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Final report on the *in vivo* investigation of the acute toxicity profiles of SWITCH (Cellfood Sport and Shape), Cellfood and NCODE (Cellfood Longevity) in Sprague Dawley Rats

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 SWITCH (Cellfood Sport and Shape), Cellfood, and NCODE (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 SWITCH (Cellfood Sport and Shape), Cellfood and NCODE (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: Dunnett's multiple comparison test – GraphPad Prism 4 statistical software program) between the control group (distilled water) and the three experimental products SWITCH (Cellfood Sport and Shape), Cellfood and NCODE (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 SWITCH (Cellfood Sport and

Shape), Cellfood and NCODE (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.

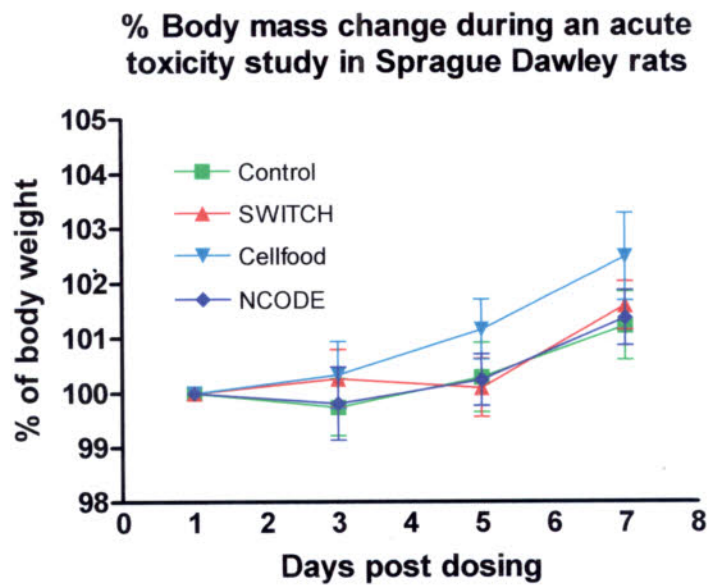


Figure 1: Changes in body mass of eighty rats undergoing a once off oral gavage dose with distilled water as a control and SWITCH, Cellfood and NCODE at 225mg/kg respectively. A slight but insignificant increase in body mass was evident in all groups for the seven day follow up period, with the Cellfood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

Comparison of Spleen mass as % of body mass

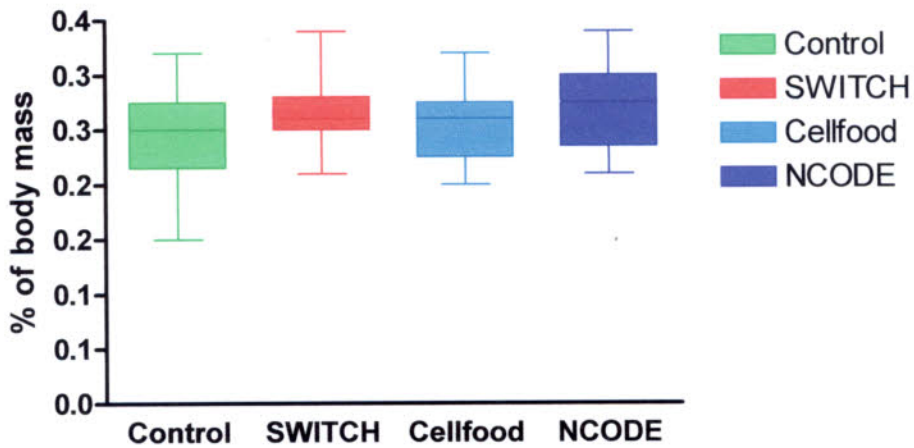


Figure 2: Comparison of the mass of the spleen as a percentage of the total body mass of eighty rats undergoing a once off oral gavage dose with distilled water as a control and SWITCH, Cellfood and NCODE at 225mg/kg respectively. No significant difference was evident in all groups for the seven day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

