Hungarian Army
Health Institute
Toxicological Research Department

HUM 057

*Research-development report*

# 60 day toxicological study of a powder humic acid product in rats

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**1998.**

## Introduction

In the studies performed so far, the examination of acute and chronic toxicity, and biological efficacy of natural humic acids and supplemented humic acid based products, was done in all cases with the active ingredient in liquid form. With the development of the manufacturing technology process, it is a step forward, that the active ingredient is produced in powder form. This production method guarantees, that in the manufacturing process, the ability to standardize the active ingredient can be increased significantly (as a result of separating on molecule sieves, the composition becomes permanent), the time until the product is storable increases.

The aim of our supplementary toxicological studies is to prove, that similarly to the prolonged, large-dose consumption of natural humic acid in liquid form and the product made from it, the new, powder active ingredient, and product does not have any toxic effects even in the case of long-term consumption.

In our present research report we summarized our results concerning the toxicity of powder humic acid.

Horizon Multiplan Ltd. manufactured the study test material for our studies and we performed the studies according to Attachment 1.

The starting date of the tests was 1998. October 1 according to the agreement, and the end date and the preparation date of the report was 1998. December 15.

Participating persons with a degree:

Dr. András Gachályi, certificated chemist engineer, certificated nuclear chemistry engineer, technical doctor

Dr. József Naményi, certificated biologist, university doctor of natural sciences

Dr. Győző Horváth, doctor of medicine, laboratory expert doctor, radiology biology and radiology health expert doctor

## Material and Method

1. ) Test animals

For the experiment WISTAR female rats were used with a 170-190 g average weight, and according to their weight they were divided into three random groups.

The animals were kept in a room with controlled temperature (26±5 °C) and humidity (70±15 %), variable illumination (12-hour light-dark cycle), in type II. plastic cages (5 animals per cage). Tap water, normal or active ingredient containing food (groups 2 and 3) was administered ad libitum.

The experimental animals were acclimatized to the test conditions for one week. General physical condition was monitored daily, the number and dates of mortalities were recorded. The weight of the animals was recorded one week before starting the experiment, then weekly.

After starting the feeding with humic acid enriched food (HS), on the $2^{nd}$, $4^{th}$, $6^{th}$, and $8^{th}$ weeks the animals were ether anesthetized, blood was taken from the abdominal aorta, then the liver, kidneys, spleen and thigh bones were removed and the organs were weighed.

## 2. ) Compounds used

The product for the experiment was supplied for us by Horizon Multiplan Ltd. (Certificate attached).

**Batch number: 014KHL1980419 potassium humate (HS)**, 50 % humic acid + fulvic acid

The effective ingredient marked with HS was mixed to normal laboratory food (powder, standard rat food, code number: 624, manufacturer: Farmer Promt Ltd., distributor: Bioplán Ltd.) and then it was given to animals. 20 g of the granulated food contained the rats' daily 60 mg (HS group 1) and 240 mg (HS group 2) humic acid need.

## 3.) Hematological studies

**Operation:**

On the experimental animals, following ether anesthetization, laparotomy was performed, the abdominal aorta was penetrated, and blood samples were taken into plastic syringes. The aliquot of the blood was distributed into so-called hematological tubes containing dried anti-coagulant (Na-EDTA). Following this, the usual hematological parameters (see the list below) were determined.

The spleen of the animals was removed and weighed, and then by adding physiological NaCl, with cautious homogenization we prepared 40 mg/ml cell suspension in such a way that the cells remained intact. Following this from the cell suspension by a Laborscale (Medicor) cell analyzator we determined the mononuclear cell count.

We prepared and removed the left thighbone (femur) of the animals, and by drilling through at the capitulum, spraying through with a 10 ml physiological NaCl, we removed the bone marrow. We centrifuged the cell suspension, and then determined the bone marrow cell count by a Laborscale (Medicor) cell analyzator.

The hematological studies were done after feeding with normal and humic acid enriched food on the $2^{nd}$, $4^{th}$ , $6^{th}$, and $8^{th}$ weeks (groups 1-3).

The hematological studies were done by TWINCELL (DIATRON Messtechnik GMBH, Wien, Austria) analyzator.

The measured parameters and abbreviatons:

| | |
|---|---|
| **FVS:** white blood cell count | ($10^9$/liter) |
| **VVT:** red blood cell count | ($10^{12}$/liter) |
| **PLT:** thrombocyte count | ($10^9$/liter) |
| **MCV:** average volume of VVT | (femtoliter) |
| **RDW:** size spectrum of VVT | (femtoliter) |
| **MPV:** average volume of PLT | (femtoliter) |
| **PDW:** size spectrum of PLT | (femtoliter) |
| **HGB:** hemoglobin concentration | (gram/deciliter ) |

Derived parameters:
**HCT:** hematocrit (the % fraction of the shaped elements) (% )
**MCH:** average hemoglobin content of VVT (picogramm )
**MCHC:** average hemoglobin concentration of VVT (gram/deciliter )

## 4. ) Experimental groups

1. Control: standard food and tap water — 16 animals
2. Feeding for 8 weeks by **60 mg**/day dosed **humic acid enriched** rat food (HS 1.) — 20 animals
3. Feeding for 8 weeks by **240 mg**/day dosed **humic acid enriched** rat food (HS 2.) — 20 animals

## Results

The effect of the feeding of different dosed humic acid raw material containing food for 8 weeks on the change of body weight is shown in Table 1 and Figure 1.

**Table 1:** The effect of the prolonged feeding of different dosed humic acid food on the change of body weight in rats (**average** ± SD)

| Duration | Control | | HS (1.) | | HS (2.) | |
|---|---|---|---|---|---|---|
| (weeks) | **average** | ± SD | **average** | ± SD | **average** | ± SD |
| 0 | **188,4** | 11,2 | **189,1** | 12,5 | **183,9** | 13,8 |
| 1 | **197,3** | 10,6 | **190,0** | 10,4 | **190,2** | 13,1 |
| 2 | **215,1** | 18,0 | **206,4** | 15,1 | **209,9** | 16,6 |
| 3 | **227,8** | 16,9 | **217,3** | 19,8 | **215,3** | 19,3 |
| 4 | **237,0** | 20,6 | **224,8** | 21,3 | **233,6** | 20,5 |
| 5 | **251,5** | 19,7 | **230,8** | 24,9 | **246,1** | 24,8 |
| 6 | **256,1** | 23,5 | **231,4** | 21,5 | **259,7** | 24,0 |
| 7 | **270,5** | 27,1 | **237,4** | 27,3 | **264,4** | 24,3 |
| 8 | **272,8** | 29,2 | **244,4** | 27,6 | **270,8** | 27,3 |

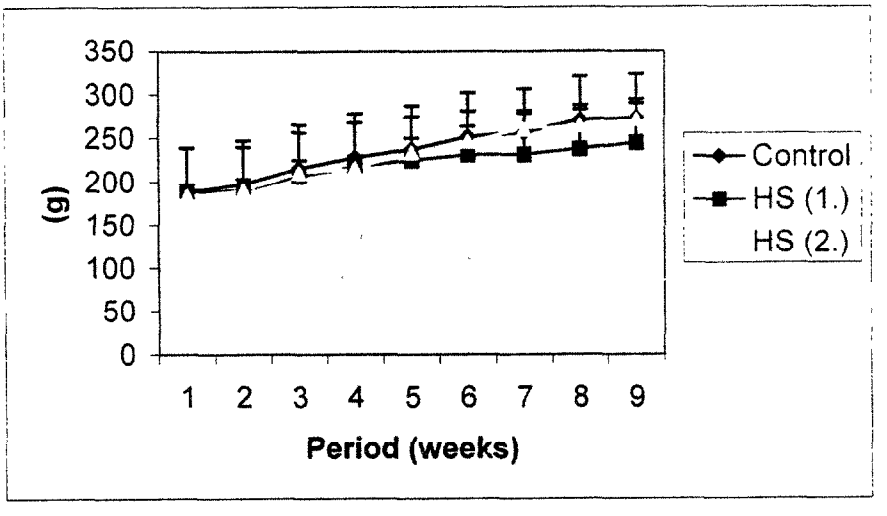


**Figure 1:** The effect of the prolonged feeding of different dosed humic acid food (HS 1. and HS 2.) on the change of body weight in rats

According to our results, until the fourth week, the humic acid treatment practically did not influence the change of body weight in the experimental animals. One month after starting the humic acid feeding, in the group with the 60 mg/day dose (HS 1.) the weight increase slowed down and this tendency continued until the end of the experiment. The weight loss can be explained by the fact, that from the food containing the active ingredient in lower concentration, the animals consumed the 60--70 % of the daily dose.

However, we have to remark that the weight deviation between the groups was not significant.

During the total duration of the experiment, mortality occurred neither in the control group, nor in the humic acid treated group.

