment of thrombotic disorders, or the increased blood clotting activity that occurs as a repercussion of myocardial reperfusion. Additional research on the cardioprotective effect of humic acid is warranted to substantiate s role in the prevention and/or treatment of cardiovascular disease.

Many of the biological actions of humic acid are thought to occur because of their complex chemical structure, consisting of numerous phenol and quinone rings [6] held together through epsilon donor acceptor complexes [32]. Epsilon donor acceptor complexes contain molecules that have electrons to donate as well as molecules, like molecular oxygen, which are considered epsilon acceptors because they accept an electron. A reactive free radical is formed during the transfer of an electron to molecular oxygen leaving the other molecule with a single unpaired electron, which will, in turn, react with other molecules within the compound. This resulting epsilon transfer produces molecular bonds between the individual molecules that are further stabilized producing intermolecular mesomery. These complexes may then form covalent, hydrogen, and epsilon bonds with macromolecules, [1] [32] inorganic compounds, and exogenous species (viruses, mutagens, etc). Therefore, it is thought that humic substances interact with a wide array of reactants in the environment, such as carbohydrates, amino acids, phenols, enzymes, minerals, free radicals, viruses, and mutagens. It is then possible to assume that these interactions also occur *in vivo* and in

humans with the potential for modulating biochemical functions; therefore, establishing a role for them in the health of these biological systems.

Safety and Toxicology

Researchers have examined the safety of humic substances because they are widespread in the environment. Contrary to the research that confirms that peat derived humic substances are safe, other research indicates that humic substances in well water may be a potential cause in the development of an endemic peripheral vascular disease, known as "blackfoot disease". Furthermore, the potential mutagenic and prooxidant effects of particular humic acids have been documented *in vitro* and *in vivo*. Because humic acids occur ubiquitously in our environment and researchers are considering supplementing the diet with ese substances, information concerning the safety of humic acid should be considered.

Blackfoot disease found in the southwestern coast of Taiwan, Republic of China is a chronic disease of infarction (death of tissue following cessation of blood supply) in blood vessel terminals [33]. Clinically, it is characterized by numbness, black discoloration, ulceration, or gangrenous changes in the extremities. Clinical analyses have revealed that reductions in hematocrit value, hemoglobin content, and red blood cell count are common among patients with this disease. Epidemiologic and geochemical studies have found the presence of a high concentration of humic acid (200 ppm) in well water from areas where Blackfoot disease is endemic, as well as a high arsenic content [34]. Inhabitants of the endemic areas are very prone to chronic arsenism [35]. Both Blackfoot disease and arsenism are limited to people drinking artesian well water with a variable but high concentration of arsenic (0.10-1.81 ppm).

Lu [33] [35] [36] hypothesized that the combined effects of the arsenic and humic acid content of well water may cause this endemic disease. Other data by Lu indicate that the shortening of prothrombin times, or the acceleration of blood clotting time, results when arsenic is present in the synthesis of humic substances. [35] [36] [37]. The researchers believe that this could then contribute to the coagulation of blood and lead to ischemic events in the vascular system. It appears that arsenic alone did not have an effect on blood coagulation; therefore, arsenic may act as an auxiliary agent when combined with high amounts of humic acid to increase blood clotting *in vitro*.

Additional *in vitro* research indicates that fluorescent humic acid is a potent inhibitor of protein C activity, even in the presence of arsenic, which enhances protein C activity [34]. Protein C is responsible for the prevention of blood coagulation or clotting and it also acts indirectly as a promoter of plasminogen activators These results do not support the data by Klocking [31] that indicate peat humic acid may be effective in promoting plasminogen activator and thus dissolving thrombi *in vivo*. The research by Yang [34] used high concentrations of arsenic and fluorescent humic acid in an *in vitro* model. Furthermore, protein C activity is only one component of a complex system of blood clotting. Additional research by Lu and Lee [33] indicates that well water humic acid inhibits human plasmin activity (inhibits fibrin clot formation) *in vitro* and therefore, may be a factor in the balance of blood coagulation and anti-coagulation.

Regardless of the various ways that humic acid appears to effect the vascular and clotting system, further delineation of the exact relationship existing between arsenic and humic acid from well water in the development of vascular disorders is warranted. Blackfoot disease is endemic to a particular area where artisan well water is used, the average daily intake of humic acid is estimated to be 400 mg [38], and high levels of arsenic intake have also been reported. Furthermore, the composition and thus physiochemical properties of humic substances vary with different geographic regions and terrestrial and aquatic environments. This is evident when comparing the results of the two studies examining fluorescent humic acid from well water in China and humic acid derived from peat and their effects on blood clotting. Although one study was an *in vitro* model and one was *in vivo*, it is apparent that the humic acid from two different sources affected the same parameter of the blood clotting system in different ways. Therefore, it can not be assumed that the same physiological effects, as in Blackfoots disease, would be seen if humic acids were isolated from a different source, used in much smaller doses, and given in the absence of arsenic. More research is needed to elucidate the role of natural humic acids from well water in vascular disorders. Specifically, quantification of protein C, plasmin, and plaminogen activator in Blackfoot disease patients might clarify the role of these substances and well water humic acid in the development of this disease.

Although there has been a great deal of attention focused on the carcinogenic nature of compounds complexed with humic acid (i.e arsenic), only one study has found humic acid to be toxic *in vivo* [39]. In this study cytogenetic methods (study of chromosomal behavior in cells) were used for studying the genotoxic (toxic to genetic material in cells) effects of humic acid [39]. Using mice, the researchers were able to analyze their intestinal and bone marrow cells for numerical and structural chromosome abnormalities. Bernacchi et al [39] found that 100 mg/kg body weight of humic acid induced structural and chromosome abnormalities in mice intestinal cells. Induction of aberrant cells was time dependent and reached a maximum after 24 hr with continual aberrations up until 72 hr after animal exposure to humic acid. The researchers hypothesize that the humic acid could become chlorinated in the gastrointestinal tract, resulting in the formation of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), which has been shown to be the main factor responsible for humic acid mutagenicity and toxic side effects in Chinese hamster ovary [28] as well as in other *in vitro* and *in vivo* studies concerning chlorinated humic acid by products [40] [41].

Zhou et al [42] state that MX is one of the strongest mutagens, but is easily converted to its isomer, EMX. EMX shows a much lower mutagenicity in a pH > 7 environment; however, there is no evidence that this is true at a lower pH as would be found in the stomach and intestines. Other research indicates that the stability of MX under physiological conditions is low [43]. Dayan, a researcher at the International Agency for Research on Cancer, suggests that, "although chlorinated humic compounds present a hypothetical risk to man, their instability *in vivo* suggests that they are unlikely to be carcinogens." In addition, LaLonde and Xie [40] found that MX was inactivated by glutathione, a molecule responsible for the detoxification of xenobiotics (foreign compounds) *in vivo*. In conclusion, the amounts of humic acid used for these cytogenetic studies are extremely high in comparison to the amount of humic acid that would be ingested from HFC. In addition, none of the before mentioned studies used peat derived humic acid for their

experiments, but humic acid that was synthetically derived in the laboratory. It is premature to state that peat derived humic acid, when ingested in reasonable physiological doses as with HFC, would contribute to the formation of chlorinated humic acid by-products. Even if the chlorination of humic acid did occur *in vivo*, the humic acid chlorinated by-products would most likely be present in insignificant amounts that are not likely to be carconogenic. Furthermore, these compounds are very unstable *in vivo* and are detoxified by inherent biological enzyme systems in the body.

Humic acid was shown to exhibit only weak mutagenicity and toxicity in human peripheral lymphocytes *in vitro* at a high dosage of 250 and 500 mcg/ml. Although a positive mutagenic response with humic acid is apparent, it was quite low when compared to alachlor and maleic hydrazide, two known herbicides used to destroy unwanted plants. It should be noted that the variable results concerning the mutagenic activity of humic substances have been attributed to the heterogenous structures of humic acids and their reactivity with various compounds, which may produce toxic by-products. Thus interpretation of their exact biological role in mutagenesis is difficult. Because most studies use high doses of humic acid *in vitro* and *in vivo*, it is not reasonable to assume that these same effects would be seen if animals or humans ingested reasonable doses of humic acid. Furthermore, extrapolating *in vitro* and *in vivo* data to human safety is often times misleading. The type and amount of humic acid used in these studies are very different than what is present in HFC. Furthermore, peat derived humic acid has been documented as non-mutagenic and safe based on a series of acute, cumulative, and mutagenic toxicology studies of HFC containing humic acid (see HFC safety and toxicology section). These data are important to consider because they provide a safety profile of the use of humic acid in animals in both reasonable physiological doses as well as amounts far exceeding the recommended dose.

There is also the concern that humic acids, due to their potential antioxidant capacity may also exhibit prooxidant characteristics when ingested by animals or humans. It is known that nutrients such as carotenoids, tocopherols or ascorbate derivatives will demonstrate an antioxidant or prooxidant characteristic depending on the individual redox potential (ability to donate or accept electrons thus determining their antioxidant potential) of the molecule, the inorganic chemistry of the cell, and the amount of the nutrient available to the cells and tissues. [44]

Research has documented that humic acids can cause a depletion of glutathione in human red blood cells *in vitro*, but with fairly high amounts of 50-100 mcg/ml of humic acid [38]. In the same study, humic acids were also shown to decrease other antioxidant enzymes (CuZN SOD, catalase, G6PD) when humic acid was present at high concentrations of 100 mcg/ml. Cells have enzymatic systems, which convert oxidants into non-toxic molecules, thus protecting the organism from the deleterious effects of oxidative stress. When enzyme systems (i.e. superoxide dismutase, catalase, glutathione) are depleted in the presence of the testing compound, such as with humic acid, this indicates that these enzyme systems are working to detoxify any of the reactive oxygen species that have been initiated by the particular test substance. This substance would then be termed a prooxidant because of its ability to initiate oxidative stress. However, the development of a beneficial or a detrimental cellular response by a nutrient will depend on the nutrient's

antioxidant or prooxidant characteristics, which in turn are a product of the cellular oxygen environment that is influenced by normal metabolic processes as well as already existing pathologies. Furthermore, the prooxidant potency of various compounds is determined by several factors, including oxygen tension, concentration of the potential prooxidant, and interactions with other antioxidants [46] [47].

