CORVINA NATURAL PRODUCTS, INC.
6633 Coyle Avenue
Carmichael, CA 95608

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Confidential and Proprietary Information Submitted to:

Office of Special Nutritionals (HFS-450)
Center for Food Safety and Applied Nutrition
US Food and Drug Administration
200 C Street SW
Washington DC 20204

Re: Notification of New Dietary Ingredient: Humifulvate

Pursuant to the Dietary Supplement Health and Education Act of 1994 (DSHEA), 21 USC 350 b(a)(2), and FDA regulations, 21 CFR 190.6, Corvina Natural Products, Inc. (Corvina), hereby submits this notification and information concerning a new dietary ingredient that Corvina intends to market for use as or in a dietary supplement. Corvina previously submitted a similar notification on December 24, 1999. This notification contains additional scientific materials to those submitted with the earlier submission. Pursuant to the provisions of DSHEA, Corvina will not introduce the ingredient or deliver the ingredient for introduction into interstate commerce until at least 75 days after the date that FDA receives this notification.

1. Name and Address of Manufacturer and Distributor of the Dietary Ingredient

The name and complete address of the manufacturer of the new dietary ingredient/supplement is as follows:

Humet Research and Development Company
(formerly known as HORIZON-MULTI-PLAN, LTD)
H-1121 Budapest
Konkoly Thege u. 29-33
Hungary
(telephone: 011361-160-1828)

The name and complete address of the distributor of the new dietary ingredient/supplement is as follows:

Corvina Natural Products, Inc.
6633 Coyle Avenue #2
Carmichael, CA 95608
(telephone: 916-961-2266)

Manufacturer of other minerals in the supplement:

SIGMA-ALDRICH, Ltd.
Budapest
Hungary

2. Name of the Dietary Ingredient

The name of the new dietary ingredient is Humifulvate.

3. Description of the Dietary Ingredient

The description of the new dietary ingredient is as follows:

Humifulvate is a chemically distinct and identifiable mixture of humic, fulvic and phenolic acids in a humate/polyphenolic complex. A tolpha torf preparate (TTP) byproduct of the incomplete natural decomposition (humification) of organic plant material, humifulvate is derived from Hungarian peat found primarily along the northern shores of Lake Balaton in Hungary. Humifulvate is processed into a concentrate for inclusion in dietary supplements in liquid and solid forms intended for oral consumption.

The natural source of humifulvate is a unique peat bog estimated to be between 3,000 and 10,000 years old; the chemical and biological properties of this bog have been studied for over 40 years.[1] This extensive scientific research has established that this peat deposit is slightly alkaline (pH 7-8), has an ash content of 28% to 43%, and contains significant quantities of two predominate humate compounds, humic acid and fulvic acid, along with minor amounts of phenolic acid. The peat deposit also contains calcium huminate, a degradation product of lignans, shell remnants, calcareous materials, sand and other minerals.

Humic and fulvic acids are multi-substituted polyaromatic heterocyclic macromolecules that incorporate protocatechic acid, vanillic acid, vanillin, resorcinol, ferulic acid, benzoic acid, and other cyclic polyphenols resulting from the degradation of the structural lignans in plant cell walls. These constituents of humic and fulvic acids are rich in carboxyl, hydroxyl, and carbonyl groups as well as in phenols, quinones and semiquinones.[2-4] Within each macromolecule, aromatic groups are linked by amino acids, amino sugars, peptides and other aliphatic carbon chains similar to those found within the human body (see Figure 1).[5] Fulvic acids obtained from peat may represent degradation products of humic acids, contain more oxygen-rich reactive groups than do humic acids, and are of smaller molecular weights than are humic acids.[5] Despite the complexity in its composition, infrared spectrosopic analyses have revealed that humifulvate is a distinct mixture of predominantly humic and fulvic acids; therefore, the humate/polyphenolic complex comprising this new dietary ingredient is referred to by the term, humifulvate.