**Table 2:** The effect of the prolonged feeding of different dosed humic acid food on the change of liver weight in rats (average ± SD)

| Duration | Control | | HS (1.) | | HS (2.) | |
|---|---|---|---|---|---|---|
| (weeks) | **average** | ± SD | **average** | ± SD | **average** | ± SD |
| 2 | **8,13** | 1,32 | **8,96** | 0,74 | **9,30** | 0,75 |
| 4 | **8,65** | 0,99 | **7,08** | 1,97 | **8,60** | 1,40 |
| 6 | **8,63** | 1,16 | **8,48** | 1,72 | **9,02** | 1,52 |
| 8 | **9,00** | 1,35 | **8,08** | 1,32 | **9,34** | 1,16 |

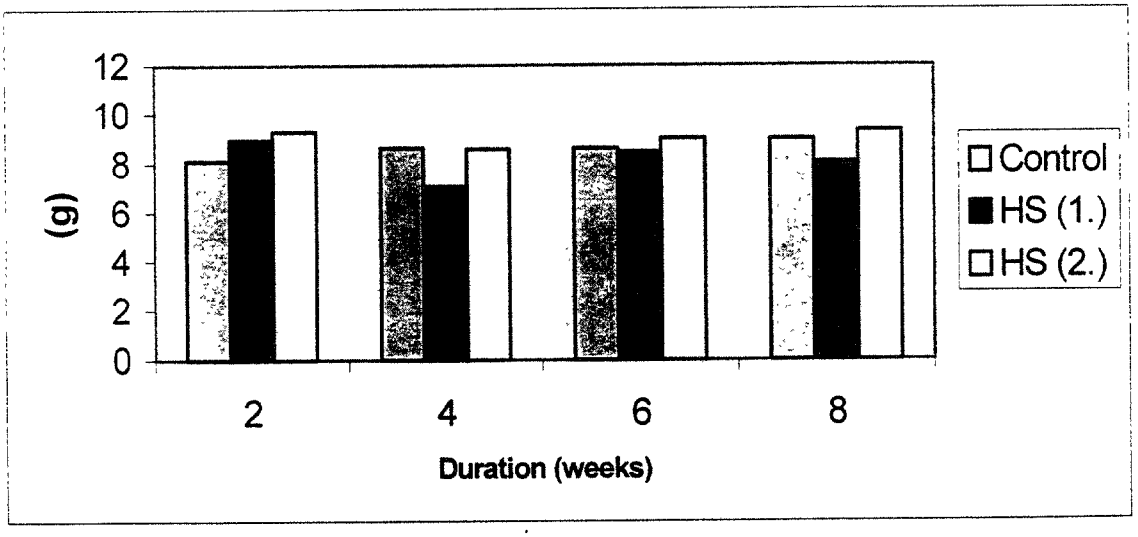


**Figure 2:** The effect of the prolonged feeding of different dosed humic acid food (HS 1. and HS 2.) on the change of liver weight in rats

**Table 3:** The effect of the prolonged feeding of different dosed humic acid food on the change of kidney weight in rats (**average** ± SD)

| Duration | Control | | HS (1.) | | HS (2.) | |
|---|---|---|---|---|---|---|
| (weeks) | **Average** | ± SD | **Average** | ± SD | **Average** | ± SD |
| 2 | **1,53** | 0,33 | **1,72** | 0,23 | **1,72** | 0,19 |
| 4 | **1,50** | 0,24 | **1,66** | 0,32 | **1,62** | 0,22 |
| 6 | **1,65** | 0,06 | **1,62** | 0,31 | **1,78** | 0,31 |
| 8 | **1,80** | 0,24 | **1,56** | 0,24 | **1,88** | 0,23 |

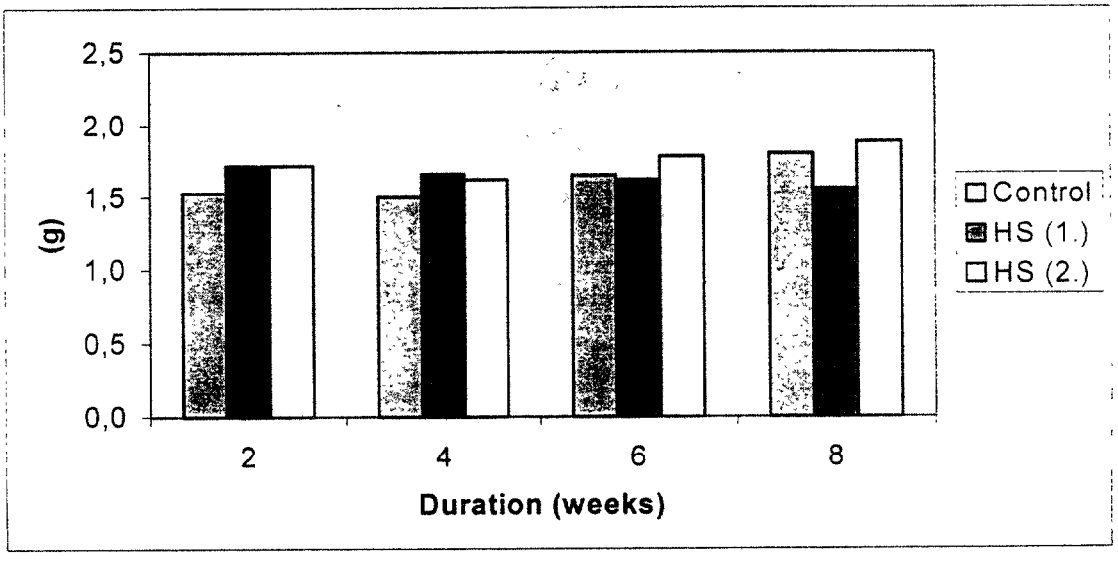


**Figure 4:** The effect of the prolonged feeding of different dosed humic acid food (HS 1. and HS 2.) on the change of kidney weight in rats

At the examination of the organs we determined that in the case of the different dosed humic acid fed groups, neither the liver, nor the kidney absolute weight deviates significantly from the similar values of the control group. Although in the humic acid group treated with 60 mg/day, corresponding to the weight decrease, from the fourth week liver weight decreased as well, however, this decrease is not significant. Therefore the results proved that the continuous feeding with humic acid containing food for 8 weeks did not influence the development of the examined organs.

We demonstrate the hematological parameters of the rats fed by humic acid containing food, the average values of the groups in Table 4. For the sake of clarity we show these data in figures as well (Figures 4-14). The individual values are given in Attachment 1.

**Table 4:** Summarizing table of the hematological parameters (**average** ± SD)

| Experimental Groups | Duration (weeks) | FVS ($10^9$/l) | | VVT ($10^{12}$/l) | | PLT ($10^9$/l) | | HGB (g/dl) | | HCT (%) | | MCH (pg) | | MCHC (g/dl) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Average** | SD | **Average** | SD | **Average** | SD | **Average** | SD | **Average** | SD | **Average** | SD | **Average** | SD |
| Control | 2 | **6,70** | 0,62 | **6,71** | 0,29 | **1449** | 77 | **14,63** | 0,83 | **34,23** | 2,14 | **21,68** | 0,91 | **44,55** | 2,60 |
| | 4 | **7,33** | 1,96 | **6,19** | 0,30 | **1392** | 84 | **13,20** | 0,35 | **30,55** | 0,70 | **21,38** | 1,45 | **43,25** | 2,04 |
| | 6 | **7,73** | 0,94 | **6,79** | 0,28 | **1429** | 194 | **14,53** | 0,76 | **36,45** | 2,30 | **19,98** | 1,34 | **39,95** | 3,14 |
| | 8 | **7,95** | 1,03 | **6,77** | 0,79 | **1281** | 162 | **15,85** | 1,07 | **32,70** | 3,74 | **23,55** | 1,34 | **48,70** | 2,80 |
| HS (1.) | 2 | **8,10** | 1,43 | **6,67** | 0,37 | **1670** | 147 | **13,98** | 0,62 | **36,92** | 1,18 | **20,96** | 0,72 | **37,86** | 1,46 |
| | 4 | **6,46** | 0,49 | **7,07** | 0,22 | **1593** | 155 | **14,96** | 0,94 | **35,86** | 2,40 | **21,12** | 1,13 | **40,88** | 2,01 |
| | 6 | **6,98** | 1,53 | **7,09** | 0,62 | **1607** | 181 | **13,84** | 0,24 | **36,30** | 2,02 | **19,72** | 1,57 | **38,42** | 2,17 |
| | 8 | **7,26** | 1,27 | **6,28** | 0,47 | **1304** | 116 | **14,58** | 1,73 | **29,70** | 2,90 | **23,98** | 1,44 | **49,06** | 2,26 |
| HS (2.) | 2 | **7,68** | 1,65 | **6,37** | 0,20 | **1639** | 202 | **14,46** | 0,15 | **34,32** | 1,93 | **22,72** | 0,68 | **42,24** | 2,28 |
| | 4 | **8,26** | 0,98 | **6,48** | 0,27 | **1287** | 66 | **13,64** | 0,47 | **33,02** | 1,44 | **21,06** | 0,78 | **41,34** | 1,40 |
| | 6 | **6,98** | 1,52 | **7,28** | 0,65 | **1371** | 53 | **15,10** | 0,75 | **37,10** | 3,43 | **20,83** | 1,06 | **40,90** | 2,22 |
| | 8 | **6,80** | 0,71 | **6,10** | 0,22 | **1227** | 41 | **15,00** | 0,42 | **28,94** | 1,41 | **25,54** | 2,14 | **51,84** | 3,75 |

