

Safety and Toxicology (of Humic Acids)

Extensive Testing of Humic Substances in Hungary

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 standardised (Humic/Fulvic Acid) 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 standardised 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 standardised (Humic/Fulvic Acid) 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 (Humic/Fulvic Acid).

Furthermore, a review of human clinical studies indicates a lack of significant side effects from the ingestion of (Humic/Fulvic Acid). The amounts of humic substances in (the Humic Acid product) are extremely low and have been documented as safe in animal and human studies.

A series of acute, cumulative and mutagenicity toxicological studies of (Humic/Fulvic Acid)-containing (product) have been carried out by investigators in Hungary. As recently as 1999, the manufacturer of (the product) commissioned an independent review of all data available to date on the toxicology and safety of (the product). 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 (Humic/Fulvic Acid) in humans is available. All animal and human toxicology studies have used (Humic/Fulvic Acid) as found in (the product) 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 (the Humic Acid) are well within safe ranges. The same independent laboratory has also reported that (Humic/Fulvic Acid) contains non-toxic levels of aluminium, lead, cadmium and arsenic. Laboratory analysis performed by the National Institute of Food Hygiene and Nutrition (OÏTI) (the authority regulating foods) in Budapest, Hungary, in 1991, did not find detectable concentrations of polycyclic aromatic hydrocarbons (PAH) in (the product), the product containing (Humic/Fulvic Acid).

Acute Toxicology Studies

In a preliminary study, 84 Wistar rats were followed for two weeks following varying doses of the standardised (Humic/Fulvic Acid) 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 (Humic/Fulvic Acid) 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 LD50 value was determined to be higher than 10gm/kg body weight. The standardised (Humic/Fulvic Acid) 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 (Humic/Fulvic Acid) two times a day in 24 hours. 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, diarrhoea, 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 haemorrhage and emphysema in the lung, haemorrhage in the thymus, and hyperaemia of the spleen, in which there was no significant difference in the number of occurrences between the two groups. The authors noted that these conditions were associated with agony. The few cases of hyperaemia and hydrometra of the uterus were connected with the neurohumoral regulation of sexual function or the cyclic physiological state of the uterus.

Results of the study indicate that the standardised (Humic/Fulvic Acid) 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 standardised 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 (LD50) of the standardised (Humic/Fulvic Acid) 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, haematological values, and thyroid hormones before and after treatment. Histological tests of tissues were administered after the treatment with the (Humic/Fulvic Acid). 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.

A subsequent repeated dose toxicity study was conducted in order to clarify the possible side effects that could occur after prolonged administration of the standardised (Humic/Fulvic Acid). Food containing the (Humic/Fulvic Acid) 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, haematology and organ weights were measured at the time of necropsy. Two groups after week three of treatment with the (Humic/Fulvic Acid) (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. 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 standardised (Humic/Fulvic Acid) 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 haematological 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 standardised (Humic/Fulvic Acid) 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 haematology 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, diarrhoea) could be observed with dose-dependent frequency.

Mutagenicity Studies

AMES-test

The standardised (Humic/Fulvic Acid) 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 standardised (Humic/Fulvic Acid) had no mutagenic activity and no bactericide effect using less than or equal to 7500 mcg of the test substance per plate.

Anti-clastogenic test

The effect of the standardised (Humic/Fulvic Acid) on a known mutagen, ionising radiation, has been studied using human peripheral blood lymphocytes. A preliminary study was conducted to confirm that the (Humic/Fulvic Acid) 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 standardised (Humic/Fulvic Acid) concentrations as compared to controls, with all of the concentrations being much higher than any physiological dose. Therefore, it was concluded that the standardised (Humic/Fulvic Acid) is not clastogenic even in very high concentrations of 200 mcl/ml. In the subsequent study concerning the anti-clastogenic effect of (Humic/Fulvic Acid), 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 standardised (Humic/Fulvic Acid) at a level of 5 mcl/ml. These results imply an *in vitro* anti-clastogenic effect of the standardised (Humic/Fulvic Acid). However, the number of di-centric ring aberrations (diagnostic value in detecting radiation effects and thus chromosome aberrations) decrease as the (Humic/Fulvic Acid) 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 standardised (Humic/Fulvic Acid) may have potential anti-clastogenic effects; however, this cannot be stated as fact due to the variable results. Considering that the standardised (Humic/Fulvic Acid) 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.

A series of studies of (Humic/Fulvic Acid) and humic acid given to mice and rats have evaluated the safety of (the product). 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 anaemia as well as the stress

caused by the administration of (Humic/Fulvic Acid). Only one death was observed in the study evaluating the effects of (Humic/Fulvic Acid) 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 (Humic/Fulvic Acid). 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 (Humic/Fulvic Acid). When high amounts of (Humic/Fulvic Acid) 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.

