HUMIFULVATE – A NATURAL ACTIVE INGREDIENT

Introduction

The inventor of HUMET®, dr. Elek Csucska, once said: "…Creation takes care of those who need care. Creation holds the materials that Nature had preserved for the ages when civilized man, losing control, would harm himself and need help… We need to discover and study these materials."

Who could deny the relevance of these sentences written decades ago! Perhaps not known generally, but it is a fact that more than half of the precious stock of human medicines today are of natural origin or are made up of close analogues of natural molecules. Sometimes a new 'breakthrough' in therapy can originate from the discovery of a completely new natural substance. Just think of the bright success of the very efficient anticancer agent, taxol. People today turn 'back' to nature in increasing numbers: we can observe a renaissance of natural products, health preparations and food supplements. It is a new discovery – recognized as a modern thought by the scientific community also – that complexity is one of the major benefits of natural health products: their demonstrated effects may not always be traced back to a single, simple and easily describable active ingredient. They are rather complex mixtures of close or distant analogue substances, where the overall effect of the mixture is more beneficial than the sum of the effects of the individual components; in other words, a synergistic action takes place. Complexity, however, carries certain risks with it: because of the unbelievable variety of nature, it is difficult – although not impossible – to guarantee absolutely identical effect for natural materials reproduced many times in sequence. Just one example: the chemical profile of extracts from individual plants collected from the same chamomile field varies – although just slightly – with individual plants and differs by the period of day. Nevertheless it is not impossible to guarantee a permanent and standardized composition: it requires strict manufacturing circumstances, adequate analytical methods and dozens of biological and human studies.

The purpose of this paper is to provide a comprehensive, chemical and biological description – reflecting all that we know today – of a new active ingredient that demonstrates a series of beneficial effects and is made of a special kind of peat, known as Humifulvate, as well as its most common product formulation that contains added essential trace elements. As you will see, Humifulvate is essentially a unique blend of natural humic and fulvic acids. For this reason we had to include a short description of humin substances as found in nature, based on scientific literature. Since Humifulvate is most commonly used as a formula supplemented with essential elements, it seemed reasonable to give a brief summary of the biological activity of these elements also.

In addition, this paper has been compiled for yet another purpose: in the tail of the popularity of natural substances, general 'magical preparations', all-curative 'wonder mixtures' healing every ailment seem to appear from time to time and unfortunately in increasing number, taking advantage of people's naivety. It must be made clear that Humifulvate itself and the products derived from it do not fall in this category. We would like to demonstrate that our claims are based on dozens of – very expensive – trials designed and carried out with definite aims in mind

as well as experience of more than eight years of human consumption rather than on gossips, believes or anecdotal evidence.

I. LITERARY REVIEW HUMIC SUBSTANCES[1]

1.1. General description of humic substances

Humic substances are the most common forms of organic carbon in the natural environment. These organic key components of the soil and sediments are extremely widespread on the surface of the earth. Most humic substances are chemically attached to inorganic components (clay and oxides), and a smaller part gets dissolved in the solutions of the soil, particularly under alkaline conditions. An important feature of humic substances is that they can combine with metal ions, oxides and clay minerals to form water soluble or insoluble complexes and can interact with organic compounds such as alkanes, fatty acids, capillary-active substances and pesticides.

The humic compounds occurring in the soil and peat deposits play a vital role in the terrestrial and aquatic ecosystems [2]. Farmers use humates to accelerate seed germination and improve rhizome growth [1]. These materials are able to stimulate oxygen transport, accelerate respiration and promote efficient utilization of nutrient by plants [1,3,4]. Livestock food fortified with humic compounds has brought about improvements in the animals' reproduction cycles, resistance to diseases and growth rate [5]. These observations prompted scientists to study the specific properties of humates and their possible benefits in improving the health and well-being of humans.

Humic substances do not correspond to a unique chemical entity in structural and chemical terms and their characterization is difficult.
Aiken and colleagues [6] have defined these substances as follows: „*Humic substances are a category of naturally occurring heterogeneous organic substances of high molecular weight that can be isolated from the environment and operationally defined in terms of their solubility.*"
Several different humic substances have been identified:

Humus: This is the fraction of humic substances that is not soluble in water at any pH value. These substances have the greatest molecular sizes, as their molecular weights can be around 300,000 dalton. The oxygen content in this substance is the lowest and falls in the range of 32-34%, while the nitrogen content is the highest, being around 4%. Because of the high molecular weight, the negative surplus charge on their surfaces is insufficient for peptizing the macromolecules even at strongly alkaline pH, and so their mobility in the soil is insignificant when in a coagulated state.

Humic acid: This is the fraction of humic substances that is not soluble in water under acid conditions (below pH 2), but becomes soluble at a greater pH. Humic acids are soluble in dilute alkaline solutions and precipitate as soon as the solution becomes slightly acidic. These substances have medium molecular size and their molecular weight is around 5,000-100,000 dalton. Oxygen represents 33-36%, while nitrogen represents 4% in this substance. Because of

their medium molecular size, sufficient negative surplus charge on their surfaces for peptizing the macromolecules will occur only in a more alkaline medium with a pH over 8 and thus their mobility in the soil is limited in neutral acidic-alkaline conditions.

Fulvic acid: This is the name of the fraction of humic substances that is soluble under all pH conditions. Fulvic acids dissolve in dilute alkaline solution and will not precipitate even if the solution turns slightly acidic. These substances have the lowest molecular size, as their molecular weight is around 2000 dalton. This is the material with the highest oxygen content (around 45-48%) and the lowest nitrogen content (less than 4%). Because of their low molecular weight their surface negative surplus charge is sufficient to peptize the macromolecules even at neutral or slightly alkaline conditions resulting in significant mobility in the soil.

Phenolic acid: This substance is not defined based on solubility but it is identified as a component of humic substances.

The two most important groups of potentially therapeutic humic substances are humic and fulvic acids [1]. Scientific literature has documented two potential therapeutic benefits of these substances: 1. Their ability to positively influence mineral and trace element absorption, and 2. The capacity to bind heavy metals and thereby decrease potential metal toxicity. These activities are attributed to their ability to chelate and facilitate the utilization of metal ions. Phenolic acids have also been widely studied for their potential therapeutic properties, specifically their metal chelating ability. However their therapeutic role compared to humic substances still needs further investigation.

1.2. The balance in the formation and degradation of humic substances

The origin of humic substances

The easily disintegrating organic matter that gets into the soil transforms quickly – in optimal circumstances – into water, carbon dioxide and elemental nitrogen or inorganic nitrogen compounds. The non-decomposing organic complexes polymerize and transform into humic substances by adsorption to the nitrogen-containing materials.

Humus is clearly the product of the activities of microorganisms in the soil (bacteria, fungi, ray fungi). Therefore the structure and elemental composition and the number of functional groups of humic substances depends greatly on the circumstances of their formation, moreover the place is also determinant, because the process is greatly influenced by the microbial flora of the given location. Since the natural formation of humic substances is a polymerization process, the age of various humic fractions probably corresponds directly to the size of the molecules. The microbes digest the more easily decomposing organic materials, and burn them, while producing humic substances as end-products from the fractions that degrade more slowly.

Humic substances originate from degrading plant residues in the soil. In scientific literature, based on molecular similarity, the lignin content of the plants is considered to be the source of humus, but in fact all organic building blocks of the vegetation, including carbohydrates, proteins, fats, waxes and resins play their roles in the formation of humus.

Degradation of humic substances

The microbes of the soil are primarily responsible for degradation. Many aerobic microorganisms that live in the soil use the humic substances as their nutritional source, as they obtain energy for life activities from the oxidation of these substances. Degradation takes place in a reverse order compared to the build-up of vegetation, in other words the materials with the lowest molecular weights will disintegrate the most speedily. This is mainly due to the solubility characteristics of humin molecules and the speed of their transport to microorganisms or their cell membranes. The fact that the decomposing enzymes working inside the microbes or at their cell membranes can less easily access the sites catalyzed by the given enzyme as the size of the molecule decreases, also reinforces this tendency.

Balance in the formation and degradation of humic substances

Approximately 3% of the humic content of the soil is renewed every year. This means that in a balanced situation, that is when circumstances remain unchanged, this is the portion of the soil that disintegrates and gets newly formed. However, this statement applies to the easily degrading humic substances of the soil only. The more stable humic substances of large molecular weights can remain the same through very long periods, i.e. centuries.

The physical and chemical properties, water regulatory conditions, vegetation and microbial fauna of a given soil determine directly the nature of humification, i.e. the quality and quantity of the humic substances in the soil.

Generally, oxidation, in other words the higher air content of the soil creates an imbalance toward reducing the quantity of humic substances. The humin content of steadily and intensively cultivated soils that have been continually stirred up and are thereby penetrated by air, is significantly lower than that of rested soils. The rate, puffer capacity against reduction and average molecular weight of oxidative functional groups (that is carboxyl and phenolic groups) of the soil increase when the soil is cultivated.

While reduction increases the amount of humic substances, the average molecular weight and the levels of oxygen-containing functional groups are lower. Under extreme reductive circumstances the plant residues decompose and humify rather slowly.

Change in the vegetation has a significant impact on the humin content of the soil. It is interesting and thought-provoking however, that reduction of humus content was found to be prevalent in experiments in which forest areas were brought under agricultural cultivation. In the United States the humin content of the soil decreased by 64% in 30 years in corn monocultures.

The impact of reverse change was found to be much lighter and statistically less manifest. This phenomenon highlights the difference in the speed of humic substance formation and degradation. Therefore, the balance of formation and degradation cannot be controlled by using simply a chemical approach.