When an inappropriate prooxidant activity develops in normal cells, the reactive oxygen metabolites generated could damage the DNA and cellular membranes. This damage to DNA is thought to be partly responsible for the process of aging, diabetes mellitus, inflammatory diseases, and liver disease [45]. Furthermore, damage to proteins can cause alterations in transport systems or enzyme activities. And the well-known event of lipid peroxidation, when reactive oxygen species damage lipids in cell membranes, is thought to be related to several pathologies such as diabetes, atherosclerosis, and liver disease [45]. Although one study has documented the potential prooxidant activity of synthetic humic acid when used in ugh amounts, none of the adverse events of prooxidation have been documented in numerous animal and human studies using peat derived humic acid in reasonable physiological doses and amounts far exceeding the recommended dose.

Prooxidant activity can induce either beneficial or harmful results in biologic systems and influence the development of human chronic diseases. Most antioxidants can act as prooxidants under certain conditions, and more research is needed to determine the occurrence and importance of this *in vivo*. As exemplified in the studies with humic acid, these prooxidant effects usually occur when the test substance is used in high amounts, far exceeding the recommended doses of humic acid in HFC. Furthermore, no *in vivo* studies have demonstrated the prooxidant effects of the humic acids found in HFC.

A small body of literature points to a potential mutagenic (induction of structural changes in cells) and prooxidant effect (oxidative damage) of humic acid when given in high doses. However, many nutrients such as vitamin C and vitamin A are also thought to be prooxidants in high amounts, which in certain conditions can influence tumor growth. On the other hand, these nutrients are also potent free radical scavengers, which help to prevent oxidative damage to cell membranes when taken in reasonable physiological doses. The mutagenic and prooxidant results from the studies with humic acid can hardly be extrapolated to human consumption of HFC at the recommended dose levels. High doses of humic acid or its chlorinated by products would not likely be found in humans given a reasonable dose of a humic acid containing supplement.

In summary, it appears that the concerns about the safety of humic acid can be somewhat misleading if careful attention is not focused on several particular issues. The confusion arises from most studies concerning its safety have used high doses of humic acid isolated from well water or synthetically derived in the laboratory. Furthermore, different isolation techniques and experimental conditions will almost surely affect the synthesis of a complete and thorough review of the safety of humic acid. Therefore, to accurately assess the safety of humic acid, one must isolate the particular humic acid of concern from its respective environment. Its safety should then be tested based on amounts that would normally be consumed in the diet from that particular environment. These results should then be compared

to a reasonable physiological amount of the humic acid to generate a pharmacodynamic profile to substantiate acceptable amounts of these substances in the body. Recent research has clarified the safety of peat derived humic acid and fulvic acid (i.e. humifulvate from Hungarian peat) for use as a dietary supplement in animals and humans. It is apparent that humifulvate isolated from peats, specifically Hungarian peat, does not react under certain test conditions as does well water, soil, or synthetic humic acid. This has been demonstrated in animal and human data that has unequivocally documented the safety of humic acid as included in HFC.

FULVIC ACID

Structural and Chemical Properties

Fulvic acid is considered a macromolecular polymer with a structure and characteristics that change ›ng with its origins and humification processes. [6]. Fulvic acids, like humic acid, occur naturally in water, soil, and peat. They are produced by the chemical and microbial decomposition of plants, also known as humification. There is conflicting evidence concerning the degradative pathway of humic substance formation, specifically fulvic acid formation. Some reports suggest that they are diagenetically downstream of humic acids. In other words, it is thought that fulvic acid may be formed after the formation of humic acid. However, two different laboratory analyses have confirmed that a complex mixture of humic and fulvic acid exist in the same Hungarian peat deposit (Flora Research Laboratory, San Juan Capistrano, CA, November, 1999).

For the most part, fulvic acids and humic acids have been thought of as two distinct entities and their characterisitcs have been described in this manner. Fulvic acids are generally known to be more oxygen-rich and carbon-poor than humic acids. Much of the increased oxygen manifests itself in carboxyl and alcohol functional groups. Similar to its humic acid counterpart, fulvic acid contains many reactive ' nctional groups, including carboxyls, hydroxyls, carbonyls, phenols, quinones, and semiquinones. These eactive groups make fulvic acid a candidate for both metal chelating and antioxidant activity. The molecular weights of fulvic acids are thought to be less than humic acids. Peat fulvic acids contain significant levels of carbohydrate-like materials, derived from decomposing plant polysaccharides Figure 3 is a model structure of fulvic acid, depicting its numerous functional groups.

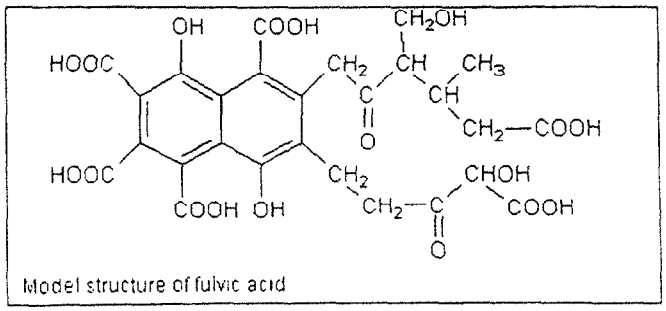


Figure 3 Model structure of fulvic acid [6]

Biological Role

Like humic acids, fulvic acids have also been shown to be effective chelators of both mineral ions and offending heavy metals [6] [18] as well as stimulators of oxidative phoshoryllation and energy production [3]. These effects have been found to favorably affect seed germination and plant growth, as well as increase the number and length of roots of plants [48]. Fulvic acid from peat has also been used for the clinical treatment of diseases induced by damage of oxygenated free radicals, such as arthritis, cancer, ulcers, and rheumatism disease [49].

The structure and chemical properties of fulvic acids are thought to be responsible for chelating mineral ions, and therefore indirectly affecting nutrient uptake and utilization of these minerals. Hence, the use of fulvic acids in enhancing seed germination and plant growth [6]. Fulvic acids may directly influence plant growth by stimulating oxidative phosphorylation, the process in plant and animal cells that generates energy. This stimulating effect of fulvic acid could be caused by the influences of fulvic acid on these respiratory processes of the plant, mainly through the stimulation effects of its quinol and phenol functional groups [48]. Fulvic acid has been shown to increase oxidative phosphorylation *in vitro*; however, the results with fulvic acid do not appear as strong as that for humic acid [3].

Furthermore, fulvic acid (as dissolved organic carbon) was found to effect the fractional uptake of cadmium in the intestinal segment of rats [18]. This is very important due to the carcinogenic and toxic nature of cadmium. Fulvic acids, like humic acids, have the ability to chelate heavy metals based on their cation exchange ability and reactive functional groups. However, the results from this study were not comprehensive enough to determine the mechanism behind the decreased absorption of cadmium in the

In summary, it appears that fulvic acids may act similarly to humic acids. This may be due to their acidic functional groups, primarily carboxylic acid and phenolic hydroxyl groups, that give them the capacity to react with various species such as free radicals, minerals, and biological enzyme systems [2] [6] [19]. However, due to the complexity of the structure and functions of fulvic acid, it is difficult to determine the exact mechanisms responsible for the effects seen *in vivo*. Further research may help to explain how these substances interact with biological systems.

Safety and Toxicology

Information on the safety and toxicology of isolated fulvic acid is minimal. However, fulvic acid is present in HFC, which has been subjected to toxicology and mutagenicity studies that have justified its safety. Furthermore, human studies have reported a limited number of adverse effects when using HFC containing fulvic acid. The importance of including data on aquatic fulvic acid and its relationship to the endemic degenerative joint disease in China further exemplifies the case with well water humic acid and the endemic vascular disease. Fulvic acid and humic acid occurring in terrestrial and aquatic environments are different in many ways. Furthermore, the fulvic acid and humic acid found in these endemic regions in China are very different from peat fulvic and humic acids. In fact, several other factors are present in determining the etiology of both endemic diseases. The following description of fulvic acid and its

proposed role in Keshan Beck disease will provide further support for the essentiality of distinguishing the humic substances in endemic regions of China from those found in peat. It will also provide additional documentation that many other factors play a role in the development of these endemic diseases.

Much research has focused on implicating isolated fulvic acid from well water in the generation of an endemic degenerative joint disorder in China. Kashin-Beck disease (KBD) is characterized by shortened stature and deformities of various joints in individuals residing in certain regions of China. Although the exact etiology of this disease has not been identified, three hypotheses for its development have been proposed: 1) organic substances (i.e. fulvic acids) in potable water, 2) mycotoxin polluted cereals, and 3) low environmental selenium levels. It is thought that removing any one of the three causes (adding extra selenium to food, reducing fulvic acid in drinking water, and providing toxin free grain) can reduce the incidence of the disease [50].

KBD has always occurred in low selenium areas [51], but it appears that selenium deficiency alone is not sufficient to be the cause of the disease. The pathology is quite complex, but in general terms the initial lesion is thought to occur as selective necrosis (death) of the cartilage cells at the base of the articular and growth plate cartilage in which the cells are subjected to damage by a toxic agent, when there is a deficiency of the required nutrient, selenium. Selenium has been shown to prevent the cartilage cells from damage when fulvic acid from water and fusarium oxysporum from grain are added to the culture medium [51]. In addition, high amounts of fulvic acid supplementation combined with selenium deficiency induced degeneration of the articular cartilage in the knee joints of mice [52]. It has been noted that the fulvic acid concentration in local drinking water in the endemic KBD areas is higher than in other areas, and it has some unique chemical properties. Furthermore, the mycotoxin polluted cereals may also induce free radical production contributing further to the degeneration of articular cartilage.

Wang et al [49] have documented research that found peat fulvic acid differed from the fulvic acid rom soil and drinking water in terms of their composition and content of elements, and content of their functional groups. Peat fulvic acid has been compared with other sources of fulvic acid, specifically from areas that fulvic acids are believed to play a part in the development of Keshan Beck Disease. The results indicated that peat fulvic acid could scavenge free radicals produced by biological and non-biological systems *in vitro*, while fulvic acid from the other sources (KBD and non-KBD regions) accelerated the generation of hydroxyl radicals in a dose dependent manner. In non-KBD regions, the toxicity of water fulvic acid is inhibited because selenium is a scavenging agent to reactive oxygen species, such as superoxide and hydroxyl radicals. Therefore, it is thought that KBD is an oxygen radical injured disease under the condition of low selenium. The differences of fulvic acid from KBD regions, non-KBD regions, and peat on free radical production and scavenging abilities indicate that fulvic acids of different origins cannot be confused or replaced by each other in the etiologic study of KBD.

HUMIFULVATE MULTIMINERAL LIQUID CONCENTRATE

Composition of HFC

The organic material content of HFC is near 50-70% (Table 1), specifically carbon arranged in aliphatic chains and aromatic moities, with hydrogen, nitrogen and oxygen contained in reactive functional groups. The humic substances found therein have peptide and saccharide chains with a protein content of about 10.5% [1]. See Table 2 below for the essential and nonessential amino acids contained in HFC.