Comparison of Heart mass as % of body mass

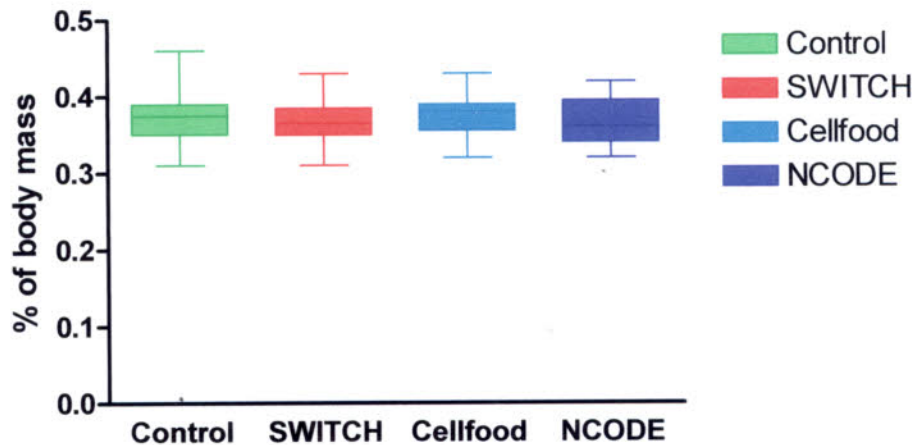


Figure 3: Comparison of the mass of the heart as a percentage of the total body mass of eighty rats undergoing a once off oral gavage dose with distilled water as a control and SWITCH, Cellfood and NCODE at 225mg/kg respectively. No significant difference was evident in all groups for the seven day follow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

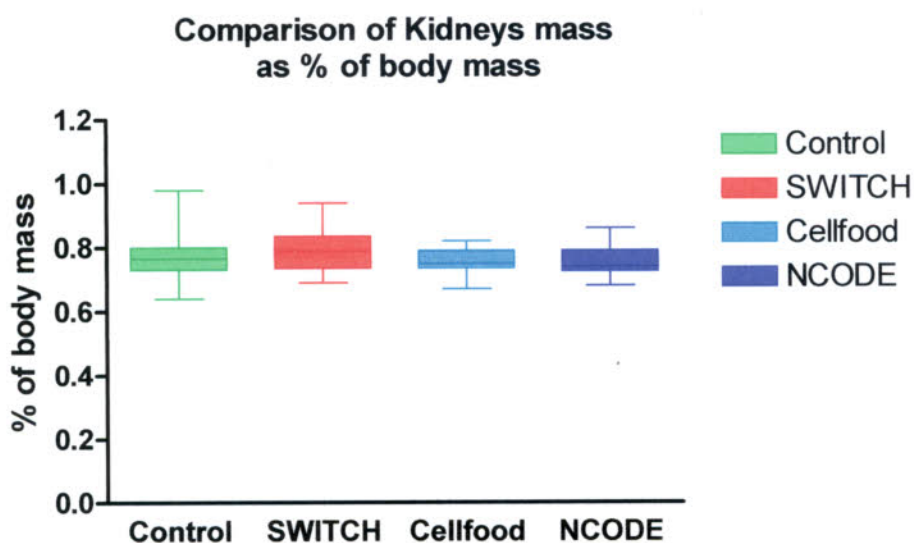


Figure 4: Comparison of the mass of the kidneys as a percentage of the total body mass of eighty rats undergoing a once off oral gavage dose with distilled water as a control and SWITCH, Cellfood and NCODE at 225mg/kg respectively. No significant difference was evident in all groups for the seven day follow up period. n= 20 rats per group