1.3 Methods for describing the humic substances

In order to be analytically characterized, humic substances first need to be isolated from their sources. Taking into consideration the complexity and sensitivity of the components, this is a rather difficult task. The **I**nternational **H**umic **S**ubstance **S**ociety (IHSS) recently approved a complex methodology suitable for obtaining the various components of humic substances. This methodology, although absolutely precise, is rather time-consuming and expensive.
The most common methods used to characterize the humic substances in the sixties were the so-called destructive testing methods (oxidation, reduction, pyrolysis). A number of non-destructive methods have been developed since then. These include elementary analysis, the assay of oxygen-containing functional groups or nitrogen-containing components. The properties of humic substances can also be described with the help of instrumental analytical methods. These include potentiometric and conductometric titration, and within the category of spectral methods, UV and visual, fluorescent, infrared, NMR and ESR spectroscopy. But other methods may also be used, including for instance X-ray diffraction, surface stress measuring, determination of molecular weight, steam pressure and membrane osmometry, size excluded chromatography (SEC), electrophoresis, ultracentrifuge, viscometry and mass spectrometry. Other methods worth mentioning include light dispersion, X-ray dispersion, electron microscope and ultrafiltration.

1.4. General chemical properties of humic compounds *Structure of humic molecules*

The skeleton of humic molecules is made up of a carbon chain of complex space formula containing a high amount of aromatic rings attached directly to one another or through oxygen and nitrogen as bridge units. The etheric, ester, keto, imine and imido groups occurring at the points where hydrocarbon particles are connected, render some parts of the molecule slightly hydrophilic, while the molecular parts consisting mainly of carbon and hydrogen are considered hydrophobic.

Functional groups
The characteristic properties of humic substances are due to the functional groups situated on the carbon chain. They could be acidic (e.g. carboxylic acid and phenol), alkaline (e.g. amine, imine) or neutral groups (alcohol, aldehyde, ketone, ether, ester and amide). The common feature of these functional groups detectable in most parts of the molecule is that they can render certain parts of the molecules hydrophilic, while other lypophilic parts are capable of binding materials that are immiscible in water. This soap-like property of humic molecules permits the binding of water-insoluble materials that do not get attached to the inorganic solid particles of the soil (fats, oils and the organic molecules soluble in them) as well as their transport in a colloidal solution through the soil, thereby facilitating their passage to plant roots and absorption to the plant. The functional groups that contain oxygen are prevalent in the humus molecules, and of all these, the carboxylic acid and phenolic groups are responsible for rendering the slightly acidic properties of humic substances. The groups that contain nitrogen primarily render certain parts of the molecule alkaline.

Colloid-chemical properties
Since relatively strong acidic and alkaline groups may occur within the humic molecule, this results in a dipolar ionic structure similar to that of proteins. This structure plays a major role in the electrostatic interactions among the various ions and the humic substances. A typical characteristic of humic substances is the isoelectric point (Point of Zero Charge, PZC), where positive and negative charges neutralize each other within the molecule. It refers to the pH value where the molecule is the least soluble, and this is where hydratation is the lowest and where the hydrophobic characteristic manifests itself the most expressively. Furthermore, the macromolecule in the soil and the root zone of the plant has the lowest mobility at this pH value. However, due to the dipolar ionic nature of the molecule, the zero charge applies only to the gross charge of the entire molecule, as there are a lot of positive and negative charges in various local sites of the molecule. If the pH value is lower, the gross charge of the molecule is positive, while if the pH is higher, it is negative. Because of the prevalence of oxygen-containing, acidic groups, the isoelectric point is in the pH = 3 - 5 range. As the set of molecules under discussion is not standard, only a pH range can be given.

Similarly to other macromolecules, the humic molecules also are capable of forming colloidal solutions only. This is important as these molecules can provide a surface for various surface-related processes, primarily adsorption in a diluted, mobilized condition. They resemble large molecular proteins in the living organisms in this respect also. Because of their extensively varied (almost random) molecular structure and continually changing spatial structure a lot of functional group combinations can be found on the surface of the humic substances that are positioned in such a way so that they are able to establish chemical bonds on the given molecules or create electrostatic interactions. Thus these groups may weaken the internal linkages of the molecule or the ionic substance and are capable of catalyzing chemical reactions, although with much lower efficiency than certain specific proteins.

Complex-chemical properties
On the same account, the humic molecules can function as ligands of complexes, similarly to chelated structures carrying appropriate central atoms. This effect is particularly important as the randomly coiling molecules can be fixed by a polyligand dative bond to stabilize the formed complex. This stabilized complex however may break up if the number of oxidation or the pH at the root of the plant changes, and so the humic molecule will deliver its central atom it had transported and make it available for absorption by the plant functioning therefore as an excellent carrier molecule.

2. THE COMPONENTS OF HUMIC SUBSTANCES

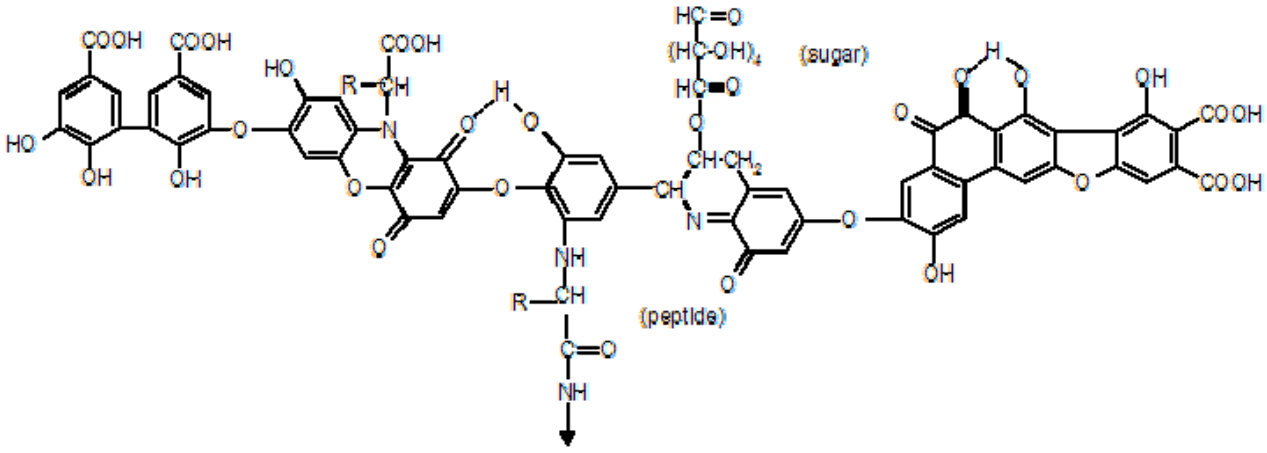
2.1. Humic acid

Structural and chemical properties
One could assume that due to the ubiquitous nature and structural and chemical properties of humic substances the environment is dependent on 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 toxicity in waters, soils, and thus possibly in plants, animals and humans as well.

Humic acids from peats show significant levels of phenolic carbons (C_6) 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, vanillin 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. Nevertheless, 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 1 below. It contains free and bound phenolic OH groups, quinone structures, nitrogen and oxygen as bridge units and carboxylic acid groups variously placed on aromatic rings.

Figure 1.



Model structure of humic acid

Because of the variable molecular composition of humic acids, a wide range of dissociation constants exists for the metals that are chelated by humic acids [1]. In addition, different metals are bound to humic acids with varying strength, and this would mean 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 microelement deficiency in the presence of Ca-humate in the 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 metals (Ca, Cd, Hg, Pb, Ba), forming insoluble complexes. The complexes formed are not available to plants and the concentration of

toxications 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 of various metals [13, 21]. Different metals have different affinities for different binding sites of the humic acid. 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 heterogeneous 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:
$Pb^{2+} \geq Cd^{2+} \geq Cu^{2+} \geq Ni^{2+} \geq Fe^{2+} \geq Co^{2+} \geq Mn^{2+} \geq Zn^{2+} \geq VO_2^{2+}$ [1].

Biological role

Although the physical and chemical properties of humic acids and their biological role in 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. In addition, 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 physicochemical properties of humic acids may also be responsible for some of the effects occurring in tissues; including the elimination of heavy metals, desmutagenic effects, antioxidant and anticoagulant activity.

Absorption and bioavailability

Studies involving rats demonstrated that the liver is the organ in which humic acids are absorbed and broken down. The breakdown of humic acids may take place also in the gut, as some 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 given 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 intestinal tract [22].

Metabolic effects

Humic acids seem to accelerate cell metabolism, the rate of breakdown of glucose, leucine and uridine. Humic acids seem to retard the rate of incorporation of these organic molecules into the liver, but once they are absorbed, humic acids appear to accelerate their metabolism. [7].
There are other data also that support the indirect influence of humic acids in enhancing the utilization of 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 following order: alkali metals (Na,

K) by 1 - 16%; alkali earth metals (Mg, Ca) by 50% and heavier metals (Mn, Fe, Zn) 80%. 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 facilitator action of transporting inorganic ions through biological membranes.

It has also been suggested 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. Humic acids can apparently 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]. As a result, more energy is generated for the individual for sustaining his normal functioning and for compensating for extra energy requirement caused by illness or some other stress.

Removal 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. 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, lead and mercury are among the most toxic and ubiquitous environmental metallic contaminants to which the population is exposed. Cadmium is known to be toxic to every body system, whether ingested or inhaled, and tends to accumulate in body tissues. The half-life of cadmium is 20 to 30 years. 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].

Long-term exposures to *cadmium* can result in renal damage [9]. Cadmium toxicity is manifested in a variety of syndromes, including kidney dysfunction [9, 10], hypertension, hepatic injury and lung damage. Furthermore, cadmium has an affinity to bind the transport proteins for minerals and displaces the ability of Cu and Zn to bind and be utilized effectively in the body [19].
The nervous system of newborns and infants is highly sensitive to *lead* exposure. The crucial effect of lead exposure on adults is hypertension: a significant direct correlation has been shown between blood lead levels and high blood pressure. This was manifested most explicitly in the blood pressure of men of 40 to 59 years of age.