The ash content or amount of inorganic minerals of HFC totals about 30%. The majority (10-18%) of the ash content includes calcium, aluminum, silicon, iron, and magnesium. One to ten percent is made up of sodium and boron. Barium, lithium, tin, manganese, copper, nickel, potassium, lead, molybdenum, berylium, and zinc make up the remaining 0.0001-1% [1]. Therefore, the peat used in the standardization of the humifulvate complex contains minute traces of naturally occurring minerals in which other minerals have been added for therapeutic purposes (Table 3).

Table 1: Composition of the peat vehicle (humifulvate)[1]

Component	% of total weight
Organic substance	55-70
Carbon	20-39
Hydrogen	3-4
Nitrogen	2
Total ProteinContent	10.5

Table 2: Distribution of amino acids [1]

Amino acid	% of total	Amino acid	% of total
Aspartate	16.9	Isoleucine	5.2
Glutamate	13.1	Lysine	4.5
Glycine	10.4	Proline	3.9
Alanine	8.4	Arginine	3.3
Valine	7.8	Phenykalanine	2.9
Threonine	7.1	Histidine	2.0
Leucine	6.1	Methionine	1.9
Serine	5.2	Tyrosine	1.3

Table 3: Mineral composition added to humifulvate [1]

Essential Minerals	mg/10 ml serving
Cobalt	0.2 mg (200 mcg)
Copper	2 mg
Iron	14 mg
Magnesium	15 mg
Manganese	3 mg
Molybdenum	.175 mg (175 mcg)
Potassium	37 mg
Selenium	.125 mg (125 mcg)
Vanadium	0.5 mg (500 mcg)
Zinc	10 mg

Biological Role

Humic and fulvic acids in water is thought to be a positive influence on biological growth in respect to phosphorus and nitrogen recycling, trace metal availability, and limiting potential metal toxicity [6]. Consequently, research has proposed that the standardized humifulvate derived from peat could positively influence trace element absorption in animals and humans by it ion exchange capacity. This unique property of humifulvate could potentially promote efficient uptake and incorporation of complexed essential minerals and trace elements into cells and tissues. Preliminary data indicate that this complex of humic substances derived from Hungarian peat in fact does affect the utilization (absorption, transport, and distribution) of essential nutrients. Although a few citations are not full scientific documents and are considered clinical observations, the data was still reviewed by the government officials and scientific experts in Hungary. The results of these clinical observations were certified for inclusion into their dietary supplement approval process indicating that they provide credible scientific data that should also be evaluated in the approval of this product. Following is a synopsis of the available research on the efficacy of this substance as a dietary supplement *in vivo* and in humans.

Elimination of Heavy Metals

Primarily investigations have focused on the ability of a microelement liquid concentrate, containing standardized humifulvate (HFC), to deliver essential minerals while also eliminating toxic heavy metals like lead, cadmium, and mercury. Oral consumption of HFC administered daily for six weeks significantly decreased blood cadmium levels and increased urine cadmium in 31 adult workers continuously exposed to occupational cadmium. [10] In the majority of subjects, initial abnormally low serum iron levels increased, and markers of kidney and liver function improved.

Research indicates that absorption of cadmium from the gastrointestinal tract and its toxicity are influenced by the supply of elements such as Zn, Cu, Fe, Se, Ca, and Vitamin C [8, 10] [19]. The ability of HFC, as an ion exchanger, may free its trace elements bound in chelate form for uptake into the tissues and bind other elements that are readily available such as Cadmium. At the same time a number of essential elements are provided that may decrease the ability of cadmium uptake and absorption in the gastrointestinal tract. The improvement of liver and kidney enzymes seen in this study population could be attributed to the effect of the preparation on the microelement status and balance in the body, which would then play a role in the functioning of these enzymes. HFC was studied for its effect on the metabolism of trace elements in 51 healthy adult volunteers. [53] Following two-weeks of oral administration of HFC, blood lead and cadmium levels decreased significantly. Furthermore, HFC decreased absorption of cadmium and lead from food or environmental exposure based on urine measures of these metals. HFC had no significant effect on blood parameters studied (i.e. hematocrit, hemoglobin, leukocyte count; SGOT, GGT, ALP; and, Na, K, Ca, and P).

Further evidence of the beneficial effects of HFC have been documented in both clinical trials evaluating occupational and environmental heavy metal exposure. In a three-week clinical observation with subjects screened for routine occupational health check-ups, 21 subjects were found to have higher than usual Pb levels (exceeding 1.0 micromol/l-health risk limit is 1.5 micromol/l) and 26 subjects had Cd levels exceeding the recommended health limit (0.08 micromol/l). Subjects given HFC showed a significant decrease in their blood Pb and Cd levels following the daily oral intake of HFC. No significant or pathological changes were obsreved in the blood chemistry of these subjects [54]. Additionally, HFC was administered orally to six adult subjects with moderately elevated lead levels that did not require pencillamine. HFC was administered to each subject for three weeks. Four of six subjects (66%) had significantly lower blood lead levels following three weeks of daily administration. The rate of decrease in lead levels in subjects was similar to that reported for penicillamine. [11] Two patients in the HFC group reported mild side effects and therapy was discontinued. The results from these clinical observations indicate that reducing toxic levels of heavy metals in humans is apparently influenced by treatment with the HFC multimineral liquid concentrate with no significant side effects resulting from its administration.

Two open clinical trials examining the effects of HFC in volunteers exposed to lead has provided further documentation of the beneficial effects of this standardized dietary supplement [55] [56]. Twenty individuals at risk for high occupational lead exposure were given 20 ml per day of HFC for six weeks. Blood levels of lead decreased markedly and significantly from the beginning of the study when compared to the control group [55]. None of the clinical or hematological parameters changed during the course of the treatment Two subjects reported mild and transitory diarrhea, which normalized without stopping treatment Four subjects reported moderate nausea and one a transitory headache Another open clinical trial in 60 subjects has demonstrated a similar but not as profound outcome [56]. At the end of a 12-week administration period, the change in serum lead parameters became significant compared to pre-administration values. The results of this trial are not as profound as the six-week administration of the

HFC in volunteers exposed to lead [55]. Although the reduction in blood lead levels was significant, a longer treatment time was needed due to the smaller dosage of HFC that was administered to the individuals. The examined laboratory parameters (i.e. serum blood, routine laboratory tests, liver and kidney function, and urine examination) exhibited no significant changes supporting the safety of HFC in the recommended dosage. Data from the two former studies indicate that the higher the serum or blood lead level, the more significant reduction in this parameter is observed. Furthermore, doses of 20 ml per day of HFC appear more effective in the treatment of occupational lead exposure.

Studies in animals have confirmed the beneficial effects of HFC on heavy metal chelation as seen in humans. Several studies using isolated humic acid have demonstrated that it does affect cadmium speciation in the intestine and thus absorption and distribution of this heavy metal (See humic acid biological role section for more detail). Additional studies using HFC in animals provide support for the ability of HFC to chelate heavy metals. A study investigating the effect of HFC on the elimination of mercury (Hg) isotope from pigs and their vital organs has been conducted. Adult pigs were fed varying doses of HFC or a control supplement. The pigs were given a mercury radioisotope. In examining the excretion patterns of the pigs, those animals that were fed HFC excreted more of the mercury isotope than did the control animals. Although the data was not significant, due to a small number of animals, this study warrants further research to document the efficacy of HFC in alleviating mercury accumulation. [57].

The effect of HFC on the absorption and incorporation of isotope labeled strontium chloride in rats has also been documented. Not only did HFC slow the strontium absorption and its incorporation into bone in these animals, it also affected the urinary excretion of this toxic element [58]. The urinary excretion of strontium in rats treated orally with the labeled strontium chloride and HFC occurred with less intensity than in those animals treated with only labeled strontium chloride solution. The authors conclude that a lower amount of the toxic element complex was absorbed when HFC was present. This same effect has been documented in humans exposed to cadmium and lead [54]. HFC decreased absorption of cadmium and lead from food or environmental exposure based on urine measures of these metals. Further data indicate that cadmium and lead urinary excretion increased in humans during the administration of HFC [10] [54] indicating the removal of this toxic element. Although it is premature to state the exact mechanism of action occurring in these animals and humans exposed to various heavy metals, it is safe to presume that the absorption and urinary excretion of heavy metals is affected by HFC.

Evidence for the protective effect of HFC bound with microelements from environmental exposure to irradiation has also been reported in the literature. The radioprotective effect of standardized HFC was tested in female wistar rats. HFC was given in one dose of 240 mg/animal (960 mg/kg body weight) and the rats were subjected to whole body irradiation. Baseline and outcome data (white blood cell, erythrocyte, platelet counts, and total serum iron binding capacity) were taken to substantiate claims of efficacy of the HFC treatment. The results showed improvements in platelet counts (leukocytes and thrombocytes) which had markedly decreased after irradiation. Platelet counts began to normalize in the

control group one week earlier than in the untreated control group of rats with just one dose of the HPC formula [59]. No side effects or toxicities were noted while administering HFC to this group of animals.

As indicated by the results of the previous data, the standardized HFC appears to be an effective chelator of offending heavy metals. Furthermore, it shows a protective effect against radiation *in vivo*. Its benefits could be utilized in the prevention of heavy metal contamination in workers in hazardous occupations, by decreasing the absorption and increasing the elimination of toxic heavy metals like cadmium. Furthermore, this standardized HFC would be beneficial in eliminating heavy metals that can be accumulated throughout a lifetime of environmental exposure, and alleviating the physiological consequences that occur with irradiation. Animal studies show a similar mechanism of action when comparing them with the studies in humans. Both indicate that HFC may work to decrease the absorption of these heavy metals as indicated by its affects on the excretion of these toxic elements in the urine.

Iron Restoration

Nutritional anemias, of which iron deficiency is the greatest cause, constitute the second most prevalent nutritional deficiency in the world, second only to protein-energy malnutrition [60]. Iron deficiency anemia affects primarily women and children and individuals with chronic disease. This nutritional deficiency respects neither social class nor geographic situation, as it is apparent in both developed and underdeveloped countries. Iron deficiency anemia is a condition in which the hemoglobin levels of red blood cells are lowered, and the red cells become smaller and deformed, thus reducing their oxygen carrying capacity. The most common cause is nutritional, including inadequate absorption of iron due to poor iron intake and reduced bioavailability. Iron loss from internal bleeding, low stomach acid and malabsorption are also important factors [19]. The standardized HFC may be an effective way to treat iron deficiency anemia and maintain adequate amounts of necessary minerals in proper balance for optimal ealth and well being.