Table 4: (continued)

| Experimental groups | Duration (weeks) | Erythrocyte parameters MCV average | (fl) SD | RDW average | (fl) SD | Thrombocyte parameters MPV average | (fl) SD | PDW average | (fl) SD |
|---|---|---|---|---|---|---|---|---|---|
| Control | 2 | **50,88** | 2,18 | **22,98** | 0,83 | **8,08** | 0,19 | **13,28** | 0,72 |
| | 4 | **49,43** | 1,39 | **21,98** | 0,62 | **7,33** | 0,10 | **13,50** | 0,40 |
| | 6 | **49,98** | 1,39 | **24,23** | 0,62 | **7,90** | 0,18 | **12,75** | 0,71 |
| | 8 | **48,33** | 0,38 | **25,05** | 0,83 | **7,78** | 0,26 | **12,35** | 0,71 |
| HS (1.) | 2 | **55,44** | 2,41 | **26,16** | 0,83 | **7,74** | 0,25 | **12,16** | 0,75 |
| | 4 | **50,64** | 2,01 | **23,02** | 1,44 | **7,70** | 0,45 | **13,70** | 1,06 |
| | 6 | **51,22** | 1,71 | **23,98** | 0,96 | **8,12** | 0,20 | **13,14** | 0,63 |
| | 8 | **48,88** | 0,88 | **23,82** | 0,38 | **7,52** | 0,13 | **11,56** | 0,36 |
| HS (2.) | 2 | **53,84** | 1,45 | **25,50** | 0,54 | **7,92** | 0,18 | **13,08** | 0,63 |
| | 4 | **50,96** | 0,87 | **22,78** | 0,78 | **7,26** | 0,15 | **13,20** | 0,44 |
| | 6 | **50,90** | 0,54 | **24,08** | 0,94 | **8,15** | 0,13 | **13,30** | 0,46 |
| | 8 | **49,30** | 0,87 | **25,26** | 0,97 | **7,70** | 0,20 | **12,18** | 0,53 |

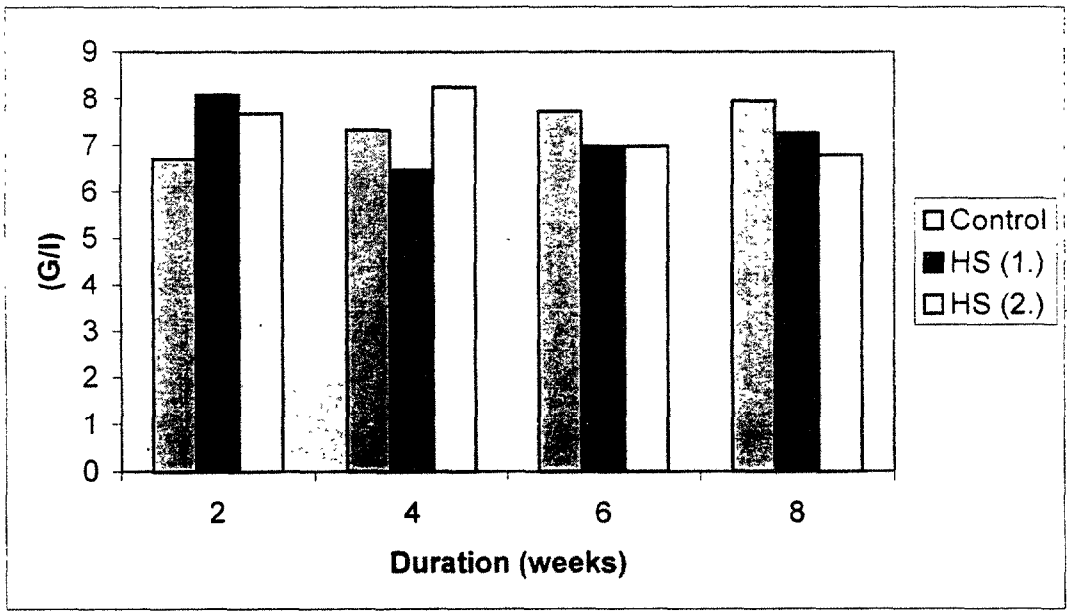


**Figure 4:** The effect of the prolonged feeding of different dosed humic acid food (HS 1. and HS 2.) on the change of the FVS count in rats

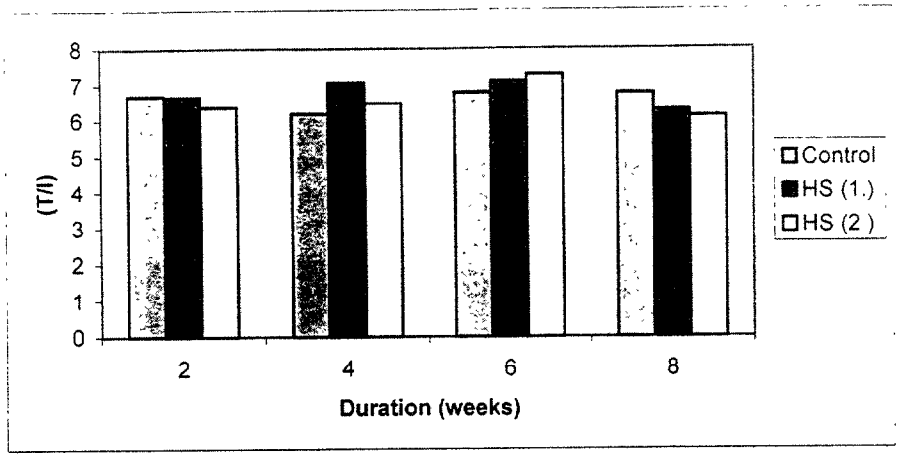


**Figure 5:** The effect of the prolonged feeding of different dosed humic acid food (HS 1. and HS 2.) on the change of the VVT count in rats

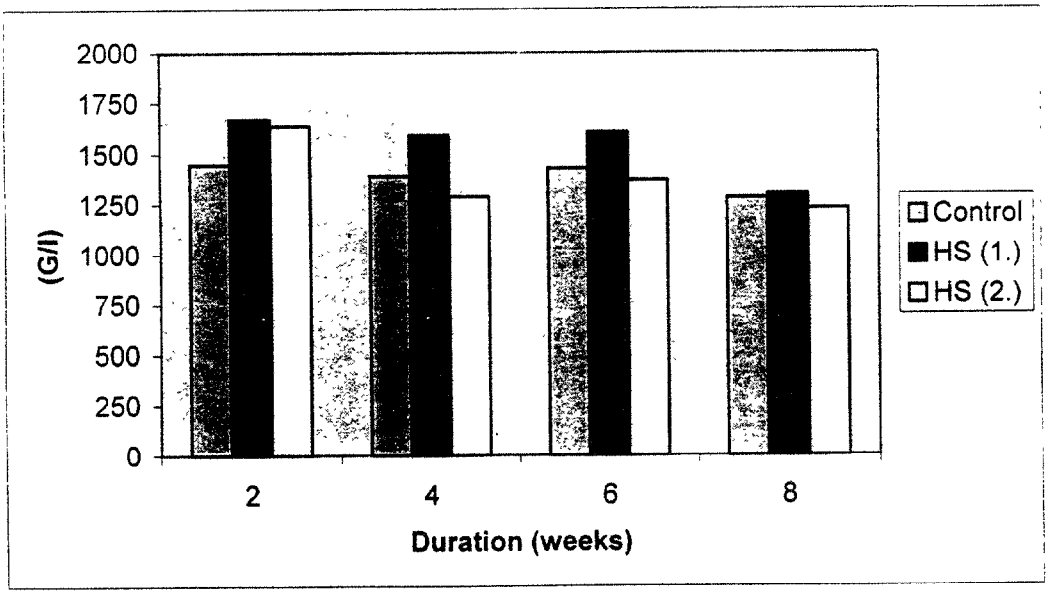


**Figure 6:** The effect of the prolonged feeding of different dosed humic acid food (HS 1. and HS 2.) on the change of the thrombocyte count in rats

Comparing the hematological parameters in the experimental groups, we can determine, that there is no significant deviation between the white blood cell, red blood cell and thrombocyte counts of the rats in the control and the rats fed continuously by two different dosed humic acid containing food (HS 1. and HS 2).