Humifulvate is a negatively-charged metal complexing ligand. There are a number of active sites where metal ions may bind to aromatic and aliphatic carboxyl and phenolic

hydroxyl groups within the humifulvate complex, allowing humifulvate to act as an ion exchanger, releasing metal ions of low atomic mass and chelating heavier metals.[6-9] These properties are abolished by either methylation or acetylation of the reactive sites.[1,10]

Humifulvate concentrate (HFC) consists of humifulvate in combination with minerals and trace elements. Research results suggest that HFC enhances mineral and trace element status, supporting the maintenance of mineral and trace element balances without bypassing normal homeostatic mechanisms for preventing mineral toxicity. Following dissociation of the minerals and trace elements delivered by HFC, the residual humifulvate complex may chelate heavy metals along the intestinal tract, thereby reducing heavy metal burdens.

A full biochemical description of humifulvate can be found in references 5,11 and 12. Interpretation of the infrared spectrological analysis of humifulvate and a description of the types, amounts and ratios of the component parts of humifulvate is provided in reference 13. A schematic rendering of the biochemical structure of humifulvate is included in reference 5.

3i. Level of Dietary Ingredient in Dietary Supplements

The new dietary ingredient, humifulvate, is intended to be supplied in a dietary supplement in combination with defined amounts of several minerals and trace elements.

The intended use of the new dietary ingredient is as a component of a dietary supplement (humifulvate concentrate; HFC). A single daily dose (10 ml) of the dietary supplement (HFC) will contain, in liquid or solid form:

New dietary ingredient:

Humifulvate ..... 75 mg (47.86%)

3ii. <u>The Conditions of Use Recommended in Labeling of or Ordinary Course of Use of Dietary Supplement containing the New Dietary Ingredient (Structure/Function Claims)</u>

1. Humifulvate concentrate (HFC) supports normal mineral and trace element homeostasis.

   Two weeks of oral administration of HFC (313.4 mg/day, providing 150 mg of humifulvate daily) was associated with increased blood copper concentrations and improved iron metabolism in 51 healthy adult volunteers.[14] In another uncontrolled study, oral consumption of HFC (156.7 mg/day) for 6 weeks resulted in significant increases in initially low serum iron concentrations.[15] Similarly, serum iron concentrations improved in 14 healthy adults consuming HFC for 3 weeks (156.7 mg/day); in all subjects with initially depressed serum iron or ferritin concentrations, those concentrations approached the respective normal physiologic ranges within the 3 weeks of treatment.[16] In addition, those subjects with supranormal prestudy serum iron concentrations exhibited decreases in serum iron concentrations during the study. In 19 pediatric patients with iron deficiency anemia, serum iron concentrations began to increase within 2 weeks of oral treatment with HFC (31.4 mg HFC/10kg body weight/day) and were significantly increased after 3 weeks of therapy.[17]

   In an investigation of the bioavailability of the trace minerals provided by HFC, adult rats that had been fed a diet deficient in trace minerals for 2 weeks were supplemented with either HFC, at a daily dose equivalent to the recommended human daily dose, or an inorganic mixture of salts, identical in composition and daily dosage to the trace minerals in HFC.[18] Following 2 weeks of replacement feeding, whole-body retentions of oral doses of radiolabeled cobalt, iron, zinc, selenium and copper were the same regardless of the form of trace minerals. In addition, the intestinal absorption of dietary iron was significantly increased by HFC.

   The benefits of HFC supplementation on mineral and trace element homeostasis appear to be transmittable to newborn progeny. In one experiment, piglets born to iron deficient sows that had been supplemented with HFC (1500 mg daily, providing 717.9 mg of humifulvate) during gestation exhibited significantly higher plasma hemoglobin concentrations than did piglets born to iron deficient sows that had received standard parenteral iron supplementation or no treatment.[19] Similarly, the pups of Sprague-Dawley rats that had been fed iron deficient diets plus HFC (10 mg/kg daily, providing 4.8 mg/kg of humifulvate) exhibited plasma hemoglobin concentrations, hematocrits, and transferrin saturation similar to those of pups born to iron deficient dams that had received supplemental iron.[20] Newborn pups of rats fed a trace mineral deficient diet supplemented throughout gestation with HFC at a daily dose equivalent to the recommended human daily dose had significantly greater whole-body contents of cobalt and zinc than did new born pups of rats fed the same trace mineral deficient diet supplemented with equivalent amounts of inorganic trace mineral salts.[18]

2. Humifulvate concentrate (HFC) supports normal physiologic utilization of minerals and trace elements.

   Improvements in appetite and general well-being in pediatric patients with iron deficiency anemia were attributed to treatment with HFC for 3 weeks (31.4 mg HFC/10kg body weight/day).[17] Daily oral administration of HFC (4.5 mg/kg body weight, providing 2.2 mg/kg of humifulvate) to nine children with chronic eczema resulted in marked improvement in the degree of eczema in eight of the children in 3 weeks.[17] Cyclic discontinuation and reinstitution of supplementation were associated with exacerbation and amelioration, respectively, of disease severity.