The ability of the standardized HFC to restore iron levels and improve hematological parameters has been documented. Serum iron improved in fourteen adult volunteers given oral doses of HFC during a three-week period.[61] Serum ferritin levels approached the desired physiological range within three weeks. It was reported that for subjects with low iron values at the beginning of the study, their iron levels increased to within the desired range for iron status; conversely, those subjects who began the study with elevated iron status their iron levels decreased to within the desired physiological range. This finding demonstrated that HFC could facilitate homeostasis of iron status in humans.

HFC was given orally as an adjuvant during cytostatic therapy to tumor patients described as a series of case reports by several authors over a six-year period. [62-64] Cytostatic therapy is used for the prevention of the growth and proliferation of cancer cells; however, damage may also occur to normal cells such as erythrocyte cells (red blood cells), which may lead to anemia (the deficiency of red blood cells, hemoglobin, and blood volume). Therefore, iron therapy is needed, because it functions as a part of hemoglobin and thus red blood cell function. One group of patients showed significant enough improvement

in their erythrocyte counts that no further need for iron therapy was required. [65] Further subjective evidence of benefits experienced by these cancer patients included: improved appetite, weight gain, reduced need for analgesics, increased general stress resistance, reduced nausea, reduced fatigue, and restoration of the capacity to work. No significant adverse side effects were reported that could be attributed to HFC.

The standardized HPC formula was used for the treatment of anemias and for faster recovery from illnesses in children. Nineteen pediatric subjects with iron deficiency anemia were studied to determine if HFC given orally would improve their general well-being, appetite, and serum iron levels. [66] Subjects reported improvements in appetite and well-being after treatment with HFC. A rise in serum iron levels was seen as early as two weeks after administration had begun. After three weeks, HFC caused a significant increase of the serum iron level. Hemoglobin levels were variable, with some rising and others decreasing, but within desired physiological levels. [66]

This same effect (variable hemoglobin levels) was also manifest in elite athletes. Hemoglobin levels were studied to determine if oral administration of HFC would effect stress resistance, and the ability to increase the intensity of exercise, following oral administration of HFC in 25 elite adult athletes. [67] Hemoglobin levels in the athletes remained within the desired (normal) physiological range. Athletes reported a subjective improvement in stress resistance and their ability to focus during exercise periods. From the two previous studies, it appears that the standardized HFC may have the ability to normalize iron, serum ferritin, and hemoglobin levels so that optimal functioning can occur. Evidence for the effect of HFCs iron normalizing capabilities has been described in the literature. Protocatechuic acid (a phenolic monomer of HFC) can form Fe^{+2}-polyphenol complexes when excess amounts of iron are available. This occurs so that excess iron (Fe^{+2}) cannot react with oxygen molecules and form reactive oxygen species [68]. This provide further support for the metal chelating activity of HFC and implies that it has the ability to normalize iron levels so that excessive oxidation does not occur in the presence of higher than usual amounts of iron.

In vivo studies have also demonstrated the effectiveness of the standardized HFC for improving iron deficiency anemia in rats and pigs. HFC was tested on the iron deficient rat model by rearing the mothers and their offspring on an iron free diet. Iron deficiency was signified by severe microcytic, hypochromic anemia, and high zinc protoporphyrin (ZP) levels indicating the lack of iron at tissue level in the bone marrow. The iron deficient rat pups also exhibited a decreased weight at birth, decreased body mass gain, and increased lethality compared to controls. [69]. HFC was compared to the effectiveness of an official medicinal preparation, Aktiferrin syrup, which is commonly used in the treatment of iron deficiency anemia. Regarding the hemopoietic and hepatic effects, measured by red blood cells (RBC), mean cell volume (MCV), hemoglobin (Hb), hematocrit (Hct), total iron binding capacity (TIBC), transferrin saturation, and liver enzymes (ALAT, ASAT, GOT, GPT) respectively, HFC exhibited equal effects compared to the Aktiferrin [69]. However, HFC proved to be superior in that body mass gain of the pups was better in this group as compared to the Aktiferrin treated group. Additionally, serum triglyceride levels

were measured, and decreased concentrations normalized in the standardized HPC formula group but not the Aktiferrin treated group.

Further support for the beneficial effects of the standardized HFC in the treatment of iron deficiency anemia has been demonstrated in iron deficient pigs. Pigs of iron deficient sows that were fed the standardized HFC while pregnant exhibited significantly higher hemoglobin levels than did the pigs of iron deficient sows that were given the standard parenteral iron supplement treatment or no treatment [5]. These results and previous *in vivo* data indicate that the standardized HFC is an effective treatment for iron deficiency and may help restore impaired metabolic processes due to iron deficiency anemia.

Trace Mineral Supplementation

A number of factors have been associated with the occurrence of mineral deficiencies in humans: deficiency in the soil, water and plants; mineral imbalances; processing of water or soil; and, inadequate dietary intake [19]. Mineral deficiencies can result in a multitude of conditions, such as hair loss, eczema, fatigue, and illness just to mention a few. A dermatological study of head hair growth was conducted in 29 adult subjects experiencing hair loss related to suspected trace element deficiencies. HFC decreased hair loss and actually increased the regeneration of hair in some subjects. [70] This was attributed to improved trace element status in subjects, particularly for iron status. Serum iron levels rose in those patients who experienced improvements in hair growth and regeneration, but not in subjects with little or no improvement. The same author reported on similar results in children but the data was inadequate to reach a conclusion.

HFC has also been shown to produce a positive response in another condition associated with mineral and trace element deficiency, chronic eczema. Eczema is an acute or chronic inflammatory condition that causes itching and burning of the skin with resulting scales, crust, or scabs. Eczema is considered more a description of a symptom rather than a disease. Eczema is synonymous with dermatitis and has various etiologic factors, such as allergic reactions, and nutrient deficiencies. For example, protein deficiency is thought to be a causal factor in chronic eczema, and manganese deficiency produces scaly dermatitis [71] Severe zinc and magnesium deficiency may produce skin lesions [9]. It has also been reported that nutrients may be beneficial in the treatment of eczema. Selenium sulfate lotions inhibit different forms of dermatitis. Free form amino acids, manganese, magnesium, zinc, and selenium have all been implicated in the treatment of eczema. The response to oral administration of HFC over a three-week period was studied in nine pediatric subjects with chronic eczema. Eight of nine subjects treated with HFC showed marked improvement in the degree of eczema.[66] After the study was concluded and subjects no longer received HFC their eczema returned. As a result, the study was continued for an additional period of two to three months with the same subjects, and again during administration of HFC the amount of eczema decreased. After discontinuing the second phase of administration, symptoms began again to relapse. HFC was then continued for six more months in the same subjects and again the amount of eczema decreased. Thus, the possibility of treating chronic eczema with HPC should be examined further for its potential role

as a long-term treatment for this condition. The effects seen in this study could possibly be due to the combination of naturally occurring amino acids attached to HFC as well as the added minerals and trace elements to the liquid concentrate.

Research in a population of 51 healthy adults supports the role of the standardized HFC in improving microelement parameters [53]. The product significantly raised the level of copper in these individuals and improved iron metabolism. HFC had no significant effect on blood parameters studied (i.e. hematocrit, hemoglobin, leukocyte count; SGOT, GGT, ALP; and, Na, K, Ca, and P). All of these laboratory parameters were still within the normal range after the administration of the HFC. Isolated humic acid has been shown to facilitate the transport of several trace elements, including copper and iron, across the intestinal membrane of rats [7]. Therefore, this data in animals provides support for the mechanism of action of humic acid and the HFC containing humic acid in improving microelement parameters in humans.

Safety and Toxicology

A series of acute, cumulative, and mutagenicity toxicological studies of HFC-containing Humet® -R have been carried out by investigators in Hungary. As recently as 1999, the manufacturer of Humet® -R commissioned an independent review of all data to date on the toxicology and safety of Humet-R. This review, by a Hungarian M.D., PhD. researcher, confirmed the safety of this product for use as an oral multi-mineral supplement. The documentation of safety data in animals is considered adequate and applicable to humans as evident by the same mechanism of action that is thought to occur in both animal and human studies.

Most importantly, clinical documentation of both the short term and long term use and safety of HFC in humans is available. All animal and human toxicology studies have used HFC as found in Humet®-R to study its safety. The substance tested complied with using Good Laboratory Practices (GLP) methods and was performed by independent laboratories using reproducible analytical methods (IR spectroscopy and fingerprinting). Furthermore, elemental analysis by an independent laboratory (Flora Research Laboratory (San Juan Capistrano, CA, November, 1999) has documented that the levels of each mineral and trace element combined with HFC are well within safe ranges. The same independent laboratory has also reported that HFC contains non-toxic levels of aluminum, lead, cadmium and arsenic. Laboratory analysis performed by the National Institute of Food and Nutrition Science (OETI) (which serves the same function as the US FDA in regulating drugs and foods) in Budapest, Hungary, in 1991, found non-detectable concentrations of polycyclic aromatic hydrocarbons (PAH) in Humet®-R containing humifulvate

Acute Toxicology Studies

In a preliminary study, 84 wistar rats were followed for two weeks following varying doses of the standardized HFC for evidence of acute oral toxicity. The rats were both male and female and were given up to 10 gm/kg body weight of the HPC formula. No death occurred even in the highest dose administered

nor were there any signs of toxicity reported based on macroscopical alterations seen in the organs of the test animals. The LD_{50} value was determined to be higher than 10gm/kg body weight. The standardized HFC was classified as belonging to the "practically non-toxic" category [72].

An additional oral acute toxicity study was designed as a 'limit test'. A limit test is often performed for relatively nontoxic chemicals. Twenty male and female wistar rats were administered 20 ml/kg (300 mg/kg) of HFC two times a day in 24 hours [73]. All animals were continuously observed for six hours initially after the treatment and then twice a day during the post treatment. Clinical observations included, the state of the skin, fur, eyes, and mucous membranes; respiratory function, circulation, autonomic nervous system function; somatomotor activity, trembling, convulsions, salivation, diarrhea, and somnolence.

There was no evidence of weight loss in either of the groups and no macroscopic alterations of the animals' organs were found. However, in the control and treatment groups, the researchers observed a few cases of hemorrhage and emphysema in the lung, hemorrhage in the thymus, and hyperaemaia of the spleen in which there was no significant difference in the number of occurrences between the two groups. The authors noted that these conditions were associated with agony. The few cases of hyperaemia and hydrometra of the uterus were connected with the neurohumoral regulation of sexual function or the cyclic physiological state of the uterus.