The toxicity of *mercury* greatly depends on the form in which it appears: the elementary form and the inorganic and organic mercury compounds show different toxicity properties. The neurotoxicity of mercury poses the highest risk for the adult population, while methyl mercury has been shown to affect the development of the fetus in pregnant women.
Several studies were conducted in order to more clearly describe the role and mechanism of humic acids in alleviating heavy metal toxicity. 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 may contribute to a lower absorption of cadmium in the intestine.

It was concluded that the complexation of cadmium to the humic acid does not happen in the intestinal lumen, but rather humic acid may be responsible for influencing the metabolism of cadmium inside the cells (as reflected by the increased distribution of cadmium to the transport proteins), instead of affecting the uptake of cadmium into the intestinal cells [18].

Another study was designed to determine if the formation of cadmium complexes with humic acid 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 cadmium in the presence of humic acid in the intestines of rats in the previous study [18], the absorption of cadmium in mice was not affected by humic compounds in this study [22]. However, the organ distribution of cadmium was affected after absorption as indicated by the decreased fractional retention in the kidneys at the highest humic acid exposure level.

The authors explained the difference in results in the two former studies. 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 cadmium humic complex could occur in the presence of other binding ligands.

Furthermore, the heterogeneous nature of humic substances and varying functional group capacity could also be responsible for different results. Further studies are warranted to clarify the role that humic substances play in the speciation of cadmium in the intestines and their role in the distribution of this metal in the body.

Apart from these studies conducted with HFC (Humifulvate multimineral concentrate) there are only sporadic references to the relationship of lead and mercury with humin substances.

Desmutagenic effects

Due to the chelating properties of humic acids, emphasis has been placed on the possible role of these substances in preventing mutagenesis. A number of medicines, chemicals and physical agents such as ionizing radiation and ultraviolet light have the ability to act as mutagens and cause genetic mutations. Some natural plant-derived materials (e.g. humic acids, gycyrrhiza 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 break down a mutagen or promutagen, thereby providing a means of defense against mutagenesis [26]. Research suggests that the desmutagenic activity of humic acids is characterized by their ability to adsorb mutagens rather then 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 [8]. This is more characteristic of humic acids than the direct protection of the DNA from damage at the intracellular level. Thus, the mechanism of action of humic acid is not by inhibition of the metabolism of the mutagen, but the humic acid is instead binding to and inactivating the mutagen. It has been found that the ability of humic acid to adsorb mutagens increases with the molecular weight of humic acid. [27].

Cardioprotective effects

The leading health care problem and cause of death in the United States is cardiovascular disease, with 733,834 deaths in 1996. In 1994 22.3 million Americans were reported to be suffering from heart disease [29]. Risk factors associated with the development of this disease include obesity, high blood pressure, diabetes, smoking and decreased antioxidant protection. Atherosclerosis, or the narrowing of the blood vessels, may lead to heart attacks. During an ischemic insult, alleviating arrhythmias upon the return of blood flow to the heart muscle (reperfusion) are 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 scavenge free radicals and thereby decrease blood-clotting activity. Studies have documented the potential cardio protective role of humic acid [30, 31].

In an experiment, in which rats were subjected to ischemic insult, the administration of standardized Humifulvate concentrate (HFC) with microelements known as the Humet®-R product manifested beneficial effects. Coronary blood flow, aortic blood flow and left ventricular and diastolic pressure were improved in the hearts of the rats [30]. More data are needed to substantiate the mechanism by which the humic acid is beneficial as a cardio protective 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.

Another potential role for humic acids as cardio protective agents has been exhibited in an *in vivo* study examining the anticoagulant effects of these humates [31]. Although a less potent anticoagulant 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.

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]. These 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 brings about 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

Contrary to a large body of research confirming that peat derived humic substances are safe, some 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 these substances, information concerning the safety of humic acid should be considered.

Blackfoot disease found in the south-western coast of Taiwan 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. A high concentration of humic acid (200 ppm) was found in well water from the 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).

Chinese researchers [33, 35, 36] hypothesized that the combined effects of the arsenic and humic acid content of the well water may cause this endemic disease. It appears that arsenic alone does 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, when 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 from other research [31] that suggest that peat humic acid may be effective in promoting plasminogen activator and thus dissolving thrombi *in vivo*. Protein C activity is only one component of a complex system of blood clotting. Additional research [33] indicates that well water humic acid inhibits human fibrin clot formation *in vitro*, and therefore may be a factor in the balance of blood coagulation and anti-coagulation. The concentrations of humic acids used in the above *in vitro* experiments were significantly higher than the normal physiological concentrations.

Blackfoot disease is endemic to a particular area where the average daily intake of humic acid is estimated to be 400 mg [38], and high levels of arsenic intake have also been reported. As the composition and thus physicochemical properties of humic substances vary with different geographic regions, fluorescent humic acid from well water in China and humic acid derived from peat and their effects on blood clotting were compared. 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 cannot be assumed that the same physiological effects, as in Blackfoot 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.

Although there has been a great deal of attention focused on the carcinogenic nature of compounds complexed with humic acid (e.g. arsenic), only one study has found humic acid to be toxic *in vivo*. This study focused on the chromosomal behavior in cells for studying any possible genotoxic 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. Induction of aberrant cells was time dependent and reached a maximum after 24 hours with continual aberrations up until 72 hours after animal exposure to humic acid. The researchers hypothesize that the humic acid could become chlorinated in the gastrointestinal tract and the resulting compound is the main factor responsible for humic acid mutagenicity and toxic side effects in Chinese hamster ovary [28], and other *in vitro* and *in vivo* studies on chlorinated humic acid seem to confirm this [40, 41].

A researcher of 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, the amounts of humic acid used for these cytogenetic studies were extremely high in comparison to the amount of humic acid that would be ingested from the HFC product. Moreover, 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 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 carcinogenic. 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 the alachlor and maleic hydrazide, two known herbicides used to destroy unwanted weeds. It should be noted that the variable results concerning the mutagenic activity of humic substances have been attributed to the heterogeneous structures of humic acids and their reactivity with various compounds, which may produce toxic by-products. Thus interpretation of the exact biological role of humic acids 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 that ingested reasonable doses of humic acid. Furthermore, extrapolating *in vitro* and *in vivo* data to human safety is often misleading. The type and amount of humic acid used in these studies are very different from what is present in HFC. 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 that contained humic acid (see the HFC safety and toxicology chapter). 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 some concern that humic acids, due to their potential antioxidant capacity may also exhibit pro-oxidant characteristics when ingested by animals or humans. It is known that nutrients such as carotenoids, tocopherols or ascorbate derivatives will demonstrate an

antioxidant or pro-oxidant characteristic depending on the individual redox potential of the molecule, the inorganic chemistry of the cell and the quality of the nutrient available to the cells and tissues [44].

Research has documented that humic acids can cause the 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 pro-oxidant 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 pro-oxidant 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 pro-oxidant potency of various compounds is determined by several factors, including oxygen tension, concentration of the potential pro-oxidant and interactions with other antioxidants [46, 47].

When an inappropriate pro-oxidant activity develops in normal cells, the reactive oxygen metabolites generated could damage the DNA and cellular membranes. This damage to DNA is believed to be partly responsible for the process of aging, diabetes mellitus, inflammatory diseases and liver disease [45]. Furthermore, damage to proteins may 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 pro-oxidant activity of synthetic humic acid when used in high amounts, none of the adverse events of pro-oxidation 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.

Pro-oxidant activity can induce either beneficial or harmful results in biologic systems and influence the development of human chronic diseases. Most antioxidants can act as pro-oxidants under certain conditions, and more research is needed to determine the occurrence and importance of this *in vivo*. These pro-oxidant effects usually occur when the test substance is used in high amounts, far exceeding the recommended doses of humic acid in HFC. So far, no *in vivo* studies have demonstrated the pro-oxidant 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 pro-oxidant 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 pro-oxidants in high amounts, which in certain conditions can influence tumor growth. The mutagenic and pro-oxidant results from these studies with humic acid can, therefore, 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.

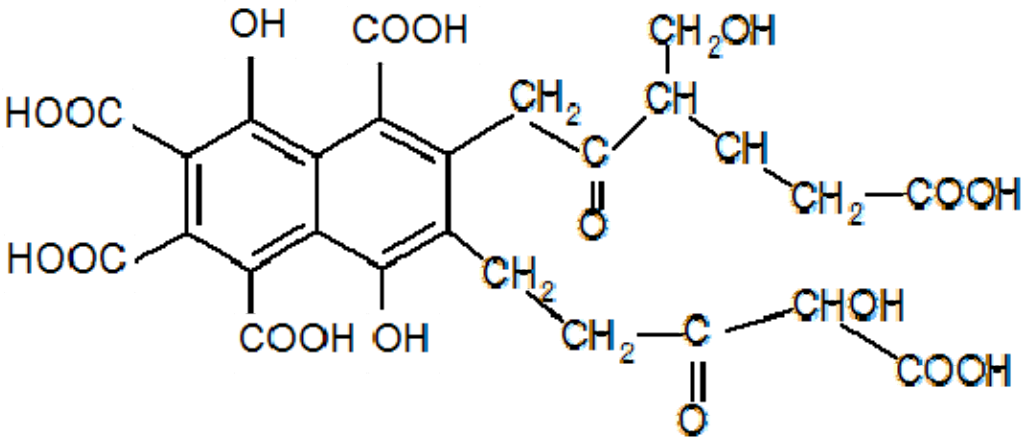
In summary, it appears that the concerns about the safety of humic acid can be somewhat misleading if careful attention is not focused on several particular issues. The confusion arises from the fact that most studies concerning its safety have used high doses of humic acid isolated from well water or 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.

2.2. Fulvic acid

Structural and chemical properties

Fulvic acid is considered a macromolecular polymer with a structure and characteristics that change along 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. 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 exists in the same Hungarian peat deposit.