   Among a set of case reports [21-24] describing the effectiveness of HFC (156.7 mg/day, providing 75 mg humifulvate daily, for varying lengths of time) as an adjuvant during cytostatic therapy in patients with confirmed tumors, one group of patients was reported to exhibit enhanced erythropoiesis during supplementation[24] and all groups of patients reported improvements in appetite, weight gain, general resistance to stress and capacity to work, while nausea, fatigue and need for analgesics were reduced. In a group of 29 adults experiencing hair loss attributed to trace element deficiencies who were treated with HFC (156.7 to 313.4 mg/day, providing 75 to 150 mg humifulvate daily, for 4 to 6 weeks), subjects who exhibited increases in serum iron concentrations also exhibited improvements in hair growth and regeneration.[25] Among a select group of 25 elite adult athletes who added HFC to their training regimens for 3 weeks (313.4 mg/day), most of the subjects reported perceptions of increased resistance to the stress of training and enhanced ability to focus during training bouts.[26]

   Rats fed HFC (10 mg/kg, providing 4.8 mg/kg of humifulvate) daily for 14 days prior to ischemic insult experienced greater coronary blood flow, aortic blood flow and left ventricular end diastolic pressure following insult than did placebo-fed rats, suggesting that HFC may be cardioprotective.[27] Female adult rats given HFC (960 mg/kg, providing 459.5 mg/kg of humifulvate) prior to whole body irradiation exhibited significantly faster recovery of platelet counts following irradiation.[28]

3. Humifulvate concentrate (HFC) supports the healthy reduction of heavy metal burdens.

   Humifulvate concentrate (HFC) contains negatively charged functional groups that contribute to the elimination of heavy metals stored in cells by organic bonding that resembles the transport of metalloproteins. Oral HFC has been demonstrated to increase urinary excretion of cadmium [15] and to decrease blood concentrations of cadmium [14,15,29] and lead [14,29-32] in adults treated with 156.7 to 313.4 mg/day (providing 75 to 150 mg/day of humifulvate) for 3 to 12 weeks. Oral HFC also has been reported to inhibit the intestinal absorption of cadmium and lead from food as well as their uptake from environmental sources.[14]

When radiolabeled strontium bound to humifulvate was fed to adult rats, the intestinal absorption and subsequent incorporation into bone of radiolabeled strontium was lower than when similar rats were fed free radioactive strontium salt.[33] In adult pigs fed 31.3 to 313.4 mg HFC daily (providing 15 to 150 mg/day of humifulvate), urinary excretion of mercury was significantly increased.[34] Isolated humic acid has inhibited the absorption of cadmium by rat intestine [35] and has reduced the accumulation of cadmium in the kidneys of rats.[36] Following 2 weeks of a trace mineral deficient diet, adult rats given oral HFC (at a dose equivalent to the recommended human daily dose) for 2 weeks exhibited significantly more rapid excretion of an oral cadmium burden, compared to similar rats given replacement trace minerals as inorganic salts.[18]

4. History of Use and Evidence of Safety

4i. History of Use of Dietary Ingredient

Humifulvate concentrate (HFC) has been marketed in Europe under the brand name of Humet®-R syrup since 1993. It is marketed as a dietary supplement for increasing overall vitality and promoting overall health. The humifulvate found in HFC has been reviewed and approved by the Hungarian National Institute of Pharmacy. Humet®-R syrup is registered in Hungary as a non-prescription preparation (GYI-430/1993). Humet®-R syrup also is registered for sale in Great Britain,[37] Taiwan, Portugal, Russia, Lithuania, and the Netherlands.