Results of the study indicate that the standardized HFC caused no toxic symptom or lethality during a fourteen-day post treatment observation period. Therefore, the maximal tolerable dose (MTD) to be administered within 24 hours was determined to be >40 ml/kg, (>600 mg/kg). This study gives a more precise demonstration of the safety profile of the standardized HFC formula thus providing a base of evidence that this product is non toxic in applicable physiological doses.

Cumulative Test of Toxicity

The initial cumulative toxicity test with ten male Wistar rats involved their treatment with 10gm/kg (LD_{50}) of the standardized HFC for four successive days in increasing percentages of the test substance for a time interval of 24 days. Upon completion of the study, the animals' organs were measured and investigated for pathological signs. Additionally, the researchers documented body weights, hematological values, and thyroid hormones before and after treatment. Histological tests of tissues were administered after the treatment with the HFC. No significant differences between the control and treatment groups were found for any of the before mentioned parameters. However, the histological examination of the spleen did reveal an increase in haemosiderosis (signifies increasing concentrations of tissue iron and additional stored iron as haemosiderin) in the treated group versus the controls. There was no mention of total body iron indicating if tissue injury would be possible at this particular dosage. In a few cases for both the control and treated group, examiners noticed a moderate change in lung tissues noted as peribronchial lymphocytic infiltration, which could not be explained [74].

A subsequent repeated dose toxicity study was conducted in order to clarify the possible side effects that could occur after prolonged administration of the standardized HFC. Food containing the HFC was fed to wistar rats for 28 days in treatment doses of 1, 3, 10, 30, and 100 mg/200 g body weight per day. Control animals were fed normal rat food. Animals were observed daily, body weights taken weekly, and parameters of clinical chemistry, hematology and organ weights were measured at the time of necropsy. Two groups after week three of treatment with the HFC (doses of 30 and 100 mg/200 g body weight) showed a decrease in weight, which the authors attributed to a decrease in appetite influenced by the joint quantity of certain trace elements in the formula [75]. However, there was no mention of a decreased amount of food intake for these animals. Examination of organs showed no significant change from the controls except at the dose levels of 30 and 100 mg/200 g body weight per day, with organ weight loss in the liver and kidneys of these two treatment groups.

The results indicate that a four-week long dose of 1, 3, and 10 mg/200g body weight per day of standardized HFC does not influence the development of the tested organs. No death occurred in any animals and no significant differences were seen in tested chemical parameters such as hematological indices and enzyme functions. Although a more complete picture could have been achieved by measuring food consumption and performing histological examinations, this study provides additional evidence that the standardized HFC is a non-toxic substance especially when used in relative doses for administration in animals and humans.

Mutagenicity

The standardized HFC containing the Hungarian humic substances has also been subjected to four mutagenic studies and under the AMES test criteria exhibit no mutagenic activity. Five Salmonella typhimurium strains were used in the presence and absence of rat liver fraction with colony number in control plates and test plates being practically the same. The results indicate that the standardized HFC had no mutagenic activity and no bactericide effect using ≤ 7500 mcg of the test substance/per plate [76]. This amount is comparable to the amount used in testing pharmaceuticals for mutagenicity.

The effect of the standardized HFC on a known mutagen, ionizing radiation, has been studied using human peripheral blood lymphocytes. A preliminary study was conducted to confirm that the HFC was not mutagenic and an additional study was administered to determine its anti-clastogenic characteristics (ability to reduce the number of chromosome aberrations) against the known mutagen [77]. No chromosome aberrations were induced by any of the standardized HFC concentrations as compared to controls, with all of the concentrations being much higher than any physiological dose. Therefore, it was concluded that the standardized HFC is not clastogenic even in very high concentrations of 200 mcl/ml.

In the subsequent study concerning the anti-clastogenic effect of HFC, the resulting data is somewhat inconsistent. A significantly lower value of aberrant cells (abnormal cells) induced by irradiation was found when the cells were treated with the standardized HFC at a level of 5 mcl/ml. These results imply an *in vitro* anti-clastogenic effect of the standardized HFC. However, the number of di-centric ring aberrations

(diagnostic value in detecting radiation effects and thus chromosome aberrations) decrease as HFC concentrations decrease. The interpretation of mechanisms responsible for these effects *in vitro* was not attempted because of the illogical results at this data collection. The results do suggest that the standardized HFC may have potential anti-clastogenic effects; however, this cannot be stated as fact due to the variable results [77]. Considering that the standardized HFC was found to have no mutagenic activity in two studies using high doses of this substance, it is appropriate to suggest its relative safety for ingestion as a dietary supplement.

Table 4: Safety of HFC and/or humic acid in amimal experiments

A series of studies of HFC and humic acid given to mice and rats have evaluated the safety of umifulvate. The following table provides a summary of this literature.

Author/Year	Sample Size	Study Design	Dose of HPC formula	Study Duration	Adverse Events (A/E)
Antal, M., Ph.D, M.D., 1990 [73]	84 wistar rats 14 controls 70 treated	Acute Oral Toxicity (LD_{50})	0, 3, 4.1, 5.5, 7 4, and 10.0 g/kg body weight	2 weeks	• No death observed • No macroscopical alterations were seen in the organs of the test animals
Kovacs, M., RPh, Ph D, 1996 [72]	40 wistar rats 20 controls 20 treated	Acute Oral Toxicity "Limit Test Method" Maximum Tolerable Dose (MTD)	20 ml/kg (300 mg/kg) 2 x per day	24 hours	• No macroscopic alterations were found in the organs of the test animals • A few cases of hemmorrhage, emphysema, and hyuperaemia in both controls and test group.
Gachalyi, A., M.D et al. 1994 [75]	60 wistar rats 10 controls 50 treated	Random grouped Prolonged Oral Feeding	1, 3, 10, 30, and 100 mg/200g body weight per day	4 weeks	• No death observed • Organ weight loss observed in the liver and kidney of groups receiving 30 and 100 mg/200 g body weight per day
Desi, I., M D., Dsc. Nagymajtenyi, L, M.D., Dsc 1993 [74]	20 wistar rats 10 controls 10 treated	Cumulative Toxicity	10 mg/kg body weight (LD_{50})	24 days	• Haemosiderosis in treated group • Peribronchial lymphocytic infiltration in two animals in both the control and treated groups
Gundy, S., M.D. 1992 [77]	200 human peripheral blood lymphocytes	Clastogenic effect of the HFC	10, 20, 100 and 200 mcl/ml		• The chromosomes showed no structural or numerical alterations

Author/Year	Sample Size	Study Design	Dose of HPC formula	Study Duration	Adverse Events (A/E)
Gundy, S., M.D. 1992 [77]	600 cultured human peripheral blood lymphocytes	Anti-clastogenic effect of the HFC against ionizing radiation	1, 2, 5, and 10 mcl/ml		• No structural or numerical alterations • Anticlastogenic effect seen at 5 mcl/ml
Olah, B., M.D. 1992 [76]	Five Salmonella typhi-murium strains in the presence and absence of rat liver fraction	Salmonella typhy-murium reverse mutation assay (AMES TEST)	12, 60, 300, 1500 and 7500 microgram per plate	Icubated for 48 hours	• No mutagenic activity observed
Szakmary, E., ol.D., Ph D. and Hudak, A., Ph.D., M.D. 1997 [69]	40 controls 40 treated with Aktiferrin syrup 160 iron deficient rat pups treated with HFC	Controlled trial of the effects of humifulvate bound with iron and microelements in iron deficient rat pups	0.66 ml/kg (3.7 mg Fe^{+2} /kg body weight) of the HFC	21 days	• During first 8 days of treatment deaths occurred in every group • Otherwise no adverse events noted
Ferdinandy, P., M D 1997 [30]	32 wistar rats 32 treated	Controlled trial of the cardioprotective effects of SHA and HA in isolated working rat heart subjected to ischmia/reperfusion	10 mg/kg of humic acid (HA) 30 mg/kg of supplemented HA (SHA)	2 weeks	• No deaths • No adverse events noted
Dallo, J., M.D 1994 [78]	8 wistar rats 8 treated	Preliminary experiment with HFC in sexually inactive rats	1 ml per animal per day	15 weeks	•No adverse events noted
arudi, I et al 1997 [57]	15 pigs 4 controls 11 treated	Controlled trial of Humet-R on the mobilization of heavy metals	2.5, 7.5 and 20 ml/day	16 days	• No adverse events noted
Gundel, J. 1995 [5]	20 pigs 10 controls 10 treated	Controlled trial of Humet-R on iron deficient pigs	100 ml per day	No time period given	• No adverse events noted
Namenyi, J et al Patent application [59]	150 animals 30 controls 120 treated	Effect of regeneration of the hemopoietic system during cobalt gamma radiation	90 or 240 mg/animal per day of humic acid	Single dose and seven day pretreatment	• No adverse events noted

Deaths occurred in only two studies. In iron deficient rats, death occurred at the beginning of the study in both the control and treatment groups, with no significant difference between the groups. This was attributed to both the severity of iron deficiency anemia as well as the stress caused by the administration of HFC. Only one death was observed in the study evaluating the effects of HFC on sexually inactive older rats. The death occurred in an older rat; therefore, it cannot be assumed that the death of this aged animal was due to treatment with HFC. Other adverse events were apparent in three of the twelve studies. However, the occurrence of adverse events was noted in both the controls and treated groups in two of these studies and were not considered significant. Only two studies reported adverse events specifically attributable to HFC. When high amounts of HFC were used during a prolonged oral feeding in rats, a decrease in organ weights were observed in the liver and kidney of these animals. Furthermore, treatment with the LD_{50} of HFC for 24 days resulted in haemosiderosis in all animals. Increasing amounts of stored .on being the only significant adverse event reported during this cumulative toxicity study is very promising.

Human Clinical Trials and Case Reports

Clinical observation of HFC given to 514 patients under medical supervision for an average of 4.3 months of administration under controlled conditions have been reported. All case reports were obtained by us and reviewed and summarized. These case reports represent patients who sought out medical treatment for specific conditions or diseases. Physicians and public health workers with advanced degrees supplied the outcome data.

Table 5 reports the rarity of adverse events that have been noted during administration of the standardized HFC to humans. Although no significant adverse events were reported for any patient, 30 out of 514 individuals (5.8%) reported transient symptoms while taking HFC, including: headache, nausea, :artburn, diarrhea, or skin reactions. Since these types of transient events may be due to random chance, it is not possible to attribute them to HFC consumption.

Furthermore, there have been no documented incidences of adverse event reports in Europe where it has been used for eight years as a nonprescription drug. Some consideration should be taken in choosing the population in which this supplement should be used. For example, individuals with iron storage diseases (i e hemochromatosis, atransferrinemia, thalassemia major, and Y-linked hypochromic anemia) could have detrimental side effects from taking a supplement that contains iron or that may affect iron utilization.