However, we have to remark that in the HS groups, on the 2nd week after starting the treatment, the average values of the white blood cell and the thrombocyte counts increased. This increase, because of the differences in the individual responding ability, is not significant.

Practically we can draw the same conclusion about the other parameters, i.e. in both treated groups they are the same as the similar values in the control animals. The only exception is the average volume of the red blood cells (MCV), for the 2nd week we found a small volume increase, the volume distribution curve widens.

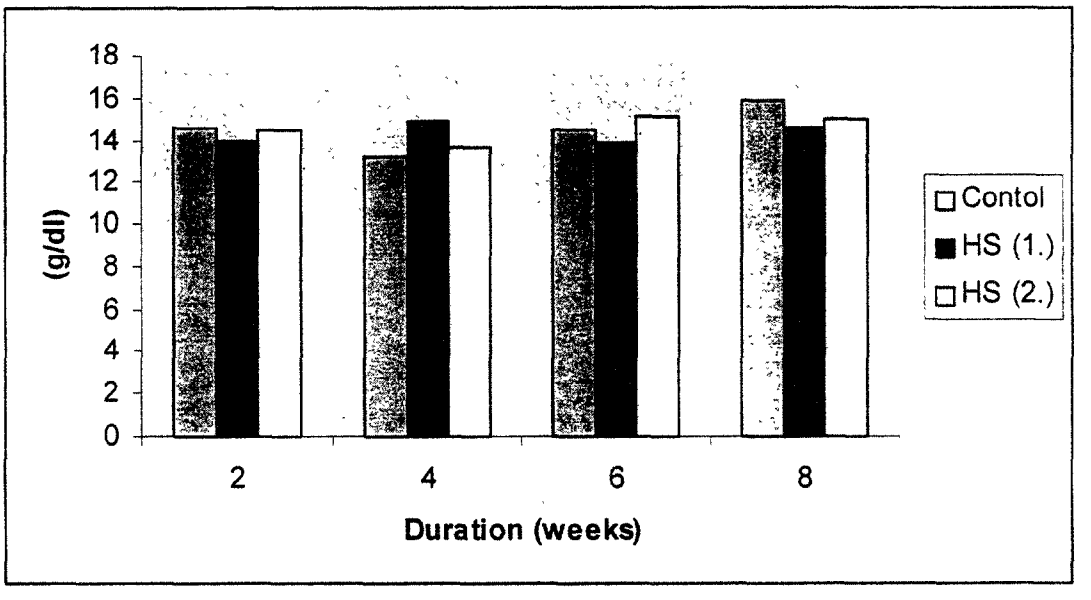


**Figure 7:** The effect of the prolonged feeding of different dosed humic acid food (HS 1. and HS 2.) on the change of the hemoglobin content in rats

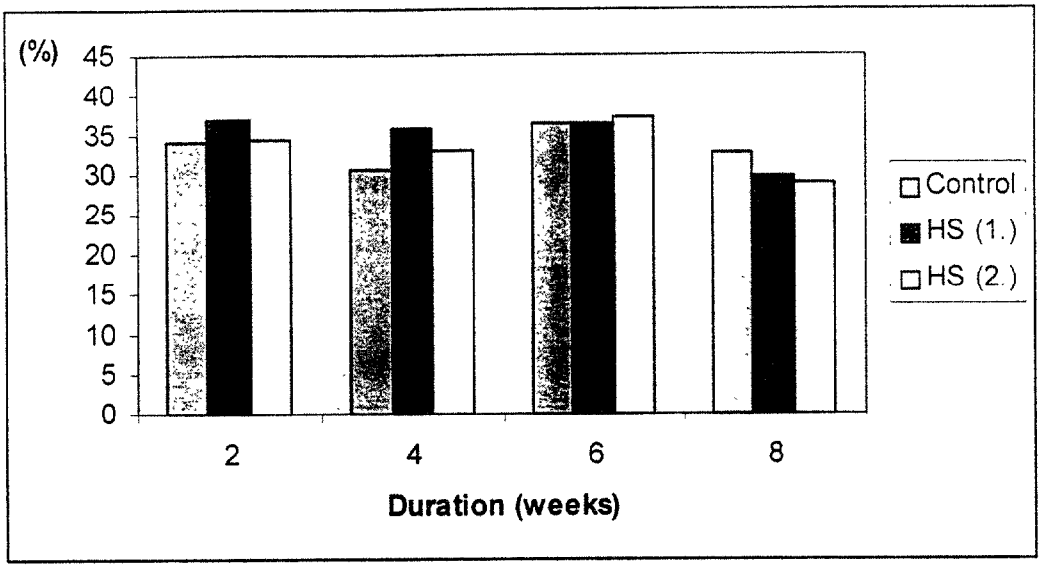


**Figure 8:** The effect of the prolonged feeding of different dosed humic acid food (HS 1. and HS 2.) on the change of the hematocrit values in rats

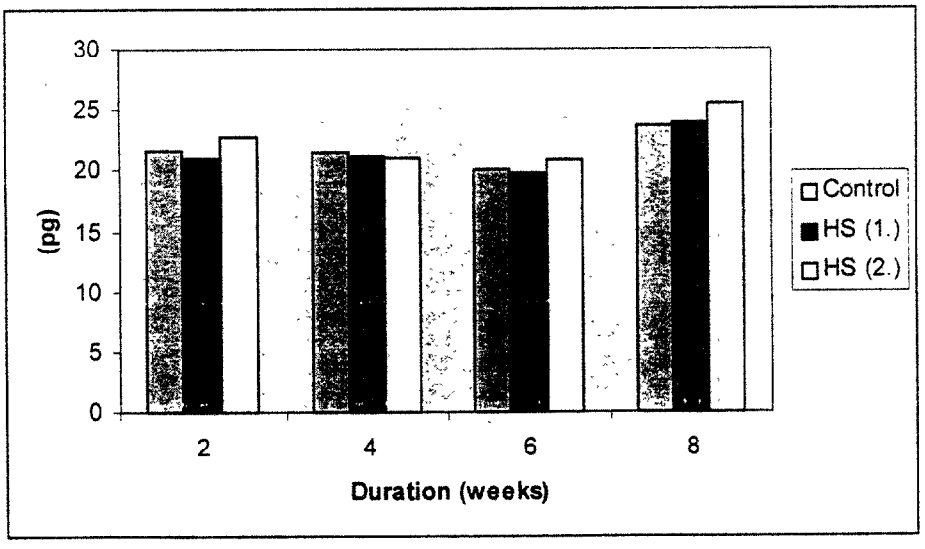


**Figure 9:** The effect of the prolonged feeding of different dosed humic acid food (HS 1. and HS 2.) on the change of the MCH values in rats

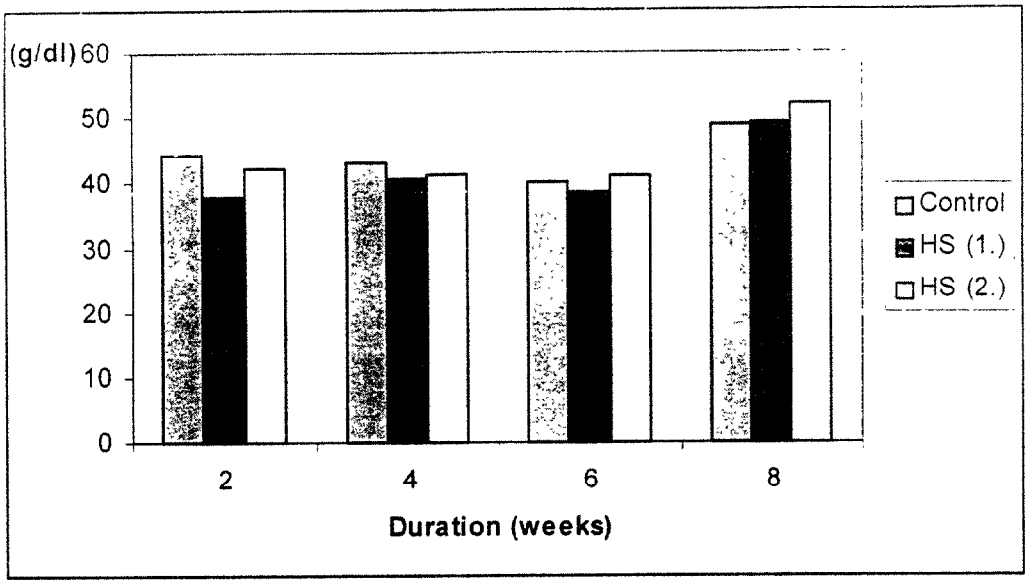


**Figure 10:** The effect of the prolonged feeding of different dosed humic acid food (HS 1. and HS 2.) on the change of the MCHC values in rats