Typical cumulative exposure to the new dietary ingredient during historical use can be estimated. Use of the dietary supplement, HFC, as recommended (156.7 mg/day of the supplement, containing 75 mg/day of the new dietary ingredient, humifulvate, for up to 2 months), results in the cumulative consumption of up to 9402 mg of humifulvate concentrate, of which 4500 mg will be the new dietary ingredient, humifulvate. In addition, use of the dietary supplement as recommended results in typical cumulative exposure of up to 2202 mg of elemental potassium, 900 mg of elemental magnesium, 840 mg of elemental iron, 600 mg of elemental zinc, 180 mg of elemental manganese, 120 mg of elemental copper, 30 mg of elemental vanadium, 12 mg of elemental cobalt, 10.5 mg of elemental molybdenum and 7.5 mg of elemental selenium.

4ii. Evidence of Safety of Dietary Ingredient

Toxicological and mutagenicity studies evaluating the safety of the dietary supplement (humifulvate concentrate; HFC) containing the new dietary ingredient (humifulvate) provide data attesting to the safety of humifulvate. All toxicological studies, mutagenicity studies and studies on laboratory animals have utilized the dietary supplement, HFC. Independent laboratory analyses utilizing infrared spectroscopy and

fingerprinting have confirmed the consistent composition of the standardized HFC used in these studies.

Documentation of the safety of the amounts of each mineral and trace element in HFC can be found in reference 12.

a. Toxicology Studies

No signs of toxicity, gross organ pathology or death have been reported in single dose toxicity tests in male and female adult rats given up to 10,000 mg of standardized HFC per kg body weight (equivalent to up to 4786 mg/kg of humifulvate and about 80 times the typical cumulative human exposure to humifulvate when HFC is used as recommended). Acute studies in rats and mice have revealed no toxicity in daily doses exceeding 1000 mg HFC per kg body weight (equivalent to 478.6 mg/kg of humifulvate and about 500 times the typical daily human exposure to humifulvate). The acute oral LD_{50} for HFC was determined by the National Institute of Food and Nutrition Science (OETI) in Budapest, Hungary, to be greater than 10,000 mg of HFC per kg body weight (about 80 times the typical cumulative human exposure to humifulvate when HFC is used as recommended).[38] An independent testing laboratory (Pharmaceutical Control and Developing Laboratory Co., Ltd., Budapest, Hungary) determined HFC to be "practically non-toxic."[39]

During a "limit test," adult male and female rats given 600 mg/kg of HFC (providing 287.3 mg/kg of humifulvate) within 24 hours exhibited no signs of weight loss or macroscopic organ pathology.[39] Isolated cases of pulmonary hemorrhage and emphysema, thymic hemorrhage, splenic hyperemia and uterine changes occurred with similar frequency in both treated and matched control rats; it was reported that these findings were consistent with indications of agonal death. No other symptoms of toxicity or lethality were observed during 14 days of post-treatment observation. From these data it was determined that the maximal tolerable dose (MTD) of HFC is greater than 600 mg/kg within a 24-hour period (containing about 300 times the recommended daily dose of humifulvate, if a dose of 150 mg of HFC is ingested by a 75-kg human).

Adult rats given a single dose of 960 mg/kg of HFC (providing 459.5 mg/kg of humifulvate, about 8 times the typical cumulative human exposure to humifulvate) exhibited no adverse reactions or signs of toxicity following sublethal whole body irradiation.[40]

Reports summarizing acute oral toxicity studies in laboratory animals are provided in references 41-43.

In controlled cumulative toxicity testing, adult rats were fed HFC at the LD_{50} (10,000 mg/kg) daily for 24 days; body weights, hematological variables, indices of thyroid function and microscopic organ histology were unaffected by the supplement.[46] However, some treated rats exhibited splenic hemosiderosis and both control and treated

rats exhibited signs of peribronchial lymphocytic infiltration. In another study, adult rats fed HFC at 5, 15 or 50 mg/kg daily for 28 days (amounting to about 1.25, 3.75 and 12.5 times the typical cumulative human exposure to humifulvate, respectively) exhibited no effects of HFC on body weights, clinical chemistry, hematological variables, enzyme functions, or organs weights.[47] However, 3 weeks of HFC at 150 or 500 mg/kg daily (providing 71.8 or 239.3 mg/kg of humifulvate daily and amounting to about 28 and 93 times the typical cumulative human exposure to humifulvate, respectively) was associated with decreases in body weights and in liver and kidney weights, which the authors attributed to undocumented reductions in appetite.

