20 November 2008

Jacques Rossouw
MNI
82 Edelvark Street
Monument Park Extention
Pretoria, 0181

Dear Jacques

Re: An in vivo investigation of the toxicity profiles of Cellfood Sport, Cellfood and Cellfood Longevity in Sprague Dawley Rats

ACUTE TOXICITY STUDY

An acute toxicity study was carried out at the University of Pretoria Biomedical Research Centre (UPBRC) in batches of 20 Sprague Dawley rats divided into four groups one control group plus one of Cellfood Sport, Cellfood, and Cellfood Longevity respectively of five rats each. A total of twenty animals were treated with each experimental product dissolved in distilled water to test the acute toxicity profile and safety by oral gavage at 225 mg/kg body weight.

On day one, rats were gavaged with approximately 400μl of either distilled water alone or with the volume equivalent to 225 mg/kg body weight (of the three experimental products) dissolved in distilled water. The animals were monitored for any signs of adverse effects or behavioural changes for seven days. The weight of the rats were recorded every second day after dosing. At the end of day 7, heparinised blood samples (1000μl/rat) were drawn by trained personnel from the UPBRC via cardiac puncture while the animals were under isoflurane anaesthesia for haematology analysis (haematocrit, red blood cell, white blood cell and haemoglobin concentration), kidney function markers (urea, creatinine) and liver marker
enzyme levels (ALT, AST, and GGT). The animals were then terminated via anaesthetic overdose.

The blood analyses were carried out immediately after collection at the Clinical Pathology Laboratories, Faculty of Veterinary Sciences, University of Pretoria.

The rat cadavers were sent for macroscopic and histopathology analysis to Golden Vetpath, a private pathology company that does our histopathology, where the relative organ weights of the heart, kidneys and livers were also determined.

Results:

All the animals in the control and the three experimental groups survived the duration of the study without any observed unusual or adverse effects. None of the animals showed any signs of stress or abnormal behaviour during this time.

The body mass of all the experimental groups showed a normal increase with no significant difference (Student t test – GraphPad Prism 4 statistical software program) between the control group (distilled water) and the three experimental products Cellfood Sport, Cellfood and Cellfood Longevity given at an orally administered dose of 225 mg/kg. There was a small but insignificantly greater increase in the mass of the group that was treated with Cellfood.

The haematology, organ mass, kidney function markers (creatinine, urea) and liver toxicity marker enzymes (ALT, AST, GGT) showed no signs of toxicity with no significantly difference, whereas within the tested electrolytes (Na, K and Ca$^{2+}$), a significant increased (p<0.05) in Ca$^{2+}$ levels was observed in the Cellfood treated group only (One Way ANOVA: Dunett’s multiple comparison test – GraphPad Prism 4 statistical software program) between the control group (distilled water) and the three experimental products Cellfood Sport, Cellfood and Cellfood Longevity given at an orally administered dose of 225 mg/kg.

Macroscopic and histopathology analysis concluded that there were no significant pathological lesions compatible with organ toxicity that could be associated with the administration of any of the three experimental products Cellfood Sport, Cellfood and Cellfood Longevity given at an orally administered dose of 225 mg/kg.
Below is a summary of the results shown graphically (Figures 1 – 9) in related groups of information obtained from the three experimental groups and compared to the control rats that were sham dosed with distilled water.

Figure 1: Changes in body mass of eighty rats undergoing a once off oral gavage dose with distilled water as a control and Cellfood Sport, Cellfood and Cellfood Longevity at 225mg/kg respectively. A slight but insignificant increase in body mass was evident in all groups for the seven day follow up period, with the Cellfood group showing the greatest increase. n = 20 rats per group

Figure 2: Comparison of the mass of the spleen as a percentage of the total body mass of eighty rats undergoing a once off oral gavage dose with distilled water as a control and Cellfood Sport, Cellfood and Cellfood Longevity at 225mg/kg respectively. No significant difference was evident in all groups for the seven day follow up period. n = 20 rats per group
**Figure 3:** Comparison of the mass of the heart as a percentage of the total body mass of eighty rats undergoing a once off oral gavage dose with distilled water as a control and Cellfood Sport, Cellfood and Cellfood Longevity at 225mg/kg respectively. No significant difference was evident in all groups for the seven day follow up period. n= 20 rats per group