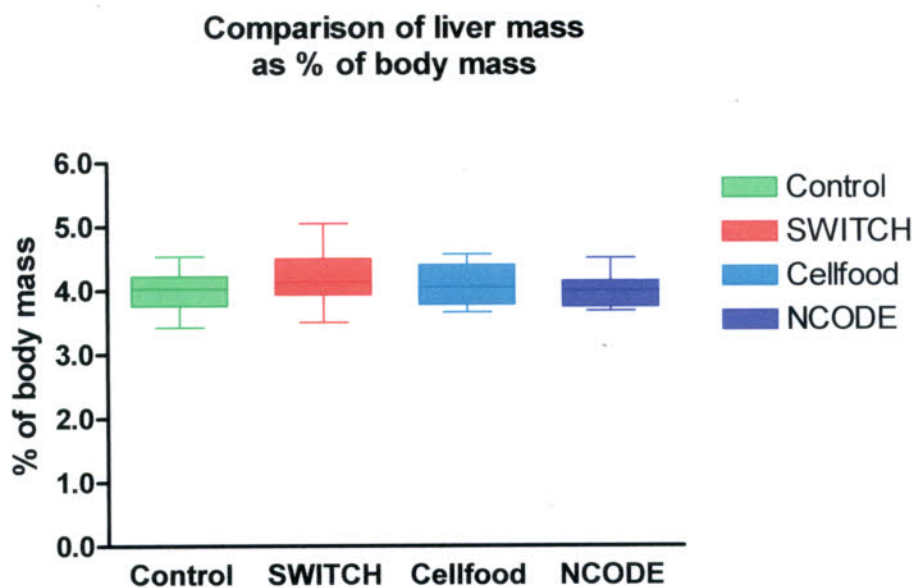


Figure 5: Comparison of the mass of the liver as a percentage of the total body mass of eighty rats undergoing a once off oral gavage dose with distilled water as a control and SWITCH, Cellfood and NCODE at 225mg/kg respectively. No significant difference was evident in all groups for the seven day follow up period. n= 20 rats per group