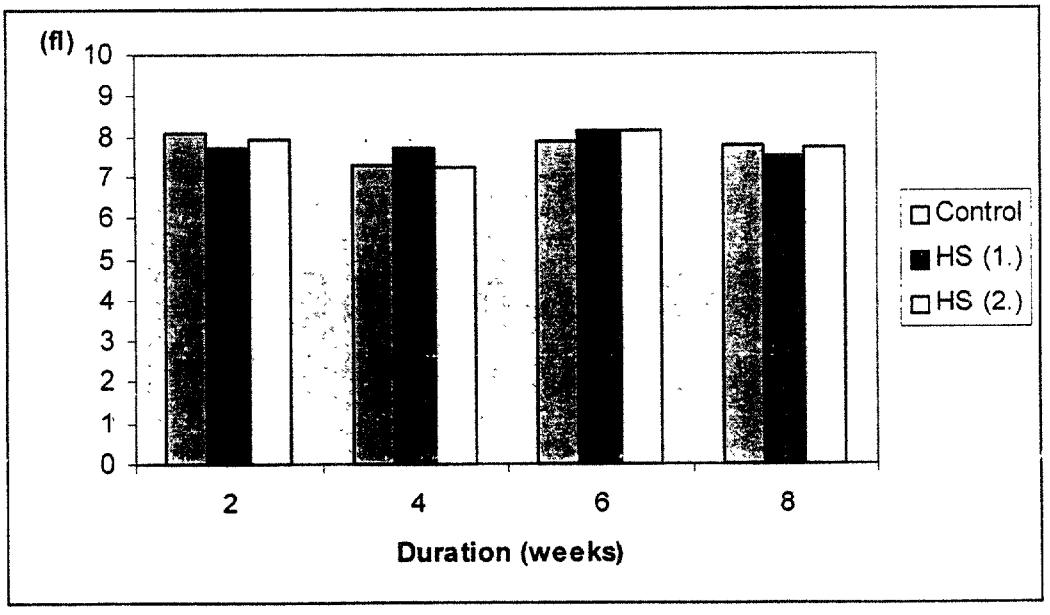


**Figure 11:** The effect of the prolonged feeding of different dosed humic acid food (HS 1. and HS 2.) on the change of the MPV values in rats

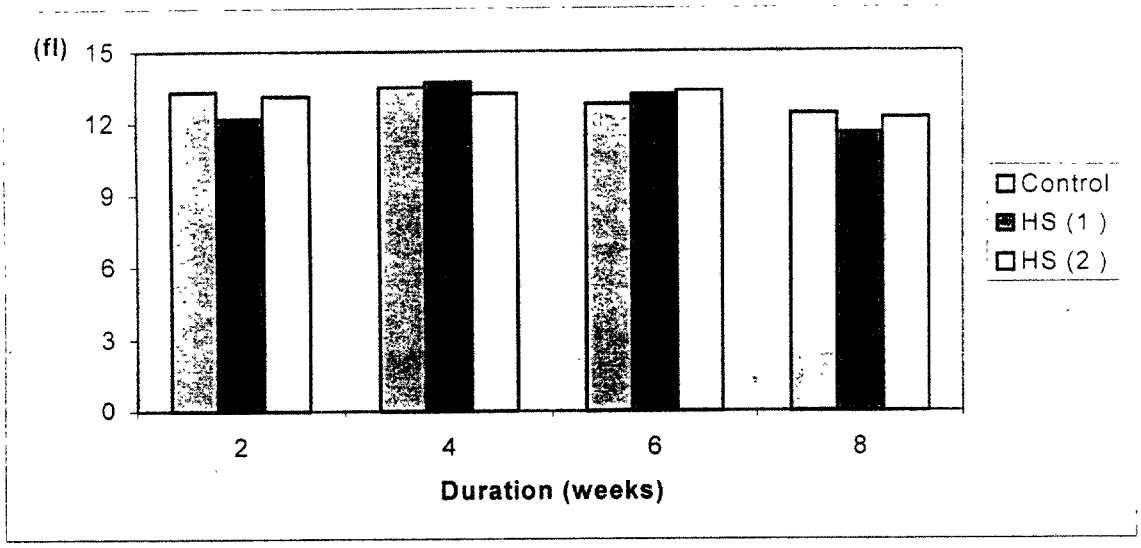


**Figure 12:** The effect of the prolonged feeding of different dosed humic acid food (HS 1. and HS 2.) on the change of the PDW values in rats

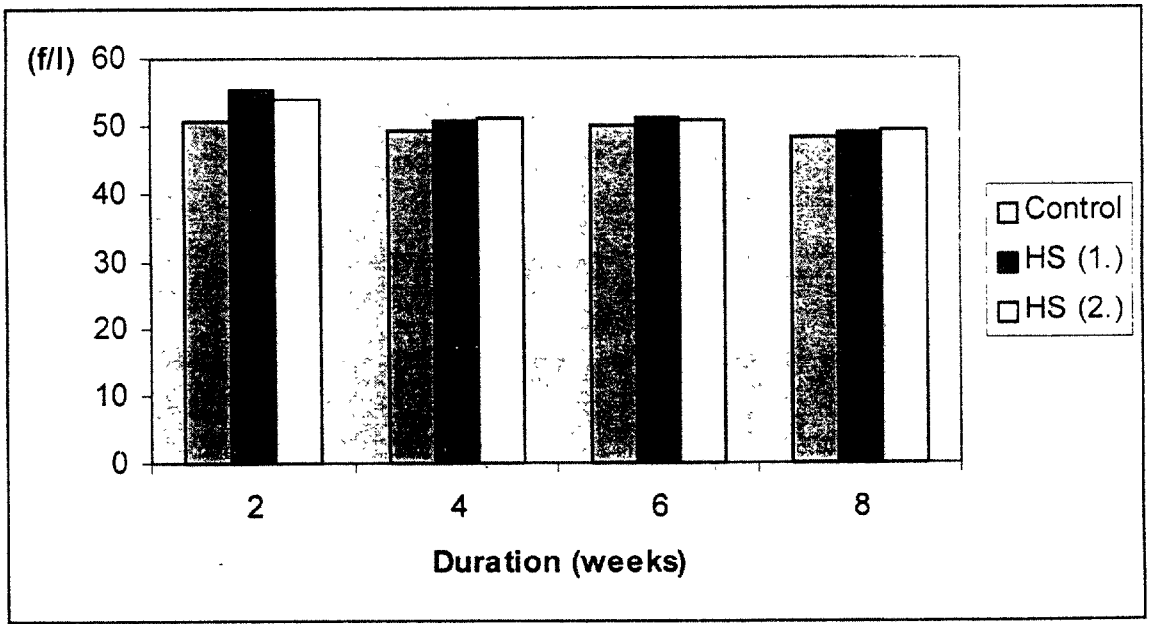


**Figure 13:** The effect of the prolonged feeding of different dosed humic acid food (HS 1. and HS 2.) on the change of the MCV values in rats

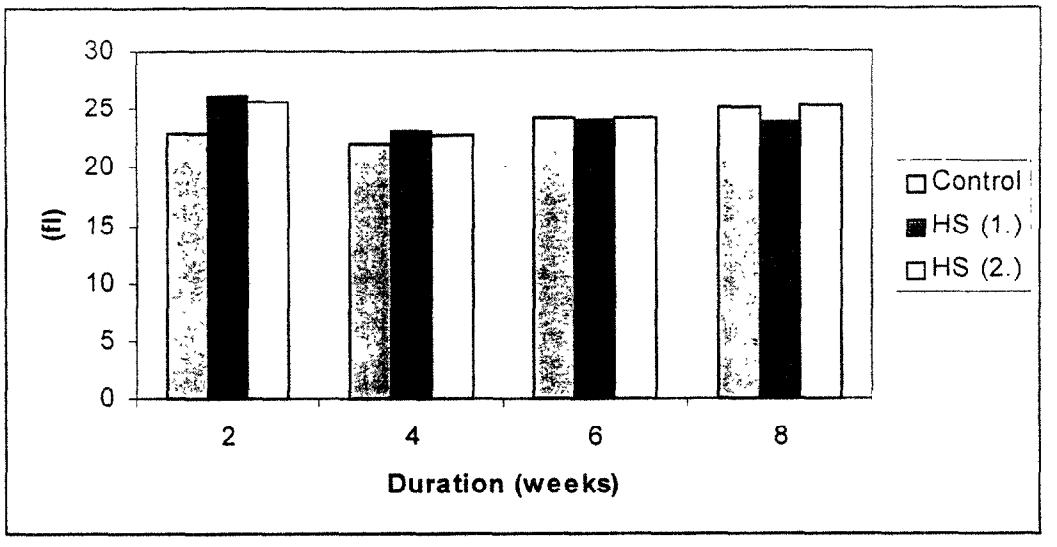


**Figure 14:** The effect of the prolonged feeding of different dosed humic acid food (HS 1. and HS 2.) on the change of the RDW values in rats

For the rats fed continuously by different dosed (HS 1. and HS 2.) humic acid active ingredient containing food, the change in spleen weight, and the time development of the spleen and bone marrow cellularity are shown in Table 5, and Figures 15-17.