Human clinical trials and case reports

Clinical observation of (Humic/Fulvic Acid) 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 summarised. 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 standardised (Humic/Fulvic Acid) to humans. Although no significant adverse events were reported for any patients, 30 out of 514 individuals (5.8%) reported transient symptoms while taking (Humic/Fulvic Acid), including: headache, nausea, heartburn, diarrhoea, or skin reactions. Since these types of transient events may be due to random chance, it is not possible to attribute them to (Humic/Fulvic Acid) 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 utilisation. In 2001 a tolerance study using EHF powder (capsules), a preparation equivalent to (Humic/Fulvic Acid), 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.

Of the altogether 514 individuals treated with (Humic/Fulvic Acid), 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 (Humic/Fulvic Acid) 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 diarrhoea).

Long-term use of (Humic/Fulvic Acid)

Long-term use of (Humic/Fulvic Acid) has been documented in 194 individuals. The average time frame for long-term treatment (defined as greater than 4 months' consumption) with (Humic/Fulvic Acid) was 12.0 months (range: 4 months to 5 years). Three patients under treatment for cancerous tumours consumed (Humic/Fulvic Acid) for 5 years. None of these three subjects required cytostatic therapy during the period in which (Humic/Fulvic Acid) was consumed and no significant adverse events were reported for any of these subjects consuming (Humic/Fulvic Acid) over a prolonged period. Improvement in well-being was reported by many of the individuals taking the (Humic/Fulvic Acid), 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 (Humic/Fulvic Acid) in the treatment

of nine paediatric eczema patients [66]. The patients were given (Humic/Fulvic Acid) for two to three months. There was a relapse of the symptoms after the treatment was stopped; therefore, treatment with (Humic/Fulvic Acid) 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 (Humic/Fulvic Acid) for greater than six months. One child reported an allergic skin reaction and one other child reported abdominal complaints and diarrhoea. Therefore, the long-term use of the standardised (Humic/Fulvic Acid) is both beneficial and safe as documented in children and cancer patients.

Safety of minerals and trace elements included in (Humic/Fulvic Acid)

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 standardised (Humic/Fulvic Acid).

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 levels of trace toxic metals in (Humic/Fulvic Acid) were compared to the amounts of each metal found in the daily diet. Food can contain about 10 ppm of aluminium. Conservative estimates indicate that at least 2-3 mg of aluminium are consumed a day. 20.7 ppm found in (Humic/Fulvic Acid) is the same as 0.188 mg of aluminium in one 10 ml serving of the standardised (Humic/Fulvic Acid). This amount is less than one-tenth the amount of aluminium a person would consume in the diet on a daily basis. The amounts of lead and arsenic found in (Humic/Fulvic Acid) are insignificant.

Cadmium toxicity is generally based on oral inhalation of ambient cadmium. Therefore, other data must be utilised to determine its safety in the amounts found in (Humic/Fulvic Acid). Average daily intakes from food in most areas not polluted with cadmium are between 10-40 mcg. Therefore, the estimated amount of cadmium in a typical diet is ~5.5 to 25 times that found in the standardised HPC formula.

It has been proposed that humic substances may bind with or absorb mutagens rendering them less toxic or less mutagenic. 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. Stream humic substances have been documented to interact with PAHs; these aromatic compounds have also been extracted from the deeper layers of peat (2.5 m). Therefore, PAHs are widely distributed in the environment and human exposure to them is unavoidable. 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 electrophilic metabolites which are not effectively further metabolised and which are probably responsible for the carcinogenic properties of these hydrocarbons. 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. 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. 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. Other investigators have stated that the sorption of PAHs to organic matter renders the PAHs non-biodegradable, which may in turn affect bio-toxicity. However, these observations and speculations will require additional data to document the specific mechanisms responsible for humic substance-PAH complex biodegradation, bioavailability, and toxicity.

Laboratory analysis performed by the National Institute of Food Hygiene and Nutrition (OŃTI) in Budapest, Hungary found non-detectable concentrations of polycyclic aromatic hydrocarbons (PAH) in (the product) that contained (Humic/Fulvic Acid). None of the following PAHs were detected by OŃTI: 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 standardised (Humic/Fulvic Acid) taken in the recommended dosage of 10ml per day is safe. Furthermore, laboratory analyses indicate that (Humic/Fulvic Acid) contains insignificant and non-toxic amounts of aluminium, lead, arsenic, and cadmium and is free of any carcinogenic compounds