Adult rats fed a diet deficient in trace minerals and supplemented with either HFC at a daily dose equivalent to that recommended for humans or an equivalent amount of inorganic trace mineral salts exhibited no differences in average body weights, organ weights (liver, lung, kidney, brain, heart, spleen), changes in these weights, total litter weights, individual birth weights of progeny, daily urine volumes and daily fecal weights.[18] However, adult rats given humic acid at the equivalent of 280 times the recommended human daily dose retained approximately 20% to 30% less dietary iron, zinc and selenium than did the rats fed HFC.

Groups of adult rats fed potassium humate providing either 60 or 240 mg/day of humic acid for 2, 4, 6 or 8 weeks exhibited growth rates, food consumption rates, physical agility, kidney and liver weights, white blood cell counts, red blood cell counts, thrombocyte counts, mean blood cell volumes, mean thrombocyte volumes, plasma hemoglobin concentrations, hematocrits, mean hemoglobin contents per red blood cell and mean red blood cell hemoglobin concentrations that were not different from those of control-fed rats.[48] There were no adverse reactions, signs of toxicity or deaths during 8 weeks of exposure to the equivalent of up to what would be 80% to 3 times the typical cumulative human exposure to humifulvate if HFC is 50% humic acid.

Reports summarizing the effects of prolonged oral intake of HFC in rats are provided in references 44 and 45.

The available scientific evidence indicates that humifulvate is not toxic or harmful when ingested by laboratory animals in amounts equivalent to between 0.8 and 500 times the typical cumulative human exposure.

b. Mutagenicity Studies

HFC has been found to exhibit no mutagenic activity under the Ames test criteria, using the *Salmonella typhimurium* reverse mutation assay, in tests conducted by the Toxicological Research Center, Ltd., Veszprem, Szabadsagpuszta, Hungary.[51] Additional studies conducted by the Medical Research Institute, Budapest, Hungary, using human peripheral blood lymphocytes, also have indicated that HFC is not mutagenic and does not increase the number or frequency of chromosome aberrations (clastogenesis) under test conditions.(44,45,52] Some data from these tests further suggest that HFC may be mildly anticlastogenic *in vitro* under certain conditions. Taken

The amounts of lead and arsenic contained in a single dose of HFC are well within the limits set by "Proposition 65" in the state of California,[69] standards for exposure to heavy metals that are used by many manufacturers of dietary supplements to ensure the safety of their products.

Although cadmium has only an oral inhalation limit, data from one study has led to the conclusion that average daily cadmium intake should be kept below 111 mcg,[70] over 60 times the amount contained in a single dose of HFC. Average daily intakes of cadmium from food in most areas that are not polluted with environmental cadmium range between 10 mcg and 40 mcg,[71] 5 to 25 times the amount contained in a single dose of HFC. The FAO/WHO Expert Committee on Food Additives and Food Contaminants recommended a tolerable weekly cadmium intake of 400 mcg to 500 mcg for an adult, or a daily average of 64 mcg to 79 mcg (about 35 to 40 times the amount of cadmium contained in a single dose of HFC).

e. Absence of Polycyclic Aromatic Hydrocarbons in HFC

The National Institute of Food and Nutrition Science (OETI) in Budapest, Hungary, reported that it was unable to detect any polycyclic aromatic hydrocarbons in samples of HFC, including benzo-(a)-pyrene, benzo-(b)-fluoroanthene, indenopyrene, benzo-(k)-fluoroanthene, fluoroanthene, or benzo-(ghi)-perylene.

f. Lack of Involvement of HFC in Endemic Chinese Diseases

Two peripheral vascular diseases, Blackfoot's Disease and Keshan-Beck Disease, endemic to regions in China have been associated with the ingestion of humic acids and fulvic acids, obtained from local aquatic sources, in conjunction with relatively large amounts of arsenic.[72,73] The clinical signs of these two diseases may well be manifestations of chronic arsenic poisoning *per se*.[72] The sources and components of HFC have never been implicated in any case of arsenism or peripheral vascular disease; the arsenic content of HFC is well below that associated with clinical disease.