The average and relative spleen weights of the control rats in the examined 8-week period were practically unchanged. In the humic acid treated animals in tendency, both the absolute and the relative spleen weights, especially on the 2$^{nd}$ week after the treatment, were higher than in the control group. During the treatment in both groups the spleen weight relative to the body weight decreased gradually, and at the end of the experiment, it was practically identical to the control values.

Two weeks after starting the treatment, in the humic acid treated group the average value of the bone marrow cellularity increased as well. This phenomenon proves obviously, that even in healthy animals a temporary increase in bone marrow cell formation occurs.

**Table 5:** The effect of the prolonged feeding of different dosed humic acid food on the change of the spleen weight, and spleen and bone marrow cellularity in rats (average ± SD)

| | | **Spleen** | | | | | | | | **Bone-marrow** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Experimental groups | Duration (weeks) | Spleen weight (mg) | | Relative spleen weight | | Mononuclear cell-count ($10^6$/lien) | | Mononuclear cell-count ($10^6$/mg lien) | | Cell-count ($10^6$/ femur) | |
| | | **average** | SD | **average** | SD | **average** | SD | **average** | SD | **average** | SD |
| Control | 2 | **453** | 54 | **2,18** | 0,15 | **473** | 59 | **1,05** | 0,13 | **111** | 5,7 |
| | 4 | **476** | 66 | **2,09** | 0,16 | **371** | 41 | **0,78** | 0,05 | **119** | 7,04 |
| | 6 | **534** | 70 | **2,17** | 0,23 | **413** | 47 | **0,78** | 0,03 | **112** | 25 |
| | 8 | **528** | 48 | **1,94** | 0,14 | **353** | 41 | **0,67** | 0,03 | **126** | 14 |
| HS (1.) | 2 | **566** | 27 | **2,68** | 0,08 | **616** | 69 | **1,09** | 0,09 | **142** | 15 |
| | 4 | **492** | 53 | **2,31** | 0,10 | **404** | 68 | **0,82** | 0,12 | **122** | 21 |
| | 6 | **533** | 79 | **2,33** | 0,29 | **444** | 75 | **0,84** | 0,13 | **107** | 22 |
| | 8 | **508** | 113 | **2,08** | 0,39 | **416** | 155 | **0,82** | 0,24 | **110** | 14 |
| HS (2.) | 2 | **568** | 49 | **2,50** | 0,13 | **592** | 84 | **1,05** | 0,15 | **137** | 34 |
| | 4 | **563** | 92 | **2,44** | 0,29 | **497** | 115 | **0,88** | 0,9 | **135** | 17 |
| | 6 | **564** | 190 | **2,21** | 0,46 | **465** | 240 | **0,8** | 0,15 | **115** | 9,3 |
| | 8 | **541** | 61 | **2,00** | 0,24 | **396** | 55 | **0,73** | 0,02 | **111** | 16 |

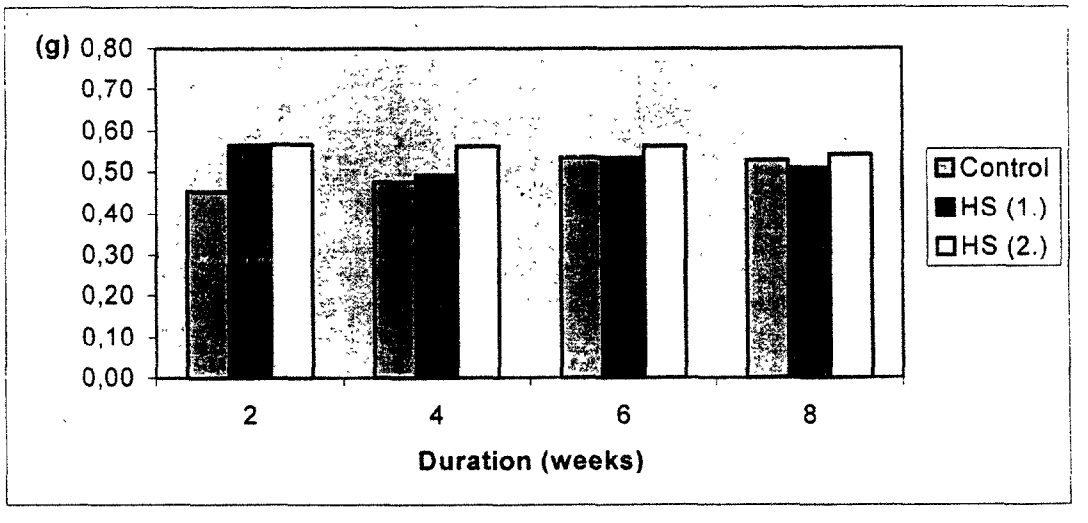


**Figure 15:** The effect of the prolonged feeding of different dosed humic acid food (HS 1. and HS 2.) on the change of the absolute spleen weight in rats

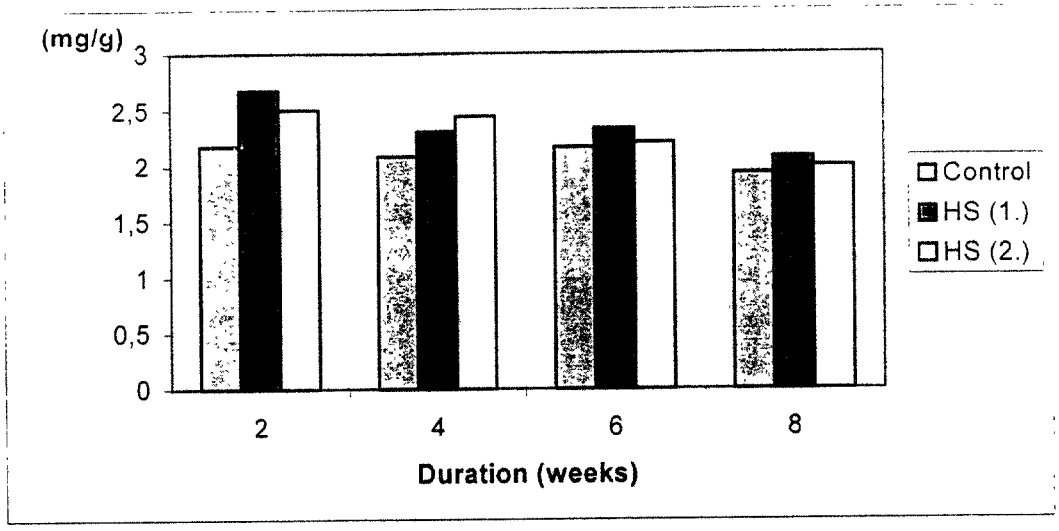


**Figure 16:** The effect of the prolonged feeding of different dosed humic acid food (HS 1. and HS 2.) on the change of the relative spleen weight in rats

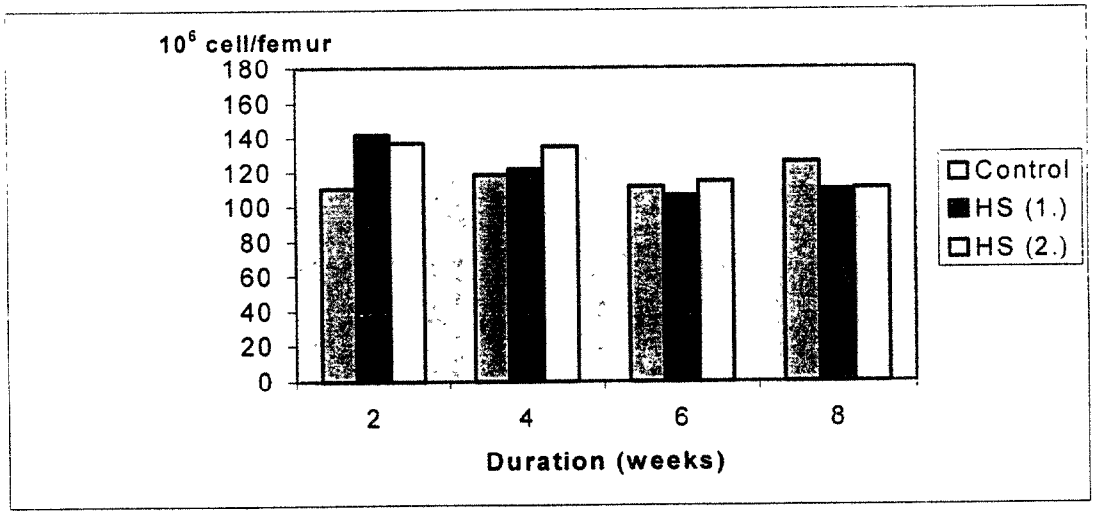


**Figure 17**: The effect of the prolonged feeding of different dosed humic acid food (HS 1. and HS 2.) on the change of the bone marrow cellularity in rats

## Summary

In our experiments we have made a comparative study in order to decide, whether potassium humate in **powder** form as **active ingredient** differs in toxicity from the **liquid** form, which has been used in the manufacturing process until now. The results of previous experiments, in which we have used humic acid products with different concentrations, have served as control at the same time.