For the most part, fulvic acids and humic acids have been thought of as two distinct entities and their characteristics have been described in this manner. Fulvic acids are generally known to be more oxygen-rich and carbon-poor than humic acids. Similarly to humic acids, fulvic acid contains many reactive functional groups, including carboxyls, hydroxyls, carbonyls, phenols, quinones and semiquinones. These reactive 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 those of humic acids. Peat fulvic acids contain significant levels of carbohydrate-like materials, derived from decomposing plant polysaccharides. Figure 2 shows a model structure of fulvic acid, depicting its numerous functional groups.



Model structure of fulvic acid

Figure 2.

Biological role

Just like humic acids, fulvic acids have also been shown to be effective chelators of both mineral ions and heavy metals, as well as stimulators of oxidative phosphorylation and the energy production. They 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 in plants. That is why fulvic acids are commonly used for 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. The quinol and phenol functional groups of fulvic acid may influence the respiratory processes of plants [48]. Fulvic acid has been shown to increase oxidative phosphorylation *in vitro*, however, the results with fulvic acid do not appear as strong as those for humic acid [3].

Fulvic acid was found to affect the 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.

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, which give them the capacity to react with various species such as free radicals, minerals and biological enzyme systems [2, 6, 9]. 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 the way 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 the standardized Humifulvate concentrate known as Humet®-R, which has been subjected to toxicology and mutagenicity studies that have justified the safety of fulvic acid also. Some human studies have reported a limited number of adverse effects when using HFC containing fulvic acid. The data on aquatic fulvic acid and its relationship to an endemic degenerative joint disease in China further exemplify the case with well water humic acid and the endemic vascular disease. Fulvic acid and humic acid that occur 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 the 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 a selective necrosis of the cartilage cells, when there is a deficiency of the required nutrient, selenium. Selenium has been shown to prevent the cartilage cells from damage in the presence of fulvic acid from water or fusarium oxysporum from grain. 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 should be 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.

It was also documented that peat fulvic acid differs from the fulvic acid from soil and drinking water in terms of their composition and content of elements, and content of their functional groups [49]. 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 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. The differences of fulvic acid from KBD regions, non-KBD regions and peat on free radical production and scavenging abilities indicate that the fulvic acids of different origins cannot be confused or replaced by each other in the etiologic study of KBD.

2.3. The role of metals

Cobalt is known 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 necessary for the biological activity of vitamin B_{12} and it is also a component of several enzymes necessary for amino acid metabolism [9, 19]. The addition of cobalt to feed-stuff of cattle and sheep has improved the digestibility of nutrients and the utilization of food. 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]. Cobalt has been also observed to increase the reproduction capacity and growth of poultry.

There is no established recommended daily allowance (RDA) or intake (RDI) for cobalt for humans from the Hungarian Academy of Sciences. Reports have noted various concentrations of cobalt in the diet, but the average daily intake can range from 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 a thousand times the concentration of cobalt in most normal diets [85]. Cobalt is excreted from the body by the kidneys; therefore any risk of toxic effects of the amount ingested as a dietary supplement (approx. 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, i.e. they remove damaging free radicals. Its deficiency may cause and increase in cadmium toxicity [19]. The estimated safe and adequate daily dietary intake for copper is 1.5 – 3.0 mg/day [86]. The optimal intake is considered to be 1.5 - 4 mg/day. Diets provide about 0.9 – 1.0 mg/day for women and approximately 1.2 mg/day for men, which is below the estimated safe and adequate intake range of 1.5 to 3.0 mg/day. Therefore, a supplement containing 2 mg of copper would assure that an individual obtains an adequate and possibly and 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]. It is 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). Men require 12 mg/day for ages 11-18 and 10 mg/day after age 18 (optimal intakes for males are 15 and 20 mg, respectively).

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 inadequate absorption of iron due to poor iron intake and reduced bioavailability. Iron loss resulting from pregnancy, internal bleeding, infections and low stomach

acid are also important factors contributing to iron deficiency. Iron can be a pro-oxidant in high doses and therefore iron supplementation should be restricted to cases of iron deficiency and anemia, vegetarians, pregnant or lactating women, or individuals with inadequate dietary intake, malabsorption or lack of stomach acid. The 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, and the recommended dietary allowance (RDA) is 15 mg/day, therefore an intake of 14 mg/day with a dietary supplement is still safe unless the individual has an iron storage problem.

Magnesium is involved in more than 3oo enzyme reactions in the body. One of its most important roles is maintaining the function of the nervous system and neuromuscular transmission and activity. Magnesium is also involved in glucose and protein metabolism and influences the metabolism of other minerals, such as calcium, phosphorus, and sodium, thereby affecting cardiac function and muscle tone of blood vessels. Magnesium is thought to help protect against the toxic effects 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 to 500 mg/day [19].

Magnesium concentrations have been observed to decrease in individuals with chronic alcoholism, diabetes and renal and intestinal disorders, hyperaldosteronism, inadequate nutritional intake 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, 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 established by the Hungarian 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 various 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 as much as 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. The Hungarian Academy of Sciences recommends an upper limit of 5 mg/day for adults, which provides an extra margin of safety. Unfortunately, few data are available to support this estimate. The consumption of 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. 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, some sources indicate that a range of 150-500 mcg per day is safe and adequate for adults [90]. Although daily intake of molybdenum ranges between 50 and 350 mcg, most diets are thought to supply only about 50-100 mcg per day [9, 86], Furthermore, a diet high in protein, copper, or sulphate 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 non-toxic element since large oral doses are necessary to overcome the homeostatic control of molybdenum.

In non-ruminants, an intake of molybdenum 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 average molybdenum levels in the diet in the United States [90]. Estimated based on the molybdenum levels of the human diet, the molybdenum variable absorption rate, its status as a relatively non-toxic 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 promote 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 biological 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 by binding these metals [19, 93]. 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. Optimal intakes range from 60-250 mcg/day depending on age and condition.

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 vegetables for food are grown. Furthermore, some minerals (e.g. zinc and copper) are antagonistic to selenium, thereby affecting its absorption [94]. Based on the available research indicating it is safe in usual and therapeutic doses, and because only intakes over 750 micrograms [19, 95] over an extended period are harmful, its use in a dietary supplement in the amount of 175 mcg per day seems reliably safe.

Vanadium 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 of 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 Hungarian 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]. The preferred range for vanadium intake in man may be 0.0007 to 2.0 mg/kg/day (or 52.5 mcg to 150,000 mcg for a 75 kg man) to show 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 (125,000mcg)/day for two weeks in insulin dependent 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 sulphate for three weeks [99].

It seems reasonable to examine some explanations for its use in these amounts. Due to the fact that certain dietary components (i.e. ascorbic acid, chromium, protein, ferrous iron, chloride, and aluminum hydroxide) affect 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+), and the same 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 physicochemical 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.

Acute or chronic toxicity of zinc is very rare. Endogenous faecal zinc losses can be increased several fold in order to maintain zinc homeostasis when there are high intakes of zinc. In addition, there is no specific zinc "store", thus it is difficult to accumulate zinc in excessive amounts in tissues. Zinc absorption decreases as an individual ages, and older adults average less than 2/3 of the RDA for zinc. 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 sulphate containing 66% of the RDA (10 mg) is considered safe [9].

3. DESCRIPTION OF HUMET®-R (HUMIFULVATE AND MULTIMINERAL) PRODUCT, TOXICOLOGICAL AND CLINICAL STUDIES

3.1. Introduction, definitions

Recently, a wide range of products containing Humifulvate as their active ingredient has become available. The classic product formula is the syrup (the HUMET-® syrup) supplemented with trace elements, also known as the Humifulvate Concentrate (HFC). Because of the sensitivity of the active ingredient, isolating it in a solid form posed substantial difficulties, and it took years of development to produce the solid product form. The solidified Humifulvate is designated as HF powder. More recently, producing a solid product that fully corresponds to the liquid HFC has also become possible, and it is going to be referred to as Enriched Humifulvate (EHF) hereafter. References to the individual substances will be made hereafter in accordance with the nomenclature given below:

HUMET-® syrup ≡ HFC: stabilized Humifulvate suspension supplemented with trace elements.
EHF ≡ solid HFC without stabilizing agent: enriched Humifulvate powder.
HF: Humifulvate powder.

As mentioned above the classic formula had been originally the HFC. In order to be able to generalize research data pertaining to this substance to apply also to the powder formulas, the two presentation forms of the products were subjected to a thorough comparison. It has been concluded that syrup made from EHF by adding water and stabilizing agent to it would be identical in all physical and chemical parameters with the original suspension product (HFC).

3.2. Description of Humifulvate

Definition and origin

Humifulvate is a standardized peat-derived humic acid, fulvic acid and phenolic acid complex intended for oral consumption. Humifulvate is the base compound to be used in combination with minerals and trace elements as a dietary supplement. Since 1993, the **standardized liquid concentrate (HFC)** has been sold in numerous European countries as an OTC product called **Humet®-R**.

The Hungarian Humifulvate to be found in this product has been derived from geologically young Hungarian peat, estimated to be 3,000-10,000 years old [1]. The composition of this substance may be due to the botanical composition of the deposit growing at the time. This unique 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. The peat deposit subjected to scientific research shows uniform geological and chemical characteristics. As it is located in a natural conservation area, no industrial or agricultural activities are performed in its environment.