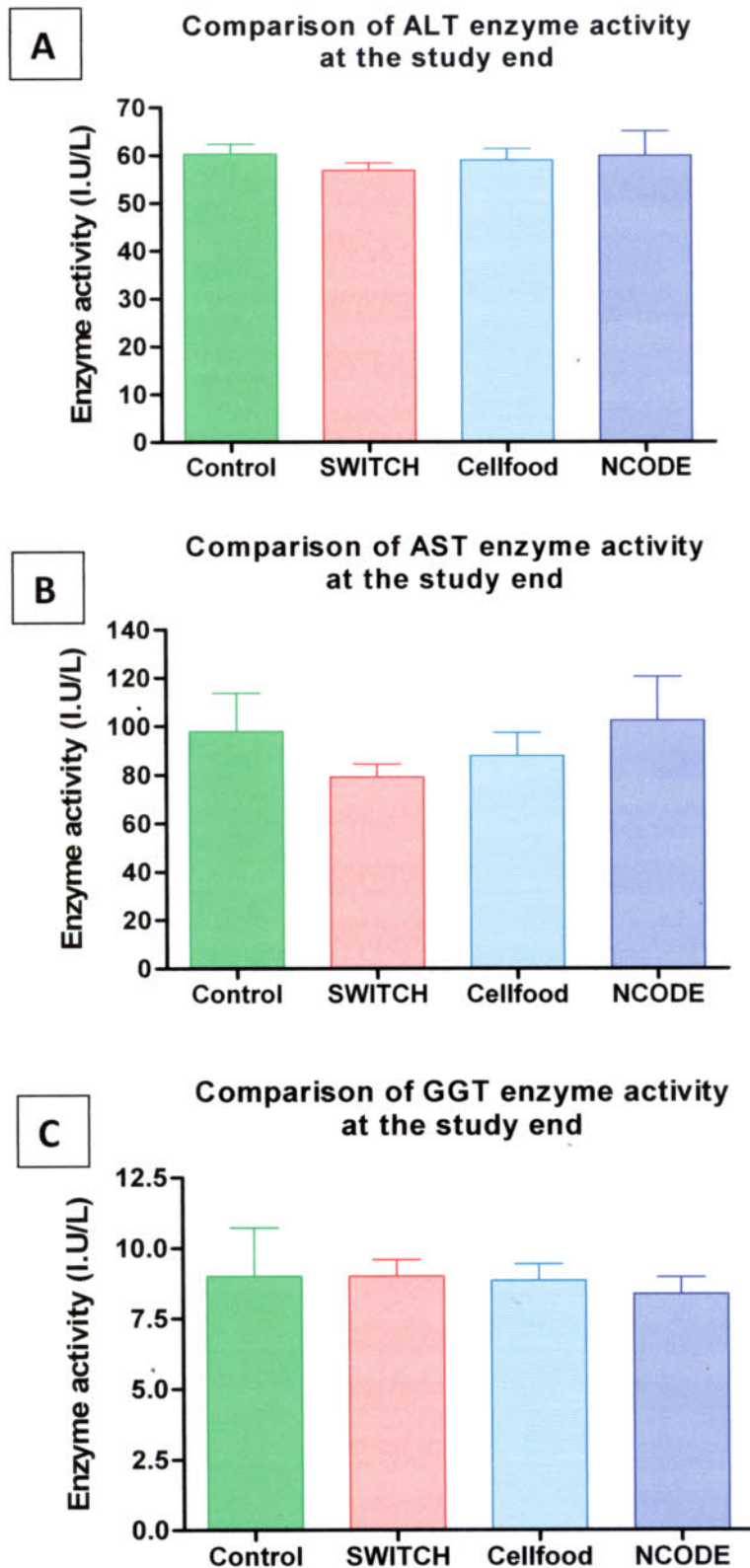
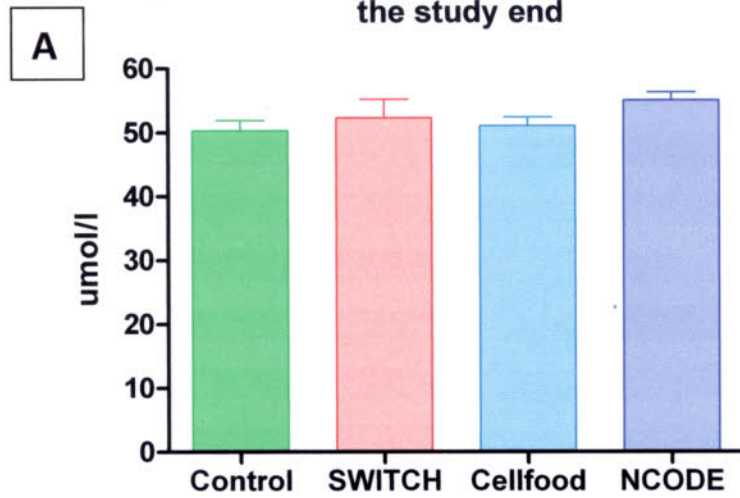


Figure 6: Comparison of liver toxicity marker (A) ALT, (B) AST and (C) GGT concentrations in the plasma as markers of liver damage for of eighty rats undergoing a once off oral gavage dose with distilled water as a control and SWITCH, Cellfood and NCODE at 225mg/kg respectively. No significant difference was evident in all groups for the seven day follow up period. n= 20 rats per group

Comparison of serum creatinine concentrations at the study end



Comparison of serum urea concentrations at the study end

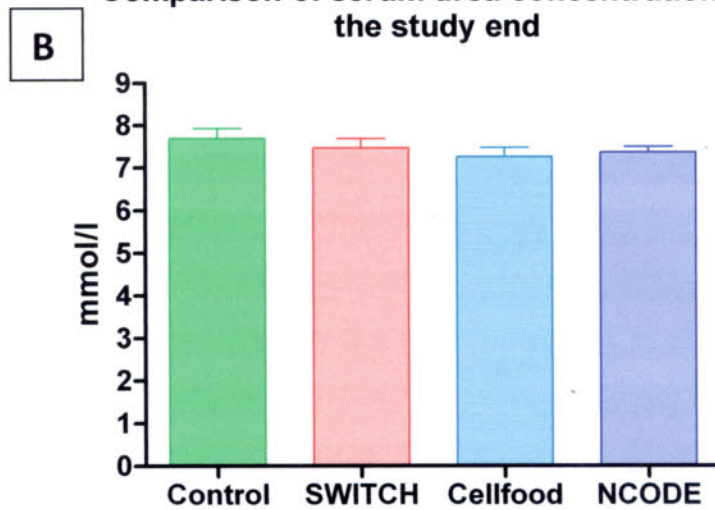


Figure 7: Comparison of (A) Creatinine and (B) Urea concentrations in the plasma as markers of kidney function of eighty rats undergoing a once off oral gavage dose with distilled water as a control and SWITCH, Cellfood and NCODE at 225mg/kg respectively. No significant difference was evident in all groups for the seven day follow up period. n= 20 rats per group