The experimental animals had been fed with humic acid enriched food for eight weeks continuously, the results are summarized below:

**1.) We have established**, that humic acid in powder form fed for a **prolonged period** (8 weeks) and in **large doses** (240 mg/day) did not change the animals' **general physical condition** (e.g.: agility, food consumption), and some **other biological parameters** (weight of the whole body and of different organs, values of hematological parameters).

The animals fed with 60mg daily dose represent an exception concerning whole body weight, because 4 weeks after starting feeding animals with active ingredient containing food, the appetite of the animals has decreased, and the increase in weight has slowed down, however, the difference is not significant.

2. ) The **survival data** of the experimental animals, proves obviously, that continuous and prolonged feeding with large doses of humic acid containing food is **non-toxic,** as there were no mortalities.

3.) The comparative study of the **hematological parameters** showed, that there is **no significant difference** between the control group and the other groups fed with different dosed **powder** humic acid containing food. We would like to draw the attention to the fact, that on the second week of the humic acid treatment the measured parameters exceeded in average the similar values of the control group.

4.) From our results it can be concluded that humic acid in **powder form** is **non-toxic**, and that it is identical in **every measured parameter** with the **toxicity of the liquid form**.

Budapest, 1998. december 14.

**Attachment 1**: The effect of the prolonged feeding with different dosed humic acid food on rats' individual hematological parameters **on the 2nd week of the treatment.**

| Experimental group | Number of animal | FVS $10^9$/l | VVT $10^{12}$/l | HGB g/dl | HCT % | MCV fl | MCH pg | MCHC g/dl | PLT $10^9$/l | MPV fl | PDW fl | RDW fl |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Control** | 11 | 7,2 | 6,7 | 14,6 | 34,2 | 50,5 | 21,7 | 44,5 | 1550 | 8,1 | 13,5 | 22,9 |
| | 12 | 6,9 | 6,3 | 13,9 | 31,8 | 50,4 | 22,1 | 43,7 | 1424 | 8,2 | 13,7 | 22,8 |
| | 13 | 6,9 | 6,8 | 15,8 | 37,0 | 53,9 | 22,5 | 48,1 | 1366 | 7,8 | 12,2 | 24,1 |
| | 14 | 5,8 | 6,9 | 14,2 | 33,9 | 48,7 | 20,4 | 41,9 | 1454 | 8,2 | 13,7 | 22,1 |
| | **Average** | **6,70** | **6,71** | **14,63** | **34,23** | **50,88** | **21,68** | **44,55** | **1449** | **8,08** | **13,28** | **22,98** |
| | SD | 0,62 | 0,29 | 0,83 | 2,14 | 2,18 | 0,91 | 2,60 | 77 | 0,19 | 0,72 | 0,83 |
| **HS (1.)** | 1 | 10,0 | 6,6 | 13,8 | 35,4 | 53,3 | 20,8 | 39,0 | 1622 | 8,0 | 12,9 | 25,4 |
| | 2 | 8,7 | 7,0 | 14,3 | 38,5 | 54,4 | 20,2 | 37,1 | 1620 | 8,0 | 12,9 | 25,4 |
| | 3 | 8,1 | 6,6 | 13,5 | 37,1 | 56,2 | 20,4 | 36,4 | 1624 | 7,5 | 11,4 | 27,3 |
| | 4 | 7,6 | 6,1 | 13,4 | 36,2 | 59,3 | 21,9 | 37,0 | 1556 | 7,7 | 12,2 | 26,7 |
| | 5 | 6,1 | 6,9 | 14,9 | 37,4 | 54,0 | 21,5 | 39,8 | 1928 | 7,5 | 11,4 | 26,0 |
| | **Average** | **8,10** | **6,67** | **13,98** | **36,92** | **55,44** | **20,96** | **37,86** | **1670** | **7,74** | **12,16** | **26,16** |
| | SD | 1,43 | 0,37 | 0,62 | 1,18 | 2,41 | 0,72 | 1,46 | 147 | 0,25 | 0,75 | 0,83 |
| **HS (2.)** | 6 | 9,7 | 6,6 | 14,4 | 37,2 | 56,3 | 21,8 | 38,7 | 1848 | 7,8 | 12,9 | 26,0 |
| | 7 | 5,4 | 6,4 | 14,5 | 34,5 | 53,6 | 22,6 | 42,0 | 1818 | 8,0 | 13,7 | 26,0 |
| | 8 | 6,8 | 6,4 | 14,4 | 33,9 | 53,0 | 22,5 | 42,5 | 1388 | 8,2 | 13,7 | 24,7 |
| | 9 | 8,0 | 6,3 | 14,7 | 34,2 | 53,7 | 23,1 | 43,0 | 1656 | 7,8 | 12,2 | 25,4 |
| | 10 | 8,5 | 6,0 | 14,3 | 31,8 | 52,6 | 23,6 | 45,0 | 1486 | 7,8 | 12,9 | 25,4 |
| | **Average** | **7,68** | **6,37** | **14,46** | **34,32** | **53,84** | **22,72** | **42,24** | **1639** | **7,92** | **13,08** | **25,5** |
| | SD | 1,65 | 0,20 | 0,15 | 1,93 | 1,45 | 0,68 | 2,28 | 202 | 0,18 | 0,63 | 0,54 |

**Attachment 2:** The effect of the prolonged feeding with different dosed humic acid food on rats' individual hematological parameters **on the 4th week of the treatment.**

| Experimental group | Number of animal | FVS $10^9$/l | VVT $10^{12}$/l | HGB g/dl | HCT % | MCV fl | MCH pg | MCHC g/dl | PLT $10^9$/l | MPV fl | PDW fl | RDW fl |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Control** | 26 | 8,9 | 5,9 | 13,7 | 29,6 | 50,5 | 23,4 | 46,3 | 1500 | 7,2 | 12,9 | 22,1 |
| | 27 | 9,1 | 6,0 | 12,9 | 30,5 | 50,7 | 21,4 | 42,3 | 1314 | 7,3 | 13,7 | 21,5 |
| | 28 | 5,3 | 6,4 | 13,1 | 30,9 | 48,6 | 20,6 | 42,4 | 1416 | 7,4 | 13,7 | 21,5 |
| | 29 | 6,0 | 6,5 | 13,1 | 31,2 | 47,9 | 20,1 | 42,0 | 1338 | 7,4 | 13,7 | 22,8 |
| | **Average** | **7,33** | **6,19** | **13,20** | **30,55** | **49,43** | **21,38** | **43,25** | **1392** | **7,33** | **13,50** | **21,98** |
| | SD | 1,96 | 0,30 | 0,35 | 0,70 | 1,39 | 1,45 | 2,04 | 84 | 0,10 | 0,40 | 0,62 |
| **HS (1.)** | 16 | 6,9 | 7,0 | 16,0 | 34,7 | 49,3 | 22,7 | 41,6 | 1480 | 8,4 | 13,7 | 22,1 |
| | 17 | 6,6 | 7,5 | 15,7 | 39,9 | 53,5 | 21,0 | 39,3 | 1786 | 7,5 | 13,7 | 25,4 |
| | 18 | 6,9 | 7,0 | 14,2 | 33,7 | 48,4 | 20,4 | 42,1 | 1722 | 7,4 | 13,7 | 22,1 |
| | 19 | 6,1 | 7,0 | 13,8 | 36,0 | 51,7 | 19,8 | 38,3 | 1428 | 7,9 | 15,2 | 23,4 |
| | 20 | 5,8 | 7,0 | 15,1 | 35,0 | 50,3 | 21,7 | 43,1 | 1550 | 7,3 | 12,2 | 22,1 |
| | **Average** | **6,46** | **7,07** | **14,96** | **35,86** | **50,64** | **21,12** | **40,88** | **1593** | **7,7** | **13,7** | **23,02** |
| | SD | 0,49 | 0,22 | 0,94 | 2,40 | 2,01 | 1,13 | 2,01 | 155 | 0,45 | 1,06 | 1,44 |
| **HS (2.)** | 21 | 7,8 | 6,8 | 13,7 | 34,7 | 51,2 | 20,2 | 39,5 | 1250 | 7,2 | 12,9 | 22,8 |
| | 22 | 9,3 | 6,3 | 13,1 | 32,4 | 51,3 | 20,7 | 40,4 | 1286 | 7,2 | 12,9 | 23,4 |
| | 23 | 7,2 | 6,2 | 13,8 | 32,1 | 51,8 | 22,3 | 43,0 | 1296 | 7,1 | 12,9 | 22,8 |
| | 24 | 7,7 | 6,8 | 14,3 | 34,4 | 51,0 | 21,2 | 41,6 | 1390 | 7,3 | 13,7 | 23,4 |
| | 25 | 9,3 | 6,4 | 13,3 | 31,5 | 49,5 | 20,9 | 42,2 | 1214 | 7,5 | 13,7 | 21,5 |
| | **Average** | **8,26** | **6,48** | **13,64** | **33,02** | **50,96** | **21,06** | **41,34** | **1287** | **7,26** | **13,2** | **22,78** |
| | SD | 0,98 | 0,27 | 0,47 | 1,44 | 0,87 | 0,78 | 1,40 | 66 | 0,15 | 0,44 | 0,78 |