5. Commercial and Confidential Designated Materials

The cover letter containing the notification is available for dissemination to the public. This expanded notification and all other materials submitted with the notification, including all attachments, are deemed proprietary and are therefore designated confidential.

6. **Conclusion and Certification**

The enclosed information and scientific studies referenced herein establish that the dietary ingredient, Humifulvate, when used under the conditions recommended, is safe.

Respectfully Submitted,

Andrew G. Pichler, M.D.
President
Corvina Natural Products, Inc.

REFERENCES TO NOTIFICATION OF NEW DIETARY INGREDIENT

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Corvina Natural Products, Inc.

HUMIFULVATE™ CHEALATED MINERAL SUPPLEMENTS,
HERBALS & VITAMINS

Andrew G. Pichler, M.D.
Exclusive U.S. Distributor for:
Humet Corporation; Budapest Hungary
6633 Coyle Avenue #2, Carmichael, CA 95608
(916) 961-2266 FAX (916) 967-7939

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Ms. Rhonda Kane
Food and Drug Administartion
Div. of Standards & Labeling Regulations
Room 2804
200 C Street, SW
Washington, D.C. 20204

Dear Ms. Kane;

Attached is the English translation of the particular article that you have indicated.

Thank you for your help and if you have any further questions please call.

Yours truly,

Andrew Pichler, M.D., F.A.C.S.
Corvina Natural Products. Inc.

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PANNON University of Agriculture
Faculty of Animal Breeding, Kaposvár, Hungary
Department of Chemistry, Biochemistry

STUDY REPORT

Effect of feeding with doted and undoted humic acid preparation on the mineral status in the rat

Kaposvár
1997.

1. Sponsor

HORIZON-MULTIPLAN Research and Development Company
Research Director: Dr. Dénes Medzihardszky, Ph.D.
Address: 1121 Budapest, Konkoly Thege út 29-33.
Phone: (36-1) 275-4868
Fax: (36-1) 275- 4869

2. Contractor

PANNON University of Agriculture, Faculty of Animal Breeding, Kaposvár, Hungary (Department of Chemistry, Biochemistry)
Project leader: Dr. Imre Sarudi professor, Department Head
Address: 7400 Kaposvár, Guba S. u. 40.
Phone: (36-82) 314-155
Fax: (36-82) 320-175

3. Contract file number

- at the Sponsor: 33-1-03
- at the Contractor: HM-97.

4. Date and place of delivery

- place: see the address in point 2 above
- duration: from March 16 to May 15, 1997
  (Feeding period: from March 25 to April 24)

5. Research work in connection with the project topic

5.1 Introduction

The active ingredient of the syrup preparation developed by the Sponsor and known as HUMET®-R consists of humic acids derived from peat as well as essential macro- and microelements (K, Mg, plus Fe, Zn, Mn, Cu, V, Co, Mo and Se). The conglomerate of these elements, known as 'doted humic acid' has been found ideal as a mineral supplement based on clinical trials and other human and animal observations and experiments conducted made to date [1-3]. Its effects have been manifested in the elimination of deficiencies of the essential elements contained therein, the reinforcement of immune reactions when needed, the increase of mental and physical capacities as well as the weight gain and improved reproduction indices in farm animals. More and more experimental data suggest that the said preparation provides certain measure of protection against heavy metals ingested per os [4-7].

Although the physiological benefits of HUMET®-R can be regarded as well-documented in many respects, research work focusing on these issues have recently intensified. This is mainly because of the very complex effect of the preparation due to its numerous components, and consequently a thorough knowledge of the exact mechanisms of action – essential for developing further products as well – may be gained only after the clarification of various details. The topic described below relates to one of these details, for it covers the effects of doted and undoted humic acid on the mineral status of the body, more precisely on the availability of bivalent cation-forming elements. The study design has been developed jointly by the representatives of the Sponsor and the Contractor in compliance with the current professional requirements.