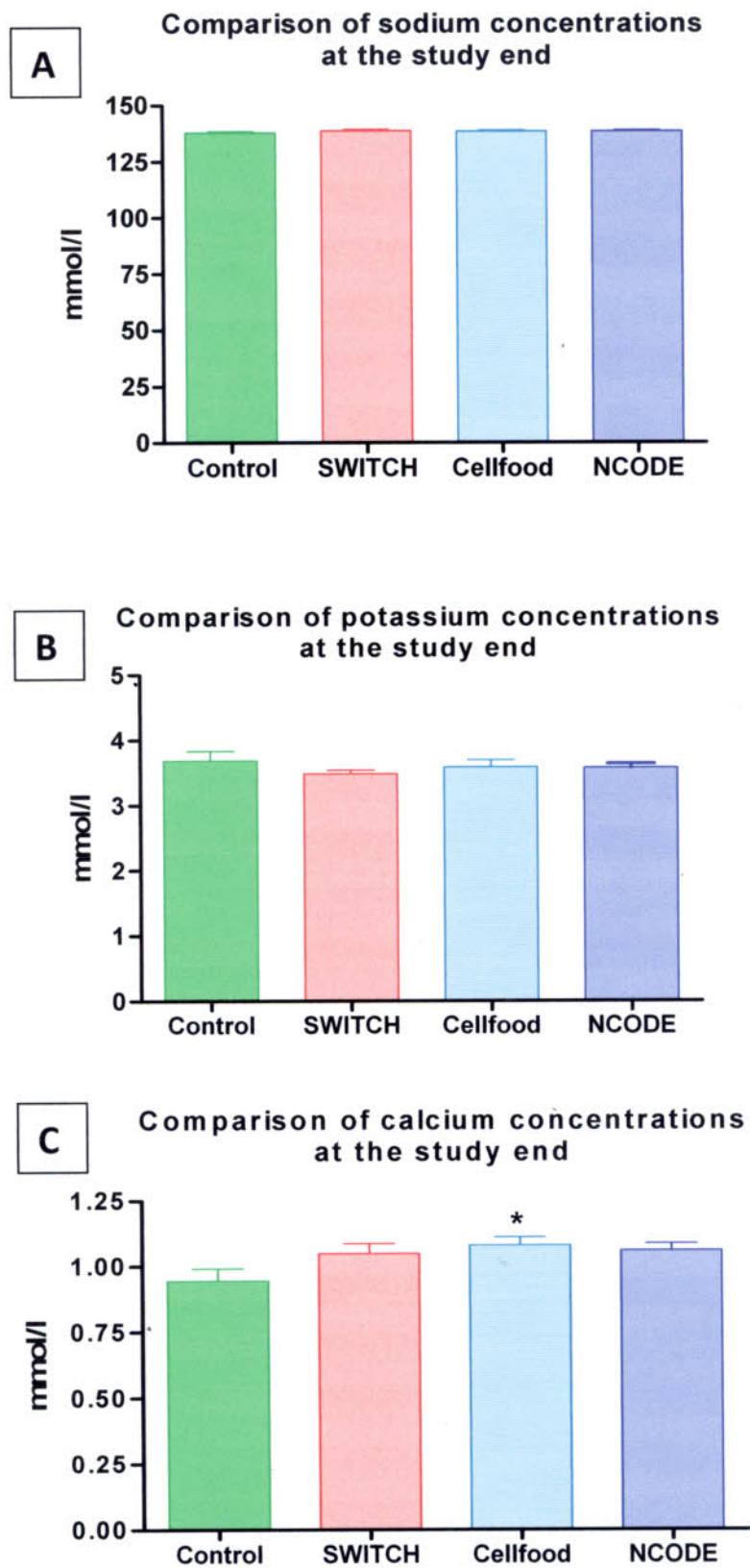
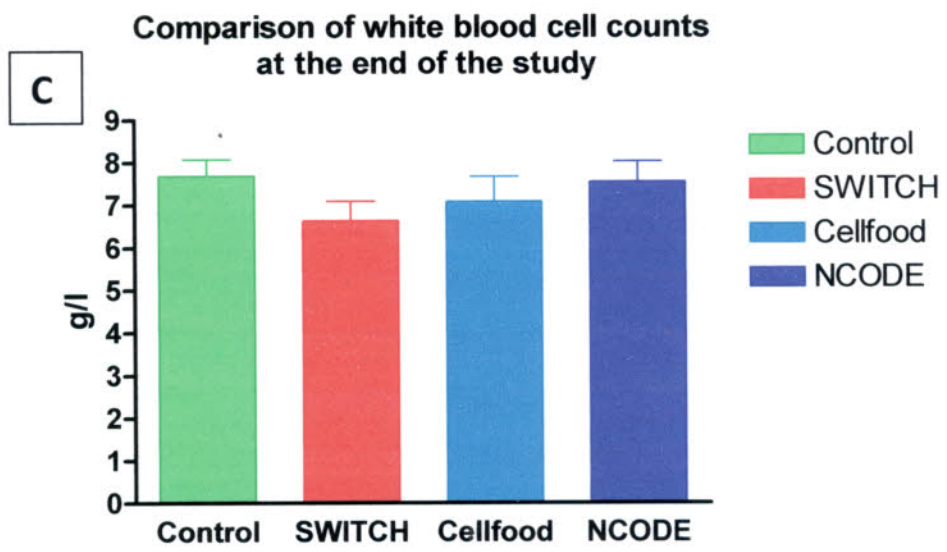