Table 5: Adverse Events Reported in HFC Human Clinical Trials and Case Reports

(Observed in less than 6% of the population in which HFC was administered)

Human Clinical Trials

Author/Year	Sample Size	Study Design	Dose of HPC formula	Study Duration	Adverse Events (A/E)
Eva Sallay, M.D. 1998 [56]	60 adults Occupational Lead Exposure	Open Clinical Trial	10 ml per day	12 weeks	Significant: None Transient: • One patient reported gastrointestinal complaints • One patient with an allergic skin reaction
Hudak, P.hD, M.D. et al. 1997 [10]	30 adults Occupational Cadmium Exposure	Open Cohort Grouped Controlled	10 ml per day	6 weeks	No A/E or complaints observed during the treatment
Peter Szuts, M.D. Peter Koszo, M.D. 1996 [66]	60 children Iron Deficiency Anemia, Alopecia, Eczema, and Serious Illness	Open Clinical Trial	3ml per 10 kg body weight	3 weeks to 6 months	Significant. None. Transient • One patient with allergic skin reaction • One patient with diarrhea and abdominal complaints
Csaba Florian, M.D 1995 [55]	35 adults Occupational Lead Exposure 20 treated 15 control	Open Labeled Group Control	20 ml per day	6 weeks	Significant: None Transient • Two subjects reported mild diarrhea • Four subjects reported nausea and transitory headache

Author/Year	Sample Size	Study Design	Dose of HPC	Study Duration	Adverse Events (A/E)
Molnar Miklos, M D. 1992 [53]	51 adults Healthy Volunteers	Open Clinical Trial	20 ml per day	2 weeks	Significant. None Transient. • Two subjects reported abdominal pressure and nausea • One subject reported softer feces
a Lenart .Sc. [79]	11 Eite Athletes	Open Clinical Trial	Dosage not given	4 weeks	Significant: None Transient • Two subjects reported transitory digestion problems and diarrhea
Miklos Molnar , M D. 51]	14 adults Health Screening	Clinical Testing of HPC	Dosage not given	3 weeks	No A/E reported
Mate Petrekanits M Sc. [67]	25 Elite Athletes	Clinical Testing of HPC	Dosage not given	3 weeks	No A/E reported
Miklos M. Inar, M.D 94]	47 adults Occupational Exposure to Lead and Cadmium	Clinical Testing of HPC	Dosage not given	3 weeks	No A/E reported
Ivan Szekely, M.D. 1994 [11]	6 adults Lead poisoning caused by adulterated paprika	Clinical Testing of HPC	Dosage not given	3 weeks	Signficant: None Transient: • Two patients reported mild side effects No specific information given

Author/Year	Sample Size	Study Design	Dose of HPC	Study Duration	Adverse Events (A/E)
Sz. Szivkovics M D. 1997 [62]	40 patients adults and children Malignant Lymphoma	Open Phase II Study	10 ml per day to adults 5 ml per day to children	Duration not given	Significant: None Transient: • One patient abandoned treatment on the fourth day due to nausea and general weakness
Sandor Dienes,), M.D. 0]	12 adults 12 children Exposure to Lead through Pottery	Clinical Testing of HPC	7.5 ml per day	2 weeks	No A/E reported
Kovacs, L., M.D. et al. [70]	63 subjects with complaints of hair loss	Double Blind Clinical Trial	10-20 ml per day	4-6 weeks	No A/E reported

Case Reports

Author/Year	Sample Size	Study Design	Dose of HPC	Study Duration	Adverse Events (A/E)
Andras Gelley, M.D 1995 [63]	64 adults Cancerous Tumors	Retrospective Evaluation	10 ml per day	3 to 18 months	Significant: None Transient: • Epigastric pain in six patients • Heartburn in one patient • Stomach complaints and nausea in five patients
Elek Csuczka, M D 1991 [64]	10 patients Cancerous Tumors	Case Reports	Dosage not given	Average length of treatment 2 6 years	No A/E reported

- 514 total number of subjects taking HPC formula • Number of individuals with adverse events=30
- Average treatment period was 4 3 months

Long term use of HFC

Long-term use of HFC has been documented in 194 individuals. The average time frame for long-term treatment (defined as greater than 4 months consumption) with HFC was 12.0 months (range: 4 months to 5 years). Three patients under treatment for cancerous tumors consumed HFC for 5 years. [63] [64]. None of these three subjects required cytostatic therapy during the period in which HFC was consumed. No significant adverse events were reported for any of these subjects consuming HFC over a prolonged period. Improvement in well-being was reported by many of the individuals taking the HFC, even during times of cytostatic therapy which may cause immunosuppression and general malaise. Therefore, for improvements of subjective well being to occur in those individuals receiving HFC, one can conclude that this supplement may be a roborant during times of illness and disease.

An open clinical trial was conducted to evaluate the long-term use of HFC in the treatment of nine pediatric eczema patients [66]. The patients were given HFC for two to three months and then treatment stopped. There was a relapse of the symptoms; therefore, treatment with HFC resumed for an additional six months. Not only did the eczema improve in these patients, but no significant adverse reactions were reported due to the administration of HFC for greater than six months. One child reported an allergic skin reaction and one other child reported abdominal complaints and diarrhea. Therefore, the long term use of the standardized HFC is both beneficial and safe as documented in children and cancer patients.

Safety of Minerals and Trace Elements included in HFC

When considering supplementation with a particular mineral or trace element, evidence regarding its biochemical fate in the organism including absorption rate, retention time, excretion route, competition with other minerals, and any potential risks for side effects must be evaluated. For example, the valence (or number of bonds an element usually forms) will affect the absorption and complexation of that particular element or mineral. In addition, the binding of elements to the metal proteins in the liver will ultimately affect its ability to become absorbed, retained, and excreted. This means that regardless of the absolute levels of an element or mineral in a product, only a fraction of this amount will enter into circulation. Furthermore, competitive site absorption occurs when several minerals are administered together in an organic complex such as with the standardized HFC.

In addition, dietary intake of many minerals is below the Recommended Dietary Allowance (RDA) or estimated safe and adequate daily dietary intake as developed by the National Academy of Sciences. Therefore, supplementation with particular minerals and trace elements is essential for health maintenance The following is a table representing the elemental amounts (absolute amounts) of each essential mineral contained in HFC In addition, the biological role of each mineral and specific consideration regarding its use in the amounts listed below will be addressed

Table 6: Mineral composition of standardized HFC

Essential Minerals	mg/10 ml serving
Cobalt	0.2 mg (200 mcg)
Copper	2 mg
Iron	14 mg
Magnesium	15 mg
Manganese	3 mg
Molybdenum	0.175 mg (175 mcg)
Potassium	37 mg
Selenium	0.125 mg (125 mcg)
anadium	0.5 mg (500 mcg)
Zinc	10 mg

•Cobalt is thought to influence iron metabolism and increase the hemoglobin concentration in red blood cells. Daily doses of 25-40 mg of cobalt have been used in blood disorders such as the anemia in renal failure and thalassemia [81]. Cobalt is the metal component of the prosthetic group of vitamin B_{12}, therefore, it is necessary for the biological activity of this vitamin. It is also a component of several enzymes necessary for amino acid metabolism [9] [19]. The addition of cobalt to feedstuffs of cattle and sheep has improved the digestibility of nutrients and utilization of food [82]. Additions of cobalt have also increased the reproductive capacity, growth and development of chickens. Growing cattle can consume up to 50 mg cobalt per 45 kg body weight without ill effects, but higher doses are thought to be injurious [82]

There is no established recommended daily allowance (RDA) or intake (RDI) for cobalt for humans from the National Academy of Sciences. Reports have noted varying concentrations of cobalt in the diet. The average daily intake can range from as little as 25 mcg up to 600 mcg without resulting side effects [81] [83] [84] [85]. The oral intake necessary to induce the toxic effects are equivalent to a dietary cobalt concentration of 250-300 mg/kg, which is approximately 1000 times the concentration of cobalt in most normal diets [85]. In addition, cobalt is excreted from the body by the kidneys; therefore, any risk of toxic effects in intakes as reported daily in the diet and when using a supplement containing a relevant amount (i.e. 200 mcg) would be minimal.

•Copper has a significant role in the formation of red blood cells. It is required to absorb, utilize, and synthesize hemoglobin, which is necessary for red blood cell formation. Copper helps to maintain the integrity of the outer covering of the nerves, metabolize vitamin C, and utilize fatty acids for energy. It influences iron and zinc balances and when copper is in proper balance with zinc, the two elements act as antioxidants by removing damaging free radicals. Its deficiency may cause an increase in cadmium toxicity [19] The estimated safe and adequate daily dietary intake for copper is 1.5-3.0 mg a day [86]. Optimal intake is considered to be 1.5-4 mg/day [19] Diets provide about 0.9 to 1.0 mg of copper per day for women and approximately 1.2 mg per day for men, which is below the estimated safe and adequate intake

range of 1.5 to 3.0 mg per day. Therefore, a supplement containing 2 mg of copper would assure that an individual obtains an adequate and possibly an optimal amount of copper for health maintenance.

• Iron is the basic component of hemoglobin, the oxygen carrying protein found in red blood cells. It is also an essential component in the electron transporting cytochromes, which are found in the mitochondrial membrane, the site of energy synthesis. Therefore, its deficiency results in fatigue, headache, loss of appetite and resulting iron deficiency anemia. Iron intake potentiates the elimination of toxic lead [87] [19]. The RDA for iron varies with gender and age [86]. Fifteen mg/day for women of all ages (optimal intake 20 mg/day for age 11-18 and over age 51 and 22 mg for ages 19-50). Males require 12 mg/day for ages 11-18 and 10 mg /day after age 18 (optimal intakes for males 15 and 20 mg, respectively) [86] [19].

Iron deficiency anemia is one of the most common deficiency diseases in the world. Even in the United States, dietary surveys indicate iron intake to be inadequate to meet the RDA [19]. The most common cause of this is nutritional, including inadequate absorption of iron due to poor iron intake and reduced bioavailability. Iron loss, resulting from pregnancy, internal bleeding, parasitic infections (i.e. hookworm), and low stomach acid are also important factors contributing to iron deficiency. Iron can be a prooxidant in high doses and therefore iron supplementation should be restricted to cases of iron deficiency and anemia, vegetarians or vegans, menstruating, pregnant, or lactating women, or individuals with inadequate dietary intake, malabsorption or lack of hydrochloric acid. Deleterious effects of daily intakes between 25 and 75 mg are unlikely in healthy persons [86]. The average daily intake of iron is about 10 mg/day, which is below the recommended dietary allowance of 15 mg/day. In addition, dietary and medicinal substances such as calcium phosphate, phytates, bran, polyphenols in tea, and antacids can decrease nonheme (plant sources or iron) iron absorption substantially [86]. The addition of 14 md/day of iron as a dietary supplement is safe unless an individual has an iron storage disease.