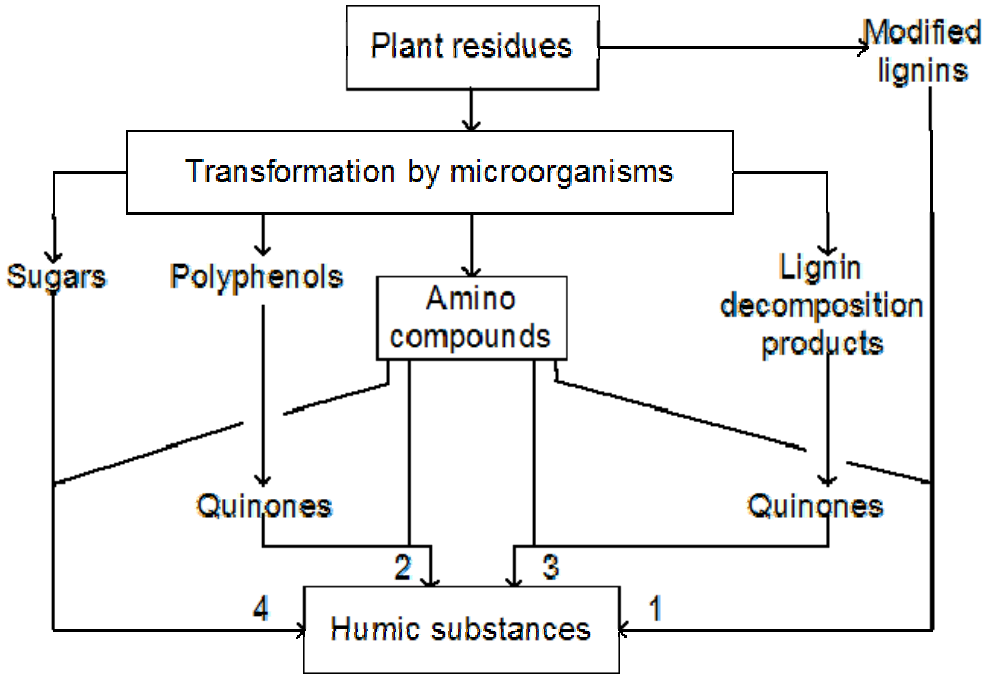
It is known that peat is a highly organic deposit, or in other words, the accumulation of plants and vegetable matter that have humified over a period of thousands of years. Due to differences in the source and nature of the surrounding aqueous environment, peat varies in its botanical origin, extent of humification, and present flora [6]. However, its increasing degradation leads to the progressive evolution of humus first, then humic acids, and finally fulvic acids [2]. Peat is therefore a storehouse of nutrients, and as such, can be exploited to produce high quality vegetable crops [6].

Humic and fulvic acids are complex organic molecules, which comprise some 60-80% of peat and soil organic matter. 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. They can be oxidized to quinones 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 (pyrocatechic acid, vanillic acid, vanillin, resorcinol, ferulic acid, and benzoic acid) would then contribute an important part to the structure of these molecules. 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 3 shows the mechanism of humic substance formation in the soil or peat.



Mechanisms for the formation of soil humic substances

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]. 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 is derived from well-defined, standardized peat using a thoroughly controlled technology. This manufacturing technology developed with a view to the stability of the raw material and the sensitivity of Humifulvate, and including interim and final product control, ensures the essential compositional consistency of the product.

Humifulvate contains oxygen-, nitrogen-, and sulphur-containing functional groups that made 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 and phenolic hydroxyl 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 and acetylation, the extent of activity and metal binding is drastically reduced [1, 2].

The percentage of the humus, 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 of soil.

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. The greater the degree of humificaton, the higher is the cation exchange capacity. The contribution from humic and fulvic acids is due largely to the ionization of carboxylic groups, although some contribution from phenolic hydroxyl and amine groups has been observed. 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. 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 co-ordination through attached metal ion (ligand exchange) [6].

Composition of HFC

The organic material content of HFC is close to 50-70% (Table 1); it is made up specifically of carbon arranged in aliphatic chains and aromatic moieties, 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]. Table 2 below shows 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, beryllium, 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 to which other minerals have been added for therapeutic purposes (Table 3).

Table 1: Composition of peat Humifulvate [1]

Component	***% of total weight***
Organic substance	55-70
Carbon	20-39
Hydrogen	3-4
Nitrogen	2
Total protein content	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	0.175 mg (175 mcg)
Potassium	37 mg
Selenium	0.125 mg (125 mcg)
Vanadium	0.5 mg (500 mcg)
Zinc	10 mg

3.3. The biological role of Humifulvate

It is evident that humic substances can affect several biological processes [7]. HFC has been demonstrated to support the normal transport, absorption, and distribution of essential nutrients in the human body.

Humic and fulvic acids in water are thought to have a positive influence on biological growth in respect to phosphorus and nitrogen recycling, trace metal availability, and the limiting of 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 its ion exchange capacity. This unique property of Humifulvate could potentially promote efficient uptake and incorporation of complex essential minerals and trace elements into cells and tissues. Preliminary data suggest that this complex of humic substances derived from Hungarian peat in fact does affect the utilization (absorption, transport, and distribution) of essential nutrients.

Elimination of heavy metals

Humifulvate has the capability of transferring metals to and from metalloproteins *in vivo*. [18]. These proteins play a role in metal storage and sequester excess metal ions, preventing toxicity. Metalloprotein concentrations are the highest in the liver where metals accumulate in the metallothionein portions of this organ. Metalloproteins can be found in many other human tissues, including small amounts in the blood plasma, which suggests that these proteins play a role in the transport of metals as well. [9].

When the free metal binding capacity of Humifulvate gets 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, helping in the excretion of these metals (i.e. in the case of toxic heavy metals like cadmium). Therefore, it may be concluded that Humifulvate may act somewhat like metalloproteins due to its chelating activity and ion exchange capacity. When metals are a part of a metalloprotein, they can modulate its biochemical reactions [19].

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 element 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 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 pieces of evidence of the beneficial effects of HFC have been documented in 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, the health risk limit being 1.5 micromol/l) and 26 subjects had Cd levels exceeding the accepted 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 observed 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 penicillamine. HFC was administered to each subject for three weeks. Four of these six subjects (66%) had significantly lower blood lead levels following three weeks of daily administration. The rate of decrease in lead levels in the 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 following its administration.

Two open clinical trials examining the effects of HFC in volunteers exposed to lead have provided further documentation of the beneficial effects of HFC [55, 56]. Twenty individuals with 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. 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, which supported 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 can be 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. Some 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 the Biological Role of Humic Acid section). Additional studies using HFC in animals provide support for the ability of HFC to chelate heavy metals. Adult pigs were fed varying doses of HFC or a control supplement and the excretion of a mercury radioisotope previously administered, was examined. 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 the 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 has also been documented. Not only did HFC slow the strontium absorption and its incorporation, it also affected the urinary excretion of this toxic element [58]. The urinary excretion of strontium was less intensive in the animals fed with HFC. The authors concluded 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]. Based on urine measures of these metals, HFC decreased the absorption of cadmium and lead from food or environmental exposure. 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 against environmental exposure to irradiation has also been provided 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 HFC formula [59]. No side effects or toxicities were noted while administering HFC to this group of animals.

As indicated by 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 effects 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 present 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 health.

The ability of the standardized HFC to restore iron levels and improve hematological parameters has been documented. Serum iron levels 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 [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 iron functions as a part of hemoglobin and thus red blood cell function. One group of patients showed significant enough improvement in their erythrocyte counts so 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 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 in 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 the oral administration of HFC would affect 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 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. Evidence for the effect of HFC's 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 amount 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 an 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 (MVC), hemoglobin (Hb), hematocrit (Hct), total iron binding capacity (TIBC), transferring 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 group. Additionally, serum triglyceride levels were measured, and decreased concentrations normalized in the standardized HFC formula group but not in 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 offers an effective treatment for iron deficiency and may help restore impaired metabolic processes due to iron deficiency anemia.

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. 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, notably chronic eczema. Eczema is an acute or chronic inflammatory condition that causes itching and burning of the skin. Eczema has various etiologic factors, such as allergic reactions, and nutrient deficiencies. For example, protein deficiency is thought to be a casual 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 sulphate 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. [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. 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 HFC 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.

3.4. Safety and toxicology

Since humic substances have existed in nature well before human existence, 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: Blackfoot disease and Keshan Beck disease. Those endemic conditions are associated 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. The standardized HFC preparation derived from Hungarian peat has been documented not to contain toxic materials and so it should not be compared to the humic substances from China. Still, as the Hungarian

preparation is to be marketed as a dietary supplement, its acceptable intakes should be determined.

The lack of toxicity of the ingredients used in this product is evident knowing that the product has been used in Europe for more than six years without any adverse event reports. Furthermore, the amounts of minerals and trace elements used in this product are considered safe, for which estimated safe and adequate daily dietary intakes and recommended dietary allowances are available. 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.

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. Cumulative and subacute toxicity and mutagenicity studies have also documented the safety of HFC. Furthermore, a review of human clinical studies indicates 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 in animal and human studies.

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 available to date on the toxicology and safety of Humet-R. This review confirmed the safety of this product for use as an oral multimineral supplement. The documentation of safety data in animals is considered adequate and applicable to humans, as is implied 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 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 Hygiene and Nutrition (OÉTI) (the authority regulating foods) in Budapest, Hungary, in 1991, did not find detectable concentrations of polycyclic aromatic hydrocarbons (PAH) in Humet®-R, the product 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 HFC formula. No death occurred even in the highest dose administered, nor were there any signs of toxicity reported based on macroscopic 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.

An additional oral acute toxicity study was designed as a 'limit test'. A limit test is often performed for relatively non-toxic 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 hyperemia 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 hyperemia 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 HPC formula, thus providing a base of evidence that this product is non-toxic in applicable physiological doses.

Cumulative toxicity test

The initial cumulative toxicity test with ten male Wistar rats involved their treatment with 10gm/kg (LD_{5O}) 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 the concentrations of tissue iron and additional stored metals 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 gram 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/200 g 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.

In a 60-day toxicology study involving rats fed with powdered Humifulvate in doses of 60mg/animal/day (~300 mg/kg body weight per day) and 240 mg/animal/day (1200 mg/kg body weight per day), respectively, no deaths were reported. The general condition and physical parameters of the animals did not change. The hematology parameters did not change, either. No deaths were observed in another 180-day subchronic toxicology study on dogs, in which the animals were fed with EHF powder even at doses fourfold the usual human dose. The dose with no observable adverse effects (NOAEL) was established as 15 mg/kg body weight, as at higher doses the expected clinical adverse effects (nausea, diarrhea) could be observed with dose-dependent frequency.