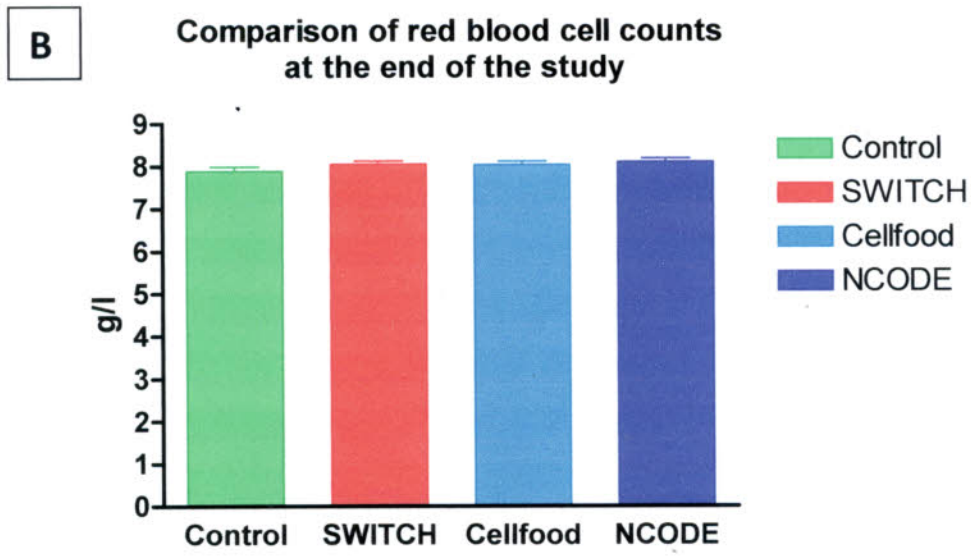
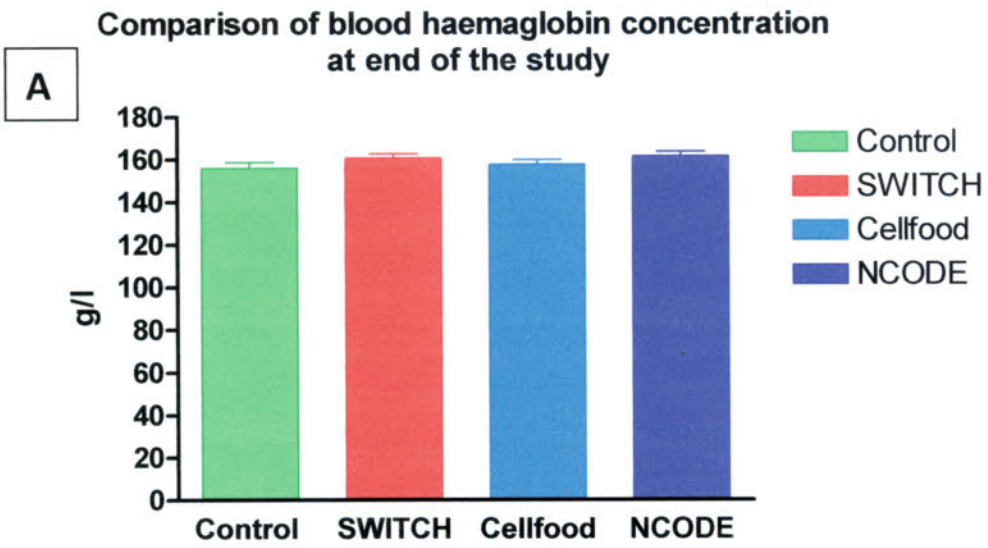