• Magnesium is involved in more than 300 enzyme reactions in the body. One of its most important functions is in maintaining the function of the nervous system and neuromuscular transmission and activity. Magnesium is involved in glucose and protein metabolism. Magnesium also influences the metabolism of other minerals, such as calcium, phosphorus, and sodium, thereby affecting cardiac function and muscle tone of blood vessels. Magnesium is also thought to help protect against the toxic affects of excess aluminum intake [19]. The RDA for magnesium is 270, 400, 350 mg/day for ages 11-14, 15-18, and 19-51+, for males, respectively. For women, 280, 300, 280 mg/day is required for the respective age groups [86] Optimal intakes range from 300-500 mg/day [19].

Magnesium concentrations are found to be decreased in individuals with chronic alcoholism, diabetes and renal and intestinal disorders, hyperaldosteronism, inadequate nutritional intake, malabsorption, and drug therapy (i.e. thiazide treatment) [19]. Average intakes for women, children and men have been reported as 207 mg, 193 mg, and 343 mg, respectively [86], which fall below safe and adequate daily dietary intake for magnesium Therefore, a supplement containing 15 mg of magnesium

sulphate would not only be considered safe, but would also help individuals meet the recommended dietary allowance as estabalished by the National Academy of Sciences.

• Manganese is involved in protein, fat, and energy metabolism. It is also required for bone growth and development, and reproduction. Its deficiency can cause dermatitis, pigment disturbances of hair, growth problems, and infertility. The estimated safe and adequate daily dietary intake for manganese is 2-5 mg/day for all adult age groups [86]. Optimal intakes are 5 mg/day and 10 mg/day in individuals over age 51 [19]. Diets high in refined carbohydrates and low in plant foods may result in an inadequate intake of this essential nutrient. Furthermore, due to the addition of supplemental iron in the diet and its effects on manganese retention time, it is imperative to also include manganese as a supplement with iron [86]. Research also documents that manganese competes with iron and cobalt for common binding sites during absorption [9]. Thus, any of these metals, can exert an inhibitory effect on the absorption of others. Furthermore, a high fiber diet and supplementation with varoius nutrients (i.e. calcium, iron, phosphorus, magnesium, copper, vitamin E, D, and certain B vitamins) are thought to reduce the absorption of manganese [9] [88].

The Total Diet Study conducted in the United States between 1982 and 1986 indicated that the mean daily dietary manganese intake was 2.7 mg and 2.2 mg for adult men and women, respectively [86]. In humans, toxicity has not been observed as a consequence of dietary intake of 8 to 9 mg of manganese per day in their food [86]. Furthermore, due to the low toxicity of manganese, an intake up to 10 mg/day by adults can be considered safe and some researchers feel that increasing the upper value to 10 mg per day should be considered [9] [89]. Manganese is often considered among the least toxic of the trace elements through oral intake. However, the National Academy of Sciences recommends an upper limit of 5 mg/day for adults for an extra margin of safety. Unfortunately few data are available to support this estimate. The values apparently were set mainly through the reasoning that most dietary intakes fall in the range recommended by the National Academy of Sciences and would not result in deficiency or toxicity signs. Furthermore, the balance data in determining the safe and adequate intake is of questionable value [9]. As additional data become available, it will be important to reevaluate the estimated safe and adequate intake of manganese. In the meantime, consuming 3 mg of manganese sulphate per day in supplement form would be considered safe and may help individuals achieve optimal intakes for health maintenance.

• Molybdenum functions as an enzyme cofactor in many biochemical reactions within the body. It also acts as an electron transfer agent in oxidation-reduction reactions. The current estimated safe and adequate daily dietary intake for molybdenum in adults is 75 to 250 mcg per day, however, other sources indicate that a range of 150-500 mcg per day is safe and adequate for adults [90]. The daily intake of molybdenum ranges between 50 and 350 mcg; however, most diets are thought to supply only about 50-100 mcg per day [9] [86]. Furthermore, a diet high in protein, copper, or sulfate can decrease molybdenum availability from the diet [91] [92]. Thus, many diets do not meet the minimum level of the suggested safe and adequate intake for molybdenum [9] In addition, the percent of absorption of ingested molybdenum falls within the range of 25 and 80% and urinary excretion is 17-80% of the total dose [90] [9] Therefore,

molybdenum is considered a relatively nontoxic element since large oral doses are necessary to overcome the homeostatic control of molybdenum.

In nonruminants, an intake of 100 to 5000 mg/kg of food or water is required to produce clinical symptoms of toxicity. When researchers apply uncertainty factors of 10 for intraspecies and 10 for interspecies differences to "no observable adverse effect levels" in animals, a tolerable daily intake (TDI) can be derived for humans when human safety studies are limited. The most recent TDI has been given a medium confidence rating and it is more than double the upper limit of adequate intake for adolescents and adults that was derived from the molybdenum content of the average diet in the United States [90]. Based on the average dietary intake of molybdenum, its variable absorption rate, its status as a relatively nontoxic element, and recent TDI estimates, a dietary supplement containing 175 mcg per day would not only help an individual meet the recommended adequate intakes of molybdenum, but also appears to be safe for human consumption.

• Potassium is an essential element in maintaining fluid balance in our cells, contributing to the transmission of nerve impulses, the control of skeletal muscle contractility, and the maintenance of normal blood pressure. There is no RDA for potassium; however, research indicates that the minimum requirement should be between 1,600 to 2,000 milligrams a day [86]. Toxicity of potassium only results from sudden enteral or parenteral increases in potassium intake to levels of about 18 grams. A high sodium diet and the use of diuretics often administered in hypertensive patients promotes potassium excretion. In addition, potassium is found primarily in fruits and vegetables which are lacking in the US diet. Therefore, supplementation with potassium may not only be necessary in some individuals, but it is also extremely safe at a level of 37 mg a day.

• Selenium is a trace element with a number of biologic effects, although it is best known as an antioxidant because of its relationship with vitamin E. There is evidence that selenium may be protective against certain cancers. Selenium is also thought to be protective against the affects of toxic elements, such as arsenic, mercury, and cadmium [19] [93] by binding these metals. The RDA for selenium is 40, 50, and 70 mcg/day for men aged 11-14, 15-18, and 19-51+, respectively. Females require 45, 50, and 55 mcg/day for the respective age groups [86]. Optimal intakes range from 60-250 mcg/day depending on age and condition [19].

An estimated average dietary intake of 108 mcg/day between 1924 and 1982 has been noted in the literature [86]. However, dietary selenium intakes are difficult to estimate because of the variation of the selenium content of the soil in which the food is grown. Furthermore, some minerals (i.e. zinc and copper) are antagonistic to selenium, thereby affecting its absorption [94] Based on the available research indicating its safety in usual and therapeutic doses, and the fact that intakes must be over 750 micrograms [19] [95] over an extended period to be harmful, its use in a dietary supplement in the amount of 175 mcg per day seems justifiable.

• Vandium has been shown to be an essential trace element in the growth of animals [9] [96]. More recent research has indicated its use in the treatment of diabetes, hypertension, and lowering serum

cholesterol [96] [97] [98] [99] [100]. Vanadium is relatively abundant in nature and is found in a variety of foods; however, there is no RDA for vanadium from the National Academy of Sciences. Most diets are thought to supply between 6 and 20 mcg daily [9]. However, other sources have indicated daily dietary intake in amounts up to 2 mg [96] [98]. Research by Mcneill and colleagues [97] indicate that the preferred range for vanadium intake in man may be 0.0007 to 2.0 mg/kg/day (0.0525 mg to 150 mg a day (or 52.5 mcg to 150,000 mcg) for a 75 kg man) for its therapeutic benefits. In fact, during the age of metallotherapy, metavanadate was given in amounts of 1-8 mg (1,000 to 8,000 mcg) by mouth without any resulting signs of toxicity [96]. In addition, it is not uncommon to see dietary supplements on the market containing vanadium in the range of 500 -150,000 mcg.

Recently, research has documented the use of sodium metavanadate in amounts of 125 mg (25,000mcg)/day for two weeks in insulin and non-insulin dependent diabetics. The data not only suggests that vanadium may have a potential role as adjunctive therapy in these patients [100] but that side effects using higher amounts of vanadium were mild (i.e. gastrointestinal intolerance). Biochemical evidence of vanadium treatment revealed no sign of toxicity based on assessment of blood electrolytes, blood urea nitrogen, creatinine, liver function studies, thyroid functions, urinalysis, and complete blood count. Furthermore, another study has documented no significant side effects observed in non-insulin dependent diabetics who were given daily doses of 100 mg (100,000 mcg)/day of vanadyl sulfate for three weeks [99].

Several explanations for its use in these amounts should be examined. Due to the fact that certain dietary components (i.e. ascorbic acid, chromium, protein, ferrous iron, chloride, and aluminum hydroxide) effect the speed at which vanadium is transformed into a usable form, the percentage of ingested vanadium absorbed is effected [9]. Furthermore, little absorbed vanadium (less than 5%) is retained under normal conditions in the body due to homeostatic regulation [9] [98] [101]. Therefore, due to the variability of vanadium concentrations in the diet, the factors affecting its complete absorption, its homeostatic regulation by controlled accumulation and its low toxicity upon oral intake in humans, dosages of 500 mcg (or 0.5 mg) in a dietary supplement appears safe.

• Zinc is essential for the functioning of over 200 enzymes in biologic systems. A critical function of zinc is its role in the structure and function of biomembranes. It is also responsible for the synthesis of DNA and RNA. Furthermore, zinc is involved in immunity, wound healing, and the functioning of the central nervous system The presence of zinc is especially important in preventing toxicity of metal ions, such as lead, arsenic, and cadmium [19]. The RDA for zinc is 15 mg/day for males and 12 mg/day for females [86]. Optimal intakes range from 15-20 mg/day, males requiring 20 mg/day as they get older (19-51+) as is the case with females but in amounts of 17 mg/day [19].

The average dietary intake for zinc in the United States has been reported as 8.6 to 14 mg per day [9] [86]. However, absorption of zinc is largely dependent upon the presence of substances in the food that alter solubility or availability of zinc at the absorption sites [9]. Plant foods contain phytic acid, which explain, in part, the lower availability of zinc from these foods In addition, several elements with similar

physiochemical characteristics as zinc compete for common pathways. For example, zinc competes with copper, cadmium and iron for binding to the same carrier protein, thus metal competition exists, which can result in reduced bioavailability of one or more of these nutrients [9]. Furthermore, the simultaneous ingestion of equal amounts of ferrous iron and zinc (as sulfates) has been shown to depress zinc absorption [86].