Mutagenicity studies

AMES-test

The standardized HFC containing the Hungarian humic substances has also been subjected to four mutagenic studies, and under the AMES test criteria exhibited 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].

Anti-clastogenic test

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 was 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 the HFC concentrations decrease. The interpretation of mechanisms responsible for these effects *in vitro* was not attempted because of the illogical results of 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.

Micronucleus test

Potential mutagenic effects of the EHF powder was studied in a standard micronucleus test on mice receiving a dose of 2000 mg/kg compared to a control group. The test substance given in a dose of 2000 mg/kg demonstrated a negative, in other words non-mutagenic effect in this test.

Table 4: The safety of HFC and/or humic acid in animal studies

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

Author/Year	*Sample Size*	*Study Design*	*Dose of HFC formula*	*Study Duration*	*Adverse Events*
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 macroscopic alterations were seen in the organs of the test animals
Kovács, 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) 2x per day	24 hours	• No macroscopic alterations were found in the organs of the test animals • A few cases of hemorrhage, emphysema, and hyperemia in both controls and test group.
Gachályi, 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/200 g 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
Dési, I., M.D., Dsc. Nagymajtényi, L, M.D., Dsc. 1993 [74]	20 wistar rats 10 controls 10 treated	Cumulative Toxicity	10 mg/kg body weight (LD_{50})	24 days	• Hemosiderosis in treated group • Peribronchial lymphocytic infiltration on two animals in both the control and treated groups
Gachályi, A. M.D. et al., 1998	16+ 20+20 Wistar rats	60-day toxicology	60 and 240 mg/animal HF powder	60 days	• No change in physical and hematological parameters
TRC, 2001	48 Beagle dogs	Subchronic, repeated dose toxicology	15, 50, 150 mg/kg	180 days	NOAEL: 15 mg/kg
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 HFC formula*	*Study Duration*	*Adverse Events*
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 • Anti-clastogenic effect seen at 5 mcl/ml
Oláh, B., M.D. 1992 [76]	Five Salmonella typhi-murium strains in the presence and absence of rat liver fraction	Salmonella typhi-murium reverse mutation assay (AMES TEST)	12, 60, 300, 1500 and 7500 microgram per plate	Incubated for 48 hours	• No mutagenic activity observed
TRC, 2001 Not published	30 + 30 mice	Micronucleus test on mice	2000 mg/kg EHF powder	24 + 48 hours	• No mutagen effect observed
Szakmáry, E., Biol.D., Ph.D. and Hudák, 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 bonded to 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 cardio protective effects of SHA and HA in isolated working rat heart subjected to ischemia/ reperfusion	10 mg/kg of humic acid (HA) 30 mg/kg of supplemented HA (SHA)	2 weeks	• No deaths • No adverse events noted

Author/Year	*Sample Size*	*Study Design*	*Dose of HFC formula*	*Study Duration*	*Adverse Events*
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
Sarudi, 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

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. Death 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 control 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 was observed in the liver and kidney of these animals. Increasing amounts of stored iron being the only significant adverse event reported during this cumulative toxicity study is very promising.

3.5. 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 collected, reviewed and summarized. These case reports represent patients who sought out medical treatment for specific conditions or diseases. Physicians and qualified public health workers supplied the outcome data.

Table 5 illustrates the rarity of adverse events that have been noted during the administration of the standardized HFC to humans. Although no significant adverse events were reported for any patients, 30 out of 514 individuals (5.8%) reported transient symptoms while taking HFC, including: headache, nausea, heartburn, 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 non-prescription drug. Some consideration should be taken in choosing the population in which this supplement should be used. For example, individuals with iron storage diseases could have detrimental side effects from taking a supplement that contains iron or that may affect iron utilization.

In 2001 a tolerance study using EHF powder (capsules), a preparation equivalent to HFC, was conducted involving 40 healthy volunteers who took 1-2-3 times the human dose for three weeks. No adverse effects were observed during the trial, and thus it may be safely assumed that the capsule containing the EHF powder is well tolerated.

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 HFC formula***	***Study Duration***	***Adverse Events (A/E)***
Sallay, Éva 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
Hudák, Ph.D., 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
Szüts, Péter M.D. Koszó, Péter M.D. 1996 [66]	60 children Iron Deficiency Anemia, Alopecia, Eczema, and Serious Illness	Open Clinical Trial	3 ml 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 complaint
Flórián, Csaba 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 HFC formula*	*Study Duration*	*Adverse Events (A/E)*
Molnár, Miklós 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
Lénárt, Ágota M.Sc. [79]	11 Elite Athletes	Open Clinical Trial	Dosage not given	4 weeks	Significant: none Transient: Two subjects reported transitory digestion problems and diarrhea
Molnár, Miklós M.D. [61]	14 adults Health Screening	Clinical Testing of HFC	Dosage not given	3 weeks	No A/E reported
Petrekanits, Máté M.Sc. [67]	25 Elite Athletes	Clinical Testing of HFC	Dosage not given	3 weeks	No A/E reported
Molnár, Miklós M.D. [54]	47 adults Occupational Exposure to Lead and Cadmium	Clinical Testing Of HFC	Dosage not given	3 weeks	No A/E reported
Székely, Iván M.D. 1994 [11]	6 adults Lead poisoning caused by adulterated paprika	Clinical Testing of HFC	Dosage not given	3 weeks	Significant: none Transient: Two patients reported mild side effects No specific information given

Author/Year	*Sample Size*	*Study Design*	*Dose of HFC formula*	*Study Duration*	*Adverse Events (A/E)*
Szivkovics, Sz. 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
Dienes, Sándor Ph.D., M.D. [80]	12 adults 12 children Exposure to Lead through Pottery	Clinical Testing of HFC	7.5 ml per day	2 weeks	No A/E reported
Kovács, 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
Gachályi, B. et al., 2001	40 healthy volunteers	EHF powder (capsule) tolerance study	1x, 2x, 3x the human dose	3 weeks	No side effects observed

Case reports

Author /Year	*Sample Size*	*Study Design*	*Dose of HFC formula*	*Study Duration*	*Adverse Events (A/E)*
Gelley, András 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
Csucska, Elek M.D. 1991 [64]	10 patients Cancerous Tumours	Case Reports	Dosage not given	Average length of treatment 2.6 years	No A/E reported

Of the altogether 514 individuals treated with HFC, adverse effects were observed in the case of only 30. The Average treatment period was 4.3 months.

A comprehensive analysis of trials with the HFC conducted under clinically controlled circumstances was carried out in 2001. The analysis of the data of 1141 subjects revealed a very low occurrence of adverse effects, representing 2.6% of the cases. Most of the adverse effects observed were gastrointestinal in nature (i.e. nausea and diarrhea).

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 and 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 immuno-suppression and general malaise. Therefore, 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. There was a relapse of the symptoms after the treatment was stopped; 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.

3.6. 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 Hungarian 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 of each essential mineral contained in HFC.

Table 6: Mineral composition of standardized HFC

Essential Elements	***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)
Vanadium	0.5 mg (500 mcg)
Zinc	10 mg

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 the HFC 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.

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. 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.

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. While many hydrocarbons are non-carcinogenic and efficiently removed from the body, small fractions of some hydrocarbons are converted to eloctrophilic metabolites which are not effectively further metabolized and which are probably responsible for the carcinogenic properties of these hydrocarbons [105]. There is speculation that humic substances, due to their binding with these compounds, may ultimately affect the fate of these carcinogenic products.

Uptake and bio-concentration factors of benzo(a) pyrene (a toxic PAH) in Atlantic Salmon were determined in water containing natural aquatic humic substances and control water. Uptake and bio-concentration of these toxic compounds were observed to significantly decrease in the presence of aquatic humic substances compared to the control water [107]. Therefore, it is apparent that humic substances in water do have a beneficial effect *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 the mechanisms responsible for the effects of humic substances on PAH biodegradation. Various PAHs could be degraded by activated sludge [20]. Furthermore, it is thought that the bacteria present in the humic acids and activated sludge may decompose the absorbed mutagens. Humic compounds were observed to be able to contribute to the enzymatic activity in activated sludge [108]. Other investigators have stated that the sorption of PAHs to organic matter renders the PAHs non-biodegradable, which may in turn affect bio-toxicity [109]. However, these observations and speculations will require additional data to document the specific mechanisms responsible for humic substance-PAH complex biodegradation, bio-availability, and toxicity.

Laboratory analysis performed by the National Institute of Food Hygiene and Nutrition (OÉTI) in Budapest, Hungary, found non-detectable concentrations of polycyclic aromatic hydrocarbons (PAH) in Humet-R that contained Humifulvate. None of the following PAHs were detected by OÉTI: benzo-(a)-pyrene, benzo-(b)-fluoro-anthene, indeno-pyrene, benzo-(k)-fluro-anthene, fluoro-anthene, or benzo-(ghi)-perylene.

To sum it up, 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 per 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.

4. CONCLUSION

Humic substances exist in all environments including soils, groundwater, streams, estuaries, and oceans. They are very reactive and are important participants in many geo-chemical 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 basis 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 rather complex issues. Therefore, further research is needed 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 than 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 so much 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 physicochemical 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 and promoters of the utilization 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 are certainly 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 on the safety of this product in respect to toxicology and mutageneseis. The diseases endemic to China (Blackfoot

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* an *in vivo* research. A number of human clinical trials 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.