**Attachment 3:** The effect of the prolonged feeding with different dosed humic acid food on rats' individual hematological parameters **on the 6th week of the treatment.**

| Experimental group | Number of animal | FVS $10^9$/l | VVT $10^{12}$/l | HGB g/dl | HCT % | MCV fl | MCH pg | MCHC g/dl | PLT $10^9$/l | MPV fl | PDW fl | RDW fl |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Control** | 41 | 6,8 | 7,2 | 13,5 | 37,4 | 51,9 | 18,8 | 36,1 | 1300 | 7,7 | 12,2 | 24,7 |
| | 42 | 8,2 | 6,6 | 14,8 | 37,0 | 48,7 | 19,5 | 40,0 | 1716 | 8,1 | 13,7 | 23,4 |
| | 43 | 7,1 | 6,8 | 15,3 | 38,3 | 49,3 | 19,7 | 39,9 | 1372 | 7,8 | 12,2 | 24,7 |
| | 44 | 8,8 | 6,6 | 14,5 | 33,1 | 50,0 | 21,9 | 43,8 | 1326 | 8,0 | 12,9 | 24,1 |
| | **Average** | **7,73** | **6,79** | **14,53** | **36,45** | **49,98** | **19,98** | **39,95** | **1429** | **7,90** | **12,75** | **24,23** |
| | SD | 0,94 | 0,28 | 0,76 | 2,30 | 1,39 | 1,34 | 3,14 | 194 | 0,18 | 0,71 | 0,62 |
| **HS (1.)** | 31 | 7,4 | 6,8 | 13,5 | 36,0 | 53,0 | 19,9 | 37,5 | 1710 | 7,8 | 12,2 | 24,1 |
| | 32 | 7,2 | 7,1 | 13,7 | 36,8 | 51,2 | 19,7 | 38,4 | 1615 | 8,1 | 13,2 | 23,5 |
| | 33 | 5,8 | 8,1 | 14,0 | 39,5 | 48,5 | 17,2 | 35,4 | 1848 | 8,3 | 13,7 | 22,8 |
| | 34 | 9,2 | 6,9 | 13,9 | 35,0 | 51,1 | 20,3 | 39,7 | 1424 | 8,3 | 12,9 | 24,1 |
| | 35 | 5,3 | 6,6 | 14,1 | 34,3 | 52,3 | 21,5 | 41,1 | 1436 | 8,1 | 13,7 | 25,4 |
| | **Average** | **6,98** | **7,09** | **13,84** | **36,3** | **51,22** | **19,72** | **38,42** | **1607** | **8,12** | **13,14** | **23,98** |
| | SD | 1,53 | 0,62 | 0,24 | 2,02 | 1,71 | 1,57 | 2,17 | 181 | 0,20 | 0,63 | 0,96 |
| **HS (2.)** | 36 | 5,2 | 7,1 | 13,4 | 34,2 | 48,4 | 19,0 | 39,2 | 1316 | 8,1 | 12,9 | 23,4 |
| | 37 | 6,3 | 6,3 | 14,1 | 32,2 | 50,9 | 22,3 | 43,8 | 1383 | 8,3 | 13,7 | 23,4 |
| | 38 | 5,2 | 7,7 | 15,3 | 39,8 | 51,6 | 19,8 | 38,4 | 1350 | 8,2 | 13,7 | 25,4 |
| | 39 | 8,6 | 7,4 | 15,1 | 37,1 | 50,3 | 20,5 | 40,7 | 1438 | 8,1 | 12,9 | 23,4 |
| | 40 | 7,8 | 7,7 | 15,9 | 39,1 | 50,8 | 20,7 | 40,7 | 1312 | 8,0 | 12,9 | 24,1 |
| | **Average** | **6,98** | **7,28** | **15,1** | **37,1** | **50,9** | **20,83** | **40,9** | **1371** | **8,15** | **13,3** | **24,08** |
| | SD | 1,52 | 0,65 | 0,75 | 3,43 | 0,54 | 1,06 | 2,22 | 53 | 0,13 | 0,46 | 0,94 |

**Attachment 4:** The effect of the prolonged feeding with different dosed humic acid food on rats' individual hematological parameters **on the 8th week of the treatment.**

| Experimental group | Number of animal | FVS $10^9$/l | VVT $10^{12}$/l | HGB g/dl | HCT % | MCV fl | MCH pg | MCHC g/dl | PLT $10^9$/l | MPV fl | PDW fl | RDW fl |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Control** | 56 | 8,9 | 7,6 | 16,6 | 36,9 | 48,4 | 21,8 | 45,0 | 1278 | 7,8 | 12,98 | 24,7 |
| | 57 | 6,6 | 5,7 | 14,3 | 27,8 | 48,7 | 25,0 | 51,4 | 1338 | 7,4 | 11,4 | 26,0 |
| | 58 | 7,7 | 6,9 | 16,5 | 32,9 | 47,8 | 24,0 | 50,2 | 1062 | 7,9 | 12,2 | 24,1 |
| | 59 | 8,6 | 6,9 | 16,0 | 33,2 | 48,4 | 23,4 | 48,2 | 1446 | 8,0 | 12,9 | 25,4 |
| | **Average** | **7,95** | **6,77** | **15,85** | **32,70** | **48,33** | **23,55** | **48,70** | **1281** | **7,78** | **12,35** | **25,05** |
| | SD | 1,03 | 0,79 | 1,07 | 3,74 | 0,38 | 1,34 | 2,80 | 162 | 0,26 | 0,71 | 0,83 |
| **HS (1.)** | 46 | 8,3 | 5,9 | 15,4 | 29,4 | 50,4 | 26,4 | 52,4 | 1450 | 7,6 | 11,4 | 23,4 |
| | 47 | 7,1 | 6,2 | 14,7 | 29,9 | 48,3 | 23,7 | 49,2 | 1334 | 7,7 | 12,2 | 24,1 |
| | 48 | 6,0 | 6,4 | 12,4 | 26,0 | 48,4 | 23,1 | 47,7 | 1155 | 7,5 | 11,4 | 24,1 |
| | 49 | 8,8 | 7,1 | 16,9 | 34,1 | 48,4 | 24,0 | 79,6 | 1222 | 7,4 | 11,4 | 23,4 |
| | 50 | 6,1 | 6,0 | 13,5 | 29,1 | 48,9 | 22,7 | 46,4 | 1358 | 7,4 | 11,4 | 24,1 |
| | **Average** | **7,26** | **6,28** | **14,58** | **29,7** | **48,88** | **23,98** | **49,06** | **1304** | **7,52** | **11,56** | **23,82** |
| | SD | 1,27 | 0,47 | 1,73 | 2,90 | 0,88 | 1,44 | 2,26 | 116 | 0,13 | 0,36 | 0,38 |
| **HS (2.)** | 51 | 6,8 | 6,2 | 14,5 | 30,7 | 49,4 | 23,3 | 47,2 | 1260 | 7,9 | 12,9 | 24,7 |
| | 52 | 6,9 | 6,4 | 15,3 | 26,8 | 50,1 | 28,6 | 57,1 | 1240 | 7,8 | 12,2 | 25,4 |
| | 53 | 5,7 | 6,1 | 14,5 | 29,3 | 47,9 | 23,7 | 49,5 | 1160 | 7,4 | 11,4 | 24,1 |
| | 54 | 6,9 | 5,9 | 15,3 | 29,2 | 49,9 | 26,1 | 52,4 | 1256 | 7,8 | 12,2 | 26,7 |
| | 55 | 7,7 | 5,9 | 15,2 | 28,7 | 49,1 | 26,0 | 53,0 | 1220 | 7,6 | 12,2 | 25,4 |
| | **Average** | **6,80** | **6,10** | **15** | **28,9** | **49,3** | **25,54** | **51,84** | **1227** | **7,7** | **12,18** | **25,26** |
| | SD | 0,71 | 0,22 | 0,42 | 1,41 | 0,87 | 2,14 | 3,75 | 41 | 0,20 | 0,53 | 0,97 |