The reason for narrowing the study topic as explained above is that among all the mineral components of HUMET®-R which humic acids form complexes

with the metal ions only, therefore effects on absorption and bio-availability can be detected primarily in regard to these elements [8-12]. It has been reported that humic acids are complex macro molecules, coming probably from lignin, to be found in the organic fraction of the soil and in dead plant remains still in the carbonisation stage (e.g. peat). They are made up of a polycyclic nucleus and the polysaccharides, polypeptides and phenols loosely bound to it. Their bio-activity is mainly based on their capacity to chelate in a reversible bond bivalent and multivalent metal ions with the help of their free functional groups (-COOH, phenolic and alcoholic –OH, $-NH_2$ etc.). The chemical formations produced this way, just like undoted humic acids, have considerable expanding capacity, while they are predominantly hydrophobic in nature, and the metal ions located in the chelate rings are clearly dehydrated. It has been assumed that the beneficial results obtained from the administration of the HUMET®-R preparation are attributable for the most part to these properties [1].

5.2 Materials and methods

The experiment was started with 30 female rats of the Wistar 579 phylum, each with a bodyweight of 80-125 g, divided into three groups of equal sizes, but due to deaths occurring for unknown reasons, results could be gained finally for only 8 animals in Group I (control group) and 9-9 animals each in Groups II and III.

The animals were let to acclimatise for four days, then experimental feeding lasted for thirty days. The animals were kept each in a standard cage of 55x28x20 cm in size, furnished with refilling water feeder. Animal keeping circumstances were otherwise fully compliant with the generally accepted recommendations [13]; lighting: diffused light through 15-16 hours a day; temperature 18-22°C; relative humidity: 50-60%; ventilation: air exchange approx. 18 times per hour. Rats were given rat feed of standard composition (Annex I.) and drinking water of excellent quality; both administered ad libitum.

This experiment focused on testing two substances in liquid form as made available to us by the Sponsor, notably a humic acid preparation (HA) and the same supplemented with various essential elements (HAD). As stated by the Sponsor, the humic acid concentration of HAD was just half of that of the HA liquid. When we had occasion we also determined the mineral content of the test materials in the appropriate detail (Annex II.).

In the course of experimental feeding the animals of Groups II and III received 320µl/body weight kg HA and 640µl/body weight kg HAD, respectively. Administration took place always by using the gastric tube technology. In an effort to guarantee the above daily dose/body weight ratio, the body weights of animals were taken by five-day intervals and the volume of the test material administered to individual animals was then adjusted to their gain in weight.

On day 31 following the administering of test materials the animals were anaesthetised with chloroform and then slaughtered, then subjected to so-called whole-body analysis for metals that form divalent cations [14, 15]. To do this, the bodies of rats – after removing the stomach and intestines – were homogenised in a mash in an equipment specially manufactured for this purpose using corrosion-proof material. The mash was then incinerated at 600±50°C. After this the ashes were diluted into a soft nitric acid solution, and concentrations of calcium, magnesium, manganese, copper, zinc and iron were determined using the atomic absorption technique [16, 17]. An PYE UNICAM SP-190 AAS instrument with air/acetylene burner was used for such assays. The following measurement wavelengths were used: Ca: 422.7 nm, Mg: 285.2 nm, Mn: 279.5 nm, Cu: 324.8 nm, Zn: 213.9 nm and Fe: 243.8 nm.

Measurement results were given for the so-called wet weight and processed using multi-factor variance analysis.

5.3 Results and discussion

Apart from the four deaths as mentioned before no abnormalities in the health condition and behaviour of the animals could be observed during the study. The weight gain in the animals was appropriate for the species and the age of the animals (Annex III.). No significant difference in body weights could be observed across the groups when compared on the same days.

The results (Tables 1 and 2) have shown that humic acid alone did not increase the absorption of the macro- and microelements examined; moreover it even caused the reduction of iron uptake (p=5%). Essentially the same applies to calcium and magnesium, though with less convincing statistical certainty (p=20%).

As we had expected, doted humic acid caused higher macro- and microelement concentrations in rat bodies compared to undoted humic acid. However, it must be noted here that the beneficial effects of doted humic acid compared to the control group could be demonstrated with statistical significance (p=5%) only in the case of calcium, magnesium and iron.

Table 1.