Figure 8: Comparison of (A) sodium, (B) potassium and (C) calcium electrolytes in the plasma as markers of damage of eighty rats undergoing a once off oral gavage dose with distilled water as a control and SWITCH, Cellfood and NCODE at 225mg/kg respectively. No significant difference was evident in all groups, except for cellfood group ($p < 0.05$) for the seven day follow up period. $n = 20$ rats per group



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Comparison of Haematocrit at the end of the study

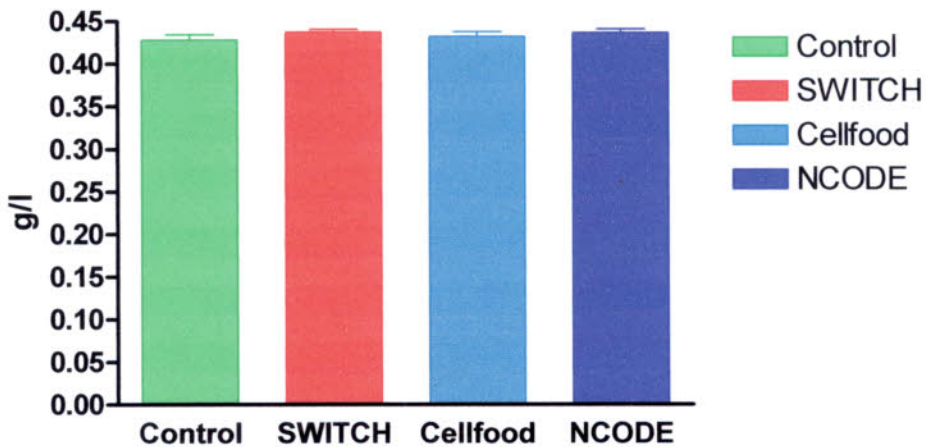


Figure 9: Comparison of (A) Hb, (B) RCC, (C) WCC and (D) HT blood parameters as markers of damage of eighty rats undergoing a once off oral gavage dose with distilled water as a control and SWITCH, Cellfood and NCODE at 225mg/kg respectively. No significant difference was evident in all groups for the seven day follow up period. n= 20 rats per group

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, SWITCH (Cellfood Sport and Shape), Cellfood, and NCODE (Cellfood Longevity) administered by oral gavage at 225mg/kg demonstrated no toxic effects in the Sprague Dawley rat model after an acute dose.

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,

Dr AD Cromarty