5. PRODUCTS CONTAINING HUMIFULVATE

Drawing from the data pool generated in the course of extensive research and development of more than a decade as described above, HUMET Plc compiled a notification on Humifulvate as a new dietary ingredient in 2000 in order to be able to market it in the form of a food supplement in the United States. The US **F**ood and **D**rug **A**dministration **(FDA)** reviewed the submission and did not object to its market introduction. Products containing Humifulvate as their ingredients have been registered in several countries of the world (Austria, most CIS states, Romania) and they has been sold within and outside Hungary (in addition to those mentioned above in the United Kingdom, Portugal and Taiwan). The following product lines have been introduced to trade to this day:

Product	***Status***	***Active ingredient***	***Notes***
HUMET-® syrup	Health product, not classified as a medicine	HFC	
HUMETTA effervescent tablets	Food supplements	EHF	With black current and blueberry flavor
HUMETTA capsules	Food supplements	EHF	Sold also in iron-free and reduced mineral levels varieties

REFERENCES

1. *Humet Product Documentation and Technical Information*. Horizon Multiplan LTD.: Budapest, 1999
2. Frimmel, F.H. and R.F. Christman, eds. *Humin Substances and Their Role in the Environment.* 1st ed. Vol.1. 1988, Wiley-Interscience Publication: New York.
3. Visser, S.A., *Effect of humic substances on mitochondiral respiration and oxidative phosphorylation*. The Science of the Total Environment, 1987. 62: p. 347-354.
4. Österberg, R. and K. Mortensen, *The growth of fractal humic acids: cluster correlation and gel formation.* Radiat Environ Biophys, 1994. 33: p. 269-276.
5. Gundel, J., Humet Document 037. 1995.
6. Aiken, G., D. McKnight, and R. Wershaw, eds. *Humic Substances in Soil, Sediment, and Water*. Vol. 1. 1985, Jon Wiley: New York.
7. Visser, S.A. *Some biological effects of humic acids in the rat*. Acta Biologica Et Medica Germanica, 1973. 31: p. 569-581.
8. Mineralab, I., *A Clinician's Guide to Toxic Metals*, 1979: Hayward.
9. Shils, O., and Shike, ed. *Modern Nutrition in Health and Disease*, 8th ed., Vol. 2. 1994, Williams and Wilkin: Baltimore
10. Hudák, A., *et al., Effect of the consumption of humic acid with bound complex micro elements in cases of occupational cadmium exposure*. Central European Journal of Occupational and Environmental Medicine, 1997. 3(3): p. 175-186.
11. Székely, I., *Lead poisoning caused by adulterated paprika*, 1994, Szt. György Hospital: Székesfehérvár (unpublshed clinical documentation).
12. Gramss, G., D. Ziegenhagen, and S. Sorge, *Degradation of soil humic extract by wood- and soil-associated fungi, bacteria, and commercial enzymes*. Microbial Ecology, 1999. 37: p. 140-151.
13. Wersahw, R.L., Application of a membrane model to the sorptive interactions of humic substances. Environmental Helath Perspectives, 1989. 83: p. 191-203.
14. Bravo, L., *Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance*. Nutrition Reviews, 1998. 56(11)? P. 317-331.
15. Bruneton, J., *Pharmacognosy, Phytochemistry Medicinal Plants*. 195, Paris, France: Lavoisier Publishing.
16. Susic, M. and K.G. Boto, *High-performance liquid chromatographic determination of humic acid in environmental samples at the nanogram level using fluroscence detection*. Journal of Chromatography, 1989. 482: p. 175-187.
17. Norden, M. and E. Dabek-Zlotorynska, *Characterization of humic substances using capillary electorphoresis with photodiode array and laser-induced flurosecence detection*. Electorphoresis, 1997. 18: p. 292-299.
18. Glynn, A.W., *Fulvic and humic acids decrease the absorption of cadmium in the rat intestine*. Archives of Toxicology, 1995. 70: p. 28-33.
19. Schauss, A., *Minerals, Trace Elements, and Human Health*. 3rd ed. 1998, Tacoma: AIBR Press.
20. Sato, T., et at., *Mechanism of the desmutagenic effect of humic acid*. Mutation Research, 1987.176: p. 199-204.

21. Cao, Y., *Competitive complexation of trace metals with dissolved humic acid*. Environmental Health Perspectives, 1995. 103(suppl 1): p. 29-32.
22. Lind, Y. and A.W. Glynn, *The influence of humic substances on the absorption and distribution of cadmium in mice*. Pharmacology and Toxicology, 1999. 84: p. 267-273.
23. Principles of Biochemistry. 2nd ed, ed. A. Lehninger, D. Nelson, and M. Cox. 1993, New York: Worth Publishers.
24. Schubert, J., E.J. Riley and S.A. Tyler, *Combined effects in toxicology – a rapid systematic testing procedure: cadmium, mercury, and lead*. Journal of Toxicology and Environmental Health, 1978. 4. P. 763776.
25. Chirstoffersen, J., *et al., Interaction of cadmium ions with calcium hydroxyapatite crystals: a possible mechanism contributing to the pathogenesis of cadmium-induced bone disease*. Calcif… Tissue Int, 1988. 42: p. 331-339.
26. Shankel, D.M., *et al., Extracellular Interception of Mutagens.* Basic Life Science, 1993. 61: p. 65-74.
27. Sato, T., *et al., Adsorption of mutagens by humic acid*. The Science of the Total Environment, 1987. 62: p. 305-310.
28. Cozzi, R. *et al. Desmutagenic activity of natural humic acids: inhibition of mitomycin C and amd… maleic hydrazide mutagenicity*. Mutation Research, 1993. 299: p. 37-44.
29. Center for Disease Control (CDC), 1999, www.cdc.gov./nhs/fastats.
30. Ferdinándy, P., *Cardioprotective effects of SHA and HA preparations in the isolated working rat heart subjected to eischaemia/reperfusion*, 1997 (unpublished).
31. Klöcking, H.-P., *Influence of natural humic acids and synthetic phenolic polymers on haemostasis*. Archives of Toxicology, 1991. suppl 14: p. 166-169.
32. Riede, U.N. *et al., Humate-induced activation of human granulocytes*. Virchows Archiv B Cell Pathol, 1991. 60: p. 27-34.
33. Lu, F.-J. and Y.-S. Lee, *Humic acid: inhibitor of plasmin*, The Science of the Total Environment, 1992. 114: p. 135-139.
34. Yang, H.-L., *et al., Plasma protein C activity is enhanced by arsenic but inhibited by floorescent humic acid associated with blackfoot disease*. Am J Hematology, 1994. 46: p. 264-269.
35. Lu, F.J., *Blackfoot disease: arsenic or humic acid?* [Letter], Lancet, 1990. 336(14 July): p. 115-116.
36. Lu, F.-J., T.S. Huang, and J.-H. Lee, *Effect of synthetic humic acid-multimetal complex on human plasma prothrombin time*. Bulletin of Environmetnal and Contamination Toxicology, 1994. 53: p. 577-582.
37. Lu, F.J., *Arsenic as promoter in the effect of humic substances on plaasma porthrombin time in vitro*. Thrombosis Research, 1990. 58: p. 537-541.
38. Cheng, M.-L., *et al., Humic acid-mediated oxidative damages to human erythrocytes: a possible mechanism leading to anemia in blackfoot disease*. Free Radical Biology and Meidcine, 1999. 27(3/4): p. 470-477.
39. Bernacchi, F., *et al, In vivo cytogenetic effects of natural humic acid*. Mutagenesis, 1996. 11(5): p. 467-469.
40. La Londe, R.T. and S. Xie, *Glutathione and N-acetycysteine inactivations of mutagenic 2(5H-furanones from the chlorination of humics in water*. Chemical Research and Toxicology, 1993. 6: p. 445-451.

41. Condie, L.W., R.D. Laurie, and J.P. Bercz, *Subchronic toxicology of humic acid floolwing chlorination in the rat*. Journal of Toxicology and Environmental Health, 1985. 15: p. 305-314.
42. Zhou, S.W., *et al. Major origin of mutagenicity of chlorinated drinking water in China: humic acid or pollutants*. The Science of the Total Environment, 1997. 196: p. 191-196.
43. Dayan, A.D., *Carcinogenicity and drinking water*. Pharmacology and Toxicology, 1993. 72(suppl 1): p. s. 108 - s. 115.
44. Schwartz, J.L., *The dual roles of nutrients as antioxidants and prooxidants: their effects on tumor cell growth.* Journal of Nutrition, 1996. 126: p. 1221S-1227S.
45. Gaté, L. *et al., Oxidative stress induced in pathologies: the role of antioxidants*. Biomedicine & Pharmacotherapy, 1999. 53: p. 169-180.
46. Decker, E.A., Phenolics: prooxidants or antioxidants? Nutrition Revews, 1997. 55(11): p. 396-398.
47. Cerutti, P.A., *Prooxidant states and tumor promotion*. Science, 1985. 227(25 Jan): p. 375-381.
48. Wang, Z., Y. Xu, and Peng, *Influences of fulvic acid on bioavailability and toxicity of selenite for wheat seedling and growth*. Biological Trace Element Research, 1996. 55: p. 147-162.
49. Wang, C., *et al., Interaction between fulvic acids of different origins and active oxygen radicals*. Science in China (Series C), 1996. 39(3): p. 267-275.
50. Peng, A., *et al., Study on the pathogenic factors of Kashin-Beck disease*. Journal of Toxicology and Environmental Health, 1992. 3: p. 79-90.
51. Peng, A. and C.L. Yang, Examination of the roles of selenium in the Kashin-Beck disease. Biological Trace Element Research, 1991. 28(1): p. 1-9.
52. Yang, C., et al., *Selenium deficiency and fulvic acid supplementation induces fibrosis of cartilage and disturbs subchondral ossification in knee joints of mice: and animal model study of Kashin-Beck disease*. Virchows Archiv A pathol Anat, 1993. 423: p. 483-491.
53. Molnár, M., *The Study of Humet-R syrup's Effect on the Metabolism of Trace Elements in Helathy Volunteers*, 1992, Hungarian State Railway Public Health Institute: Budapest (unpublished).
54. Molnár, M., *Blood lead and blood cadmium levels*, Institution of Public Helath of the Hungarian Railways: Budapest (Unpublished clinical documentation).
55. Florián, C., *The treatment of olunteers continually exposed to high doses of lead with the Humet-R syrup*, 1995, Primary Medical care System Outpatient Clinic, Ajka Crystal Ltd.: Ajka (unpublished).
56. Sallay, E., *Open-Labeled Prospective Clinical Research on Volunteers Exposed to Lead,* 1998, Humet and Trade, Research and Development Company: Budapest (unpublished).
57. Sarudi, I., T. Rétfalvy, and I. Lassú, *Effect of Humet-R on the mobilization of a toxic heavy metal in pigs*, in *H-M Doc. 45-1-12. 1997*.
58. Magyar, K. and J. Lengyel, *Pharmaco-kinetics of Strontium Ruthenium humic acid complexes*, Semmelweis Medival University, Central Isotope Laboratory: Budapest.
59. Naményi, *Effect of humic acid on the regeneration of the haemopoietic system during and after cobalt gamma radiation*: Hungary
60. *Nutrition and Diet Therapy*, 7th ed, ed. S. Rodwell-Williams. 1993, St. Louis? Mosby-Year Book, Inc.