**Figure 4:** Comparison of the mass of the kidneys as a percentage of the total body mass of eighty rats undergoing a once off oral gavage dose with distilled water as a control and Cellfood Sport, Cellfood and Cellfood Longevity at 225mg/kg respectively. No significant difference was evident in all groups for the seven day follow up period. n= 20 rats per group
Figure 5: Comparison of the mass of the liver as a percentage of the total body mass of eighty rats undergoing a once off oral gavage dose with distilled water as a control and Cellfood Sport, Cellfood and Cellfood Longevity at 225mg/kg respectively. No significant difference was evident in all groups for the seven day follow up period. n= 20 rats per group.
Figure 6: Comparison of liver toxicity marker (A) ALT, (B) AST and (C) GGT concentrations in the plasma as markers of liver damage for eighty rats undergoing a once off oral gavage dose with distilled water as a control and Cellfood Sport, Cellfood and Cellfood Longevity at 220mg/kg respectively. No significant difference was evident in all groups for the seven day follow up period. n= 20 rats per group.
Figure 7: Comparison of (A) Creatinine and (B) Urea concentrations in the plasma as markers of kidney function of eighty rats undergoing a once off oral gavage dose with distilled water as a control and Cellfood Sport, Cellfood and Cellfood Longevity at 225mg/kg respectively. No significant difference was evident in all groups for the seven day follow up period. n= 20 rats per group.
Figure 8: Comparison of (A) sodium, (B) potassium and (C) calcium electrolytes in the plasma as markers of damage of eighty rats undergoing a once off oral gavage dose with distilled water as a control and Cellfood Sport, Cellfood and Cellfood Longevity at 225mg/kg respectively. No significant difference was evident in any of the groups, for the seven day follow up period. n= 20 rats per group
A

Comparison of blood haemoglobin at end of the study

B

Comparison of red blood cell counts at the end of the study

C

Comparison of white blood cell counts at the end of the study
It can be concluded, when all the data collected during the one week acute toxicity study (behavioural observations, body mass, haematology, organ mass, kidney function markers (creatinine, urea), electrolytes (Na, K and Ca$^{2+}$), liver toxic marker enzymes (ALT, AST, GGT), histopathology and macroscopic pathology) is considered, that all three test compounds, namely, Cellfood Sport, Cellfood, and Cellfood Longevity administered by oral gavage at 225 mg/kg demonstrated no toxic effects in the Sprague Dawley rat model after an acute dose.

**CHRONIC TOXICITY STUDY**

A chronic toxicity study was carried out at the University of Pretoria Biomedical Research Centre (UPBRC) in two batches of 40 Sprague Dawley rats divided into four groups (one control group plus one of Cellfood Sport, Cellfood, and Cellfood Longevity respectively) of ten rats each. A total of twenty animals were treated with each experimental product dissolved in distilled water, to test the chronic toxicity profile and safety by oral gavage at 125 mg/kg body weight/day.

Rats were gavaged with approximately 400 µl per day of either distilled water alone or with the volume equivalent to 125 mg/kg body weight (of the three experimental products).
dissolved in distilled water. The animals were monitored for any signs of adverse effects or behavioural changes for forty five days. The weight of the rats were recorded every fifth day after dosing. At the end of day 45, heparinised blood samples (1000 µl/rat) were drawn by trained personnel from the UPBRC via cardiac puncture while the animals were under Isofluorane anaesthesia for haematological analysis (haematocrit, red blood cell count, white blood cell count and haemoglobin concentration), kidney function markers (urea, creatinine), electrolytes (Na⁺, Ca²⁺, K⁺) and liver marker enzyme levels (ALT, AST, and GGT). The animals were then terminated via anaesthetic overdose.

The blood analyses were carried out immediately after collection at the Clinical Pathology Laboratories, Faculty of Veterinary Sciences, University of Pretoria.

The rat cadavers were sent for macroscopic and histopathology analysis to Golden Vetpath, a private pathology company that performs animal autopsies and histopathology, where the macro-anatomical analysis and relative organ weights of the heart, kidneys and livers were determined.

Results:

All the animals in the control and the three experimental groups survived the duration of the study without any observed unusual behaviour or adverse effects. None of the animals showed any signs of stress or abnormalities during this time.

The body mass of all the experimental groups showed a normal increase with no significant difference (Student t test – GraphPad Prism 4 statistical software program) between the control group (distilled water) and each of the three experimental products Cellfood Sport, Cellfood and given at an orally administered dose of 125 mg/kg/day. There was a small insignificant decrease in the mass of the group that was treated with Cellfood Longevity. There was a small but insignificant greater increase in the mass of the group that was treated with Cellfood Sport and Cellfood.

The haematological analysis, kidney function markers (creatinine, urea), electrolytes (Na⁺, K⁺ and Ca²⁺), and liver toxicity marker enzymes (ALT, AST, GGT) showed no signs of toxicity with no significantly differences.
The organ mass data indicated:

- a small but significant (p<0.001) increase in spleen mass in the Cellfood and Cellfood Longevity treated groups
- a significant increase (p<0.05) in liver mass was observed in the Cellfood treated group only (One Way ANOVA: Dunett's multiple comparison test – GraphPad Prism 4 statistical software program), when compared to the control group (distilled water) after the three experimental products were orally administered a daily dose of 125 mg/kg/day.

Macroscopic and histopathology analysis concluded that there were no significant pathological lesions compatible with organ toxicity that could be associated with the administration of any of the three experimental products Cellfood Sport, Cellfood and Cellfood Longevity given at an orally administered dose of 125 mg/kg/day.