Results of whole-body analyses in the rat (g/kg wet weight)

- macroelements -

Group	Test material	Ca		Mg	
		x	SD	x	SD
I. (n=8)	-	29.22[a□]	3.05	1.02[a□]	0.06
II. (n=9)	HAx	26.48[a□]	3.01	0.97[a□]	0.05
III. (n=9)	HADxx	32.98[b]	4.03	1.10[b]	0.06

[x] undoted humic acid preparation: 320/µl/bodyweight kg/day

[xx] preparation containing doted humic acid: 640/µl/bodyweight kg/day

Legend of indices:

- identical small Latin letters in the same column: no significant difference at probability level p=5%;
- different Greek letters in the same column: significant difference at probability level p=20%.

Table 2.

Results of whole-body analyses in the rat (g/kg wet weight)

- microelements -

Group	Test material	Mn		Cu		Zn		Fe	
		x	SD	x	SD	x	SD	x	SD
I. (n=8)	-	2.22[a□□]	0.21	4.54[abAB]	0.94	80.56[abAB]	2.50	133.3[a]	6.75
II. (n=9)	HAx	2.11[a□]	0.28	3.94[aA]	0.58	79.03[aA]	3.83	121.1[b]	10.8
III. (n=9)	HADxx	2.29[a□]	0.20	4.99[bB]	0.37	82.74[bB]	5.33	148.0[c]	13.2

Footnotes:

same as for Table 1, plus: the various Latin capitals (Zn) refer to the fact that the difference exists at the level of p=10%.

5.4 Conclusions

Given a sufficient macro- and microelement supply, undoted humic acid caused some deterioration in the iron, calcium and magnesium status of the body, while the beneficial effects of doted humic acid were manifested in regard to these same elements. In respect of manganese, copper and zinc, the effects of neither the doted, nor the undoted humic acid could be shown with statistical significance.

Having compared these results with the favourable results obtained with regard to the HUMET®-R preparation earlier, it is suggested that the macro- and microelement supplementation capacity of the doted humic acid depends largely on the original availability of these elements to the body. In this situation the macro- and microelement content of the rat feed was satisfactory, and that is why supplementation in general was not reflected in the whole-body analysis results with a sufficiently convincing level of significance. It is advisable, therefore, to perform the tests on rats that receive during, or received in the weeks preceding, the experimental rat feed that is indeed poor in macro- and microelements. Furthermore, it seems reasonable to use in such experiments also animal groups that receive supplementation without humic acids, i.e. in the usual inorganic forms ($ZnSO_4$, $MnSO_4$ etc.).

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Kaposvár, June 10, 1997.

Dr. Imre Sarudi

university professor, head of department

Annex I.

Composition of rat feed

Component	g/kg	mg/kg
Dry substance	919	-
Crude protein	237	-

Crude fat	53	-
Crude fibre	105	-
Crude ash	90	-
Nitrogen-free material	434	-
Sodium (Na)	0.31	-
Potassium (K)	10.20	-
Calcium (Ca)	4.60	-
Magnesium (Mg)	2.64	-
Phosphor (P)	4.28	-
Manganese (Mn)	-	43
Copper (Cu)	-	8.5
Zinc (Zn)	-	36
Iron (Fe)	-	200

Annex II.

Mineral content of test materials
(mg/kg liquid)

Element	Humic acid (HA)	Doted Humic acid (HAD)
Calcium	2065	532
Magnesium	248	2490
Manganese	9.5	517
Copper	0.8	338
Zinc	1.9	1769
Iron	257	2127

- Doted humic acid (and probably undoted humic acid, too) contains other minerals as well, but for this project it seemed unnecessary to determine these elements.
- Assays were made from dilute nitric acid ash solution using the AAS measurement technology.
- Dry substance: - HA: 6.1%; HAD: 5.4%.

Annex III.

Development of rat body weights during the experiment (g)

day	Group I.		Group II.		Group III.	
	(n=8)		(n=9)		(n=9)	
	x	SD	x	SD	x	SD
1.	95.75	14.4	96.9	10.9	98.4	11.5
6.	109.3	15.1	106.3	11.5	104.4	10.8
11.	117.8	13.9	115.5	12.0	123.5	12.2
16.	124.5	13.0	126.2	15.6	129.1	14.0
21.	137.5	14.2	138.6	16.3	134.0	15.7
26.	147.2	14.7	148.4	17.3	140.8	16.1
31.	153.9	18.8	153.1	20.1	147.8	14.2