61. Molnár, M. and G. Szabó, *Serum iron*, Institution of Public Health of the Hungarian Railways: Budapest (unpublished clinical documentation).
62. Szivkovics, S., *The application of Humet-R product on patients suffering from malignant lymphoma in combination with cytostatic therapy*, 1997, Ukraine Oncology and Radiology Science Research Institute, Department of Tumourous Diseases (test report excerpts).
63. Gelley, A., *Retrosepctive evaluation of the data of patients treated with humic acid metal complex*, 1995, Hospital of the Hungarian Railways: Budapest.
64. Csuczka, E., *Cases of patients sufferting from cancer treated with a preaparation called „Humet",* 1991.
65. Győrfy, *Tumor patients*, Part II, Kaposi Mór Hospital: Kaposvár (unpublished clinical documentation).
66. Szűts, P. and P. Koszo, *The application of Humet-R Roborant Syrup in Paediatrics* (Open Clinical Test Findigs), 1996, Erzsébet Hospital: Hódmezővásárhely.
67. Petrekanits, M., *Effects on teh performance of elite athletes*, Hungarian School of Physical Education: Budapest
68. Yoshino, M. and K. Murakami, *Interaction of iron with polyphenolic compounds: application to antioxidant characterization*. Analytical Biochemistry, 1998. 257: p. 40-44.
69. Szakmári, É. and A. Hudák, *Study of the effects of different formulations of humic acid bound with iron and other micro elements in iron deficient rap pups*, 1997.
70. Kovács, L. and F. Kőhegyi, *Alopecia patients* (unpublished clinical documentation).
71. Kirschmann, G.J. and J.D. Kirchmann, *Nutrition Almanac*. 1996, New York: McGraw Hill.
72. Kovács, M.e.a., Final Report: Oral Acute *Toxicity Study of Supplementaed Humic Acid (DHS) in Mice with „Limit Test" method*, 1996, Pharmaceutical Control and Developing Laboratory Co., Ltd: Budapest.
73. Antal, M., Humet: *Acute oral toxicity study in the rat*, 1990, National Institution of Food and Nitrition Science: Budapest.
74. Dési, I. and Magymajtényi, L., *Cumulative Toxicological Investigation of Humet®-R Solution*, 1993, SZOTE, Department of Public Health in Hungary.
75. Gachaly, A. *et al., Effect of the prolonged oral dose of Humet®-R in rats,* 1994: Budapest.
76. Oláh, B., *The testing of „HUMET" with Salmonella typhi-murium reverse mutation assay (Ames test)*, 1992, Toxicological Resesarch Centre Ltd: Veszprém, Szabadságpuszta, Hungary.
77. Gundy, S., *The study of the potential anti-calstogenic effect of HUMET®-R in human peripheral blood lymphocytes,* 1992, Medical Research Genetics, Department of Human Gebetics: Budapest.
78. Dallo, J., *Observation of the effect of Humet derivatives on male rat's sexuality*, 1994: Budapest.
79. Lénárt, A., *Effects on the psychic activity of elite athletes*, Hungarian School of Physical Education: Budapest.
80. Dienes, S., *Observations about workers exposed to lead in connection with the application of the HUMET®-R syrup,* University of Medicine and Pharmacy, Marosvásárhely, Occupational Medicine Department (unpublished clinical documentation).

81. Mucklow, E., et al., *Cobalt pisoning in a 6-year-old*. The Lancet, 335: p. 981.
82. Frieden, E., ed. *Biochemistry of the Essential Ultratrace Elements*, Biochemistry of the Elements, ed. E. Frieden. Vol. 3. 1984, Plenum Press: New York. 426.
83. Waldron, H., *Cobalt, in Metals in the Environment*. 1980, Academic Press: New York. P. 133-153.
84. Valber, L., J. Ludwig, and D. Olatunbosum, *Alteration in Cobalt Absorption in Patients with Disorders of Iron Metabolism*. Gastroenterology, 1969. 56(2): p. 241-251.
85. Organization, W.H., *Trace elements in Human Nutrition*. Vol. 532. 1973, Geneva: World Health Organization.
86. Subcommitte on the 10th edition of the RDAs, F.a.N.B.N.R.C. *Recommended Dietary Allowances*. 10 ed. 1989, Washington D.C.: National Academy Press.
87. Wright, R.O., et al., *Association between iron deficiency and low-level lead pisoning in an urban primary care clinic*. American Journal of Public Health, 1999. 89(7): p. 1049-1053.
88. Watts, D., *The Nutritional Relationship of Manganese*, Journal of Orthomolecular Medicine, 1990. 5(4): p. 219-222.
89. Schroeder, H., J. Balassa, and I. Tipton, *Essential Trace Metals in man: Manganese*. Journal of Chronic Disease, 1966. 19: p. 545-571.
90. Vyskocil, A., and C. Viau, *Assessment of molybdenum toxicity in humans*, Journal of Applied Toxicology, 1999. 19: p. 185-192.
91. *Molybdenoum, in Handbook of Vitamins, Minerals, and Hormones*. p. 167-176.
92. Bandyopadhyay, S. *et al., Biochemical Studies on Molybdenum Toxicity in Rats: Effects of High Protein Feeding*, International Journal of Vitamin and Nutrition Research, 1981. 51: p. 401-409.
93. Jamba, L., B. Nehru, and M.P. Bansal, *Selenium supplementation during cadmium exposure: changes in antioxidant enzymes and the ultrastructure of the kidney*. The Journal of Trace Elements in Experimental Medicine, 1997. 10: p. 233-242.
94. Watts, D., *The Nutritional Relationships of Selenium*. Journal of Orthomolecular Medicine, 1994. 9(2): p. 111-117.
95. Chen, J. and L.C. Clark, *Proposed supplemental dosages of selenium for a phase I trial based on dietary and supplemental selenium intakes and episodes of chronic selenosis*. Journal of the American College of Toxicology, 1986. 5(1): p. 71-78.
96. Schroeder, H., J. Balassa, and I. Tripton, *Abnormal trace elemnets in man – vanadium*. Jorunal of Chronic Disease, 1963. 16: p. 1047-1071.
97. Mcneil, J. and C. Orvig, *Vanadium Compositions*, in *United States Patent*. 1999: Canada. P. 1-21.
98. Badmaev, V., S. Prakash, and M. Majeed, *Vanadium: a review of its potential role in the fight against diabetes*. Journal of Alternative and Complementary Medicine, 1999. 5(3): p. 273-291.
99. Cohen, N., et al., *Oral vanadyl sulphate improves hepatic and peripheral insulin sensitivity in patients with non-insulin-dependent diabetes mellitus*. J Clin Invest, 1995. 95: p. 2501-2509.
100. Goldine, A., et al., *Metabolic effects of sodium metavandate in humans with insulin-dependent and noninsulin-dependent diabetes mellitus in vivo and in vitro studies.* Journal of Clinical Endocrinology and Metabolismm, 1995. 80(11): p. 3311-3320.

101. Llobet, J. and J. Domingo, *Acute toxicology of vanadium compounds in rats and mice*. Toxicology Letters, 1984. 23: p. 227-231.
102. State of *California: Proposition 65, The Safe Drinking Water and Toxic Enforcement Act of 1986*, www.prop65news.com.
103. Kido, T., et al., *Dose-response realtionshipt betwwen dietary cadmium intake and metallothioneinuria in a population from a cadmium-polluted area of Japan*. Toxicology, 1991. 66: p. 271-278.
104. World Health Orgaization., *Environmental Health Criteria 134 Cadmium*. 1992, Geneva: WHO.
105. Grover, P.L., *Pathways involved in the metabolism and activation of polycyclic aromatic hydrocarbons*, Xenobiotica, 1986. 16(10): p. 915-931.
106. Phillips, D.H., *Policyclic aromatic hydrocarbons in the diet*. Mutation Research, 1999. 443: p. 139-147.
107. Johnsen, S., J. Kukkonen, and M. grande, *Influence of natural aquatic humic substances on the bioavailability of benzo(a)pyrene to Atlantic salmon*. The Science of the Total Environment, 1989. 81/82: p. 691-702.
108. Frolund, B., T. Griebe, and P.H. Nielsen, *Enzymatic activity in the activated-sludge floc matrix.* Applied Microbiological Biotechnology, 1995. 43: p. 755-761.
109. Weissenfells, W., H. Klewer, and J. Langhoff, *Adsorption of polycyclic aromatic hydrocarbons (PAHs) by soil particles: influence on biodegradability and biotoxicity*. Applied Microbiology Biotechnology, 1992. 36(5) p. 689-96.

1 In the Hungarian language the English expression "humic substances" refers to both humus and humin substances.