Below is a summary of the results shown graphically (Figures 10 – 18) in related groups of information obtained from the three experimental groups and compared to the control rats that were sham dosed with distilled water.

![% Body mass change during a chronic toxicity study in Sprague Dawley rats](image)

Figure 19: Changes in body mass of eighty rats undergoing a daily oral gavage dose with either distilled water as a control, Cellfood Sport, Cellfood and Cellfood Longevity at 125mg/kg respectively. A slight but insignificant increase in body mass was evident in Cellfood Sport and Cellfood groups for the six weeks follow up period. n= 20 rats per group.
Figure 11: Comparison of the mass of the spleen as a percentage of the total body mass of eighty rats undergoing a daily oral gavage dose with either distilled water as a control, Cellfood Sport, Cellfood and Cellfood Longevity at 125mg/kg/day respectively. A significant difference (p<0.001) was evident in the Cellfood and in the Cellfood Longevity treated group respectively, after the six week follow up period. n= 20 rats per group.

Figure 12: Comparison of the mass of the heart as a percentage of the total body mass of eighty rats undergoing a daily oral gavage dose with either distilled water as a control, Cellfood Sport, Cellfood and Cellfood Longevity at 125mg/kg/day respectively. No significant difference was evident in all groups for the six weeks follow up period. n= 20 rats per group.
Figure 13: Comparison of the mass of the kidneys as a percentage of the total body mass of eighty rats undergoing a daily oral gavage dose with either distilled water as a control, Cellfood Sport, Cellfood and Cellfood Longevity at 125mg/kg/day respectively. No significant difference was evident in all groups for the six weeks follow up period. n=20 rats per group.

Figure 14: Comparison of the mass of the liver as a percentage of the total body mass of eighty rats undergoing a daily oral gavage dose with either distilled water as a control, Cellfood Sport, Cellfood and Cellfood Longevity at 125mg/kg/day respectively. A significant difference (p<0.05) was evident in the Cellfood treated group only for the six weeks follow up period. n=20 rats per group.
Figure 18: Comparison of liver toxicity marker (A) ALT, (B) AST and (C) GGT concentrations in the plasma as markers of liver damage for eighty rats undergoing a daily oral gavage dose with either distilled water as a control, Cellfood Sport, Cellfood and Cellfood Longevity at 125mg/kg/day respectively. No significant difference was evident in all groups for the six weeks follow up period. n= 20 rats per group.
Figure 16: Comparison of (A) Creatinine and (B) Urea concentrations in the plasma as markers of kidney function of eighty rats undergoing a daily gavage dose with either distilled water as a control, Cellfood Sport, Cellfood and Cellfood Longevity at 125mg/kg/day respectively. No significant difference was evident in all groups for the six weeks follow up period. n= 20 rats per group.
Figure 17: Comparison of (A) sodium, (B) potassium and (C) calcium electrolytes in the plasma of eighty rats undergoing a daily oral gavage dose with either distilled water as a control, Cellfood Sport, Cellfood and Cellfood Longevity at 125mg/kg/day respectively. No significant difference was evident in all groups for the six weeks follow up period. n= 20 rats per group.
**Figure 18:** Comparison of (A) Hb, (B) RCC, (C) WCC and (D) HT blood parameters as markers of toxicity in eighty rats undergoing a daily oral gavage dose with either distilled water as a control, Cellfood Sport, Cellfood and Cellfood Longevity at 125mg/kg/day respectively. No significant difference was evident in all groups for the six weeks follow up period. n= 20 rats per group.
It can be concluded, when all the data collected for the six weeks chronic toxicity study (behavioural observations, body mass, haematology, organ mass, kidney function markers (creatinine, urea), electrolytes (Na, K and Ca$^{2+}$), liver toxic marker enzymes (ALT, AST, GGT), histopathology and macroscopic pathology) is considered, that the test compounds namely Cellfood, Cellfood Sport and Cellfood Longevity demonstrated no significant toxic effects. The origin of the slight spleen mass increase should be determined to rule out any subtle long term toxic effects.

Cellfood, Cellfood Sport and Cellfood Longevity administered by oral gavage at 125mg/kg/day for six weeks has not demonstrated any significant toxic effects in the Sprague Dawley rat model.

The combination of the acute toxicity assay that involved a single high dose (225mg/kg) and the six week chronic toxicity assay at a lower dose (125mg/kg/day) without any observed toxic effects would indicate that the three test compounds namely Cellfood, Cellfood Sport and Cellfood Longevity are non toxic in the rodent model commonly used for the initial in vivo testing of the safety of new products destined for use in humans.

Should you have any queries please do not hesitate to contact me either by e-mail duncan@med.up.ac.za or on 073 3064220.

Yours truly,

OD Cromarty

Dr AD Cromarty