Acute or chronic toxicity of zinc is very rare. Endogenous fecal zinc losses can be increased several fold to maintain zinc homeostasis with high intakes of zinc. In addition, there is no specific zinc "store", thus it is difficult to accumulate zinc in excessive amounts in tissues. Finally, zinc absorption decreases as an individual ages, and older adults average less than 2/3 of the RDA for zinc. Therefore, a dietary supplement may be necessary in these individuals. Levels of zinc supplements as low as 25 mg per day have been reported to induce copper deficiency, thus it is recommended to consume supplements containing less than this amount of zinc. Due to the fact that several factors affect zinc absorption and its toxicity is rare, a dietary supplement of zinc sulfate containing 66% of the RDA (10 mg) is considered safe [9].

The lack of toxicity of the ingredients used in this product is evident based on its use in Europe for more than six years without any adverse event reports, in addition to its documentation of safety in animal and human trials. Furthermore, the amounts of minerals and trace elements used in this product are considered safe as reported by the National Academy of Sciences-National Research Council for which estimated safe and adequate daily dietary intakes and recommended dietary allowances are available. In addition, the before mentioned parameters regarding the biochemical fate of minerals and trace elements in humans will ultimately affect the absorption, distribution, and retention of the various minerals used in this product. A cumulative body of evidence points to the safety of each ingredient in the standardized humifulvate based multimineral liquid concentrate for its use as a dietary supplement by humans.

Independent Laboratory Analysis of HFC

Independent laboratory analysis by Flora Research Laboratory (San Juan Capistrano, CA, November, 1999) reported that HFC contains non-toxic levels of aluminum, lead, cadmium and arsenic: 20.7 ppm, 0.07 ppm, 0.02 ppm, and 0.07 ppm, respectively. This has been translated into the amount that could occur in a single oral dose of the standardized HPC formula (Table 7).

Table 7: Trace Elements in HPC formula	mg/ 10 ml serving
Aluminum	0.18 mg (20.7 ppm)
Arsenic	0.0006 mg (0.07 ppm)
Cadmium	0.0018 mg (0.02 ppm)
Lead	0.0006 mg (0.07 ppm)

The levels of trace toxic metals in HFC were compared to the amounts of each metal found in the daily diet. Food can contain about 10 ppm of aluminum. Conservative estimates indicate that at least 2-3

mg of aluminum are consumed a day [9]. 20.7 ppm found in HFC is the same as 0.188 mg of aluminum in one 10 ml serving of the standardized HFC. This amount is less than one-tenth the amount of aluminum a person would consume in the diet on a daily basis. The amounts of lead and arsenic found in HFC are insignificant. Manufacturers of dietary supplements in the United States typically look to the standards for maximum exposure to heavy metals set by the State of California under "Proposition 65 [102]. The daily exposure of lead and arsenic from HFC complies with limits permitted by Proposition 65 in California.

Cadmium toxicity is generally based on oral inhalation of ambient cadmium. Therefore, other data must be utilized to determine its safety in the amounts found in HFC. One toxicology study concluded that the average cadmium intake should be kept below 110 mcg per day [103]. The FAO/WHO Expert Committee on Food Additives and Food Contaminants provides a tolerable weekly cadmium intake of 400-500 mcg per week for an adult, which translates into an average of 64 mcg per day. Average daily intakes from food in most areas not polluted with cadmium are between 10-40 mcg [104]. Therefore, the estimated amount of cadmium in a typical diet is ~5.5 to 25 times that found in the standardized HPC formula. The tolerable daily dietary cadmium intake as set by the FAO/WHO Expert Committee is 35 times the amount found in the HPC formula.

It has been proposed that humic substances may bind with or absorb mutagens rendering them less toxic or less mutagenic [20]. Among these mutagens are polycyclic aromatic hydrocarbons (PAH). PAHs are generated through inefficient or incomplete combustion of organic matter and, while initially released largely into the atmosphere, they are subsequently deposited in soil and water [105]. Stream humic substances have been documented to interact with PAHs [6]; these aromatic compounds have also been extracted from the deeper layers of peat (2.5 m)[2]. Therefore, PAHs are widely distributed in the environment and human exposure to them is unavoidable [106]. The food chain appears to be the dominant pathway of human exposure to toxic and mutagenic PAHs. The metabolic fate of hydrocarbons in mammalian systems has been extensively studied and, while many hydrocarbons are noncarcinogenic and efficiently detoxified, small fractions of some hydrocarbons are converted to electrophilic metabolites which are not effectively further metabolized and which are probably responsible for the carcinogenic properties of these hydrocarbons [105]. There is also speculation that humic substances, due to their binding with these compounds, may ultimately affect the fate of these carcinogenic products.

Evidence for the ability of humic substances to influence the fate of PAHs is provided by Johnsen and colleagues [107]. Uptake and bioconcentration factors of benzo(a) pyrene (a toxic PAH) in Atlantic Salmon were determined in water containing natural aquatic humic substances (AHS) and control water with low humic content. Uptake and bioconcentration of BaP were observed to significantly decrease in the presence of AHS compared to the control water. The rate for uptake of AHS bound with BaP was found to be 30 percent of that of free dissolved BaP [107]. Therefore, it is apparent that humic substances in water do have some effect on the uptake and concentration of PAHs *in vivo*. However, more research is needed to determine whether this is also true with terrestrial humic substances.

Some attempts have been made to understand mechanisms responsible for the effects of humic substances on PAH biodegradation. Sato [20] has reported that various PAHs were degraded by activated sludge. Furthermore, it is thought that the bacteria present in the humic acids and activated sludge may decompose the absorbed mutagens. Frolund et al. [108] reported that humic compounds could contribute to the enzymatic activity in activated sludge originating from a microbiological response. Other investigators have stated that the sorption of PAHs to organic matter renders the PAHs non-bioavailable and thus non-biodegradable, which may in turn affect biotoxicity [109]. However, these observations and speculations will require additional data to document the specific mechanisms responsible for humic substance-PAH complex biodegradation, bioavailability, and toxicity.

Laboratory analysis performed by the National Institute of Food and Nutrition Science (OETI) (which ·rves the same function as the US FDA in regulating drugs and foods) in Budapest, Hungary, in 1991, ,ound non-detectable concentrations of polycyclic aromatic hydrocarbons (PAH) in Humet-R containing humifulvate. None of the following PAH's were detected by OETI: benzo-(a)-pyrene, benzo-(b)-fluoro-anthene, indeno-pyrene, benzo-(k)-fluro-anthene, fluoro-anthene, or benzo-(ghi)-perylene.

In summary, when considering the toxicological data compiled from *in vitro* and *in vivo* laboratory tests and the lack of a significant amount of side effects reported in human subject studies, one can conclude that the standardized HFC taken in the recommended dosage of 10ml/day is safe. Furthermore, laboratory analyses indicate that HFC contains insignificant and non-toxic amounts of aluminum, lead, arsenic, and cadmium and is free of any carcinogenic compounds that may be hazardous if consumed in the diet.

CONCLUSION

Humic substances exist in all environments including soils, groundwater, streams, estuaries, and oceans. They are very reactive and are important participants in many geochemical reactions and processes. The functions they perform are multiple and varied and include, but are not limited to, the mobilization and transport of metal ions, contribution to the cation-exchange capacity of peat, soil, and water and binding of various organic molecules such as carbohydrates, lipids, and proteins. Furthermore, they may also reduce the toxicity of certain toxic compounds found in soils and waters. These characteristics provide a good base for the substantiation of the role that these substances can have in animal and human health.

However, the data on humic substances and an accepted conceptual framework of their role in the environment remain to be thoroughly integrated. Therefore, the elucidation of their role in animal and human health continues with attempts to discern their actual physiological significance. It seems that the observed differences and variable results in the literature may be due to differences in humic substances or the experimental conditions used in the collecting of data. Such differences emphasize the care that must be used in attempting to draw conclusions from data collected by different workers using different humic substances and experimental conditions. Thus, it is imperative to characterize humic substances in terms of

their origin and environment, rather in definite terms of chemical composition and/or properties. Especially when investigating their potential therapeutic activity *in vitro, in vivo,* and in human clinical trials. This is why such emphasis has been placed on the particular origin and environment of the standardized humifulvate complex.

Based on the available data on humic substances and HFC it can be concluded that these compounds have physiochemical properties that may lend them to be beneficial agents in the health and well being of animals and humans. Due to the formation and chemical structure of humic acid and fulvic acid from plant lignins, polyphenolic compounds contribute partly to the functional capacity of these humic substances. Polyphenols are known chelators of various metal ions; therefore, supporting the specific functional properties of humic acid and fulvic acid as effective chelators of metal ions and promoters of the ilization of essential minerals and trace elements in the body. Their combination with a bound multimineral complex was shown to restore iron and copper levels and hemopoietic profiles as well as reduce blood levels of heavy metals. In addition, this standardized HFC showed positive effects in regenerating hair loss, treating chronic eczema, and roborating individuals with serious illness and disease, which may be attributable to its ability to restore the necessary minerals that are lacking in these conditions. Minerals and trace elements bound to this standardized HFC should be absorbed very efficiently as compared to inorganic mineral supplements. This has been attributed to their binding with the organic compounds humic acid and fulvic acid, which support the function of transport proteins in the body; thereby promoting the effective uptake of these nutrients into cells and tissues.

The available literature on the safety of HFC for its use in dietary supplements appears very promising. Studies with the standardized HFC provide documentation of the safety of this product in respect to toxicology and mutagenesis. The diseases endemic to China (Blackfoots disease and Keshan Beck disease) appear to be related to an accumulation of other factors rather than just humic substances acting alone. Furthermore, the source of humifulvate used in HFC is different in respect to its origin, amount ingested and the lack of combined toxic elements that are found in humic substances in the endemic regions in China.

Expert opinions have documented the safety of the standardized humifulvate mulitmineral liquid concentrate based on *in vitro* and *in vivo* research. A number of human clinical trials evaluating its safety and efficacy have been documented and should be considered as important data justifying the use of HFC as a dietary supplement. Furthermore, the effects of this standardized HFC on the long-term use in pediatric patients and those with cancer are promising. The available literature on the standardized HFC confirms the safety and efficacy of this new dietary supplement as a source of essential minerals and trace elements as well as a facilitator of the proper utilization of these nutrients.

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