ACUTE ORAL TOXICITY IN RATS – LIMIT TEST

TEST METHOD NO.:	P203
STUDY NUMBER:	12797
SPONSOR	NuScience Corporation 43102 Business Center Pkwy Lancaster, CA 93535
TEST SUBSTANCE IDENTIFICATION:	Cellfood
TEST SUBSTANCE DESCRIPTION:	Light amber colored liquid
DATE RECEIVED:	October 10, 2002
REFERENCE NO.:	021010-1D
DATE OF TEST:	October 15-29, 2002
NOTEBOOK NO.:	02-56: pages 287-291

1. PURPOSE

To provide information on health hazards likely to arise from short-term exposure to Cellfood by the oral route.

2. PROCEDURE

A group of Sprague-Dawley derived, albino rats was received from Ace Animals, Inc., Boyertown, PA. The animals were singly housed in suspended stainless steel caging with mesh floors. Litter paper was placed beneath the cages and was changed at least three times per week. The animal room was temperature controlled and had a 12-hour light/dark cycle. The animals were fed Purina Rodent Chow #5012 and filtered tap water was supplied *ad libitum* by an automated watering system.

Following acclimation to the laboratory, a group of animals was fasted overnight by removing feed from their cages. After the fasting period, ten rats (five male and five female) were selected for test based on health and initial bodyweights. Individual doses were calculated based on these bodyweights, taking into account the specific gravity of the test substance. Each animal received 5,000 mg/kg of the test substance by intubation using stainless steel ball-tipped gavage needle attached to an appropriate syringe. After administration, each animal was returned to its designated cage. Feed was replaced approximately 3 hours after dosing.

The animals were observed for mortality, signs of gross toxicity and behavioral changes at approximately on hour post dosing and at least once daily for 14 days. Bodyweights were recorded prior to initiation and at termination. All animals were euthanized by CO_2 inhalation at termination.

3. RESULTS

Individual bodyweights and doses are presented in Table 1. Cage-side observations are presented in Table 2.

All animals survived, gained weight and appeared active and healthy. There were no signs of gross toxicity, adverse pharmacological effects or abnormal behavior.

4. CONCLUSION

Under the conditions of this study, the single dose acute oral LD_{50} of Cellfood is greater thank 5,000 mg/kg of bodyweight in male and female rats.

TABLE1: INDIVIDUAL BODYWEIGHTS AND DOSES

Animal No.	Sex	Bodyweight (g)		Dose[1]
		Initial	Day 14	ml
5413	M	270	369	1.3
5414	M	261	388	1.3
5415	M	262	374	1.3
5416	M	273	380	1.3
5417	M	257	366	1.2
5418	F	200	247	0.97
5419	F	193	239	0.93
5420	F	184	236	0.89
5421	F	180	244	0.87
5422	F	174	232	0.84

TABLE: INDIVIDUAL CAGE-SIDE OBSERVATIONS

Animal Number	Findings	Day of Occurrence
MALES		
5413-5417	Active and healthy	0-14
FEMALES		
5418-5422	Active and healthy	0-14

University of Pretoria

23 October 2006

Whom it concerns,

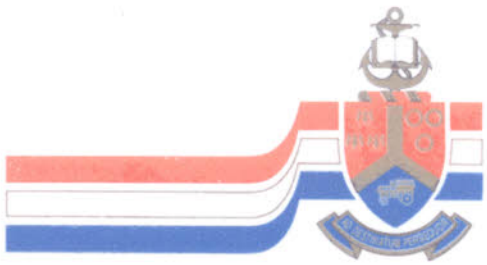
RE: Research on products OFL

Acute toxicity studies were carried out by the Department of Pharmacology in collaboration with University of Pretoria's Biomedical Research Centre (UPBRC) at Onderstepoort.

Cellfood®, Cellfood DNA/RNA (known as NCODE® in RSA), and Cellfood Natural Weight Loss Formula (known as SWITCH® in RSA) showed no toxicity as evaluated on lymphocytes at the dilutions recommended by the supplier.

Yours faithfully

OD Gomarty



University of Pretoria

Toxicity test results: Lymphocyte survival/proliferation assay using varying concentrations of three tested compounds: Cellfood, NCODE and Switch.

Introduction:

Toxicity testing in an *in vitro* situation is a prerequisite for any potential therapeutic compound to allow further testing by *in vivo* or *ex vivo* experiments such as in rodents or other lower animals. The compound to be tested should be tested for direct toxicity to cells in culture as well as by tests that would ascertain the carcinogenicity or mutagenicity using the classical Ames tests.

This report is the summary of the cell culture toxicity test using lymphocytes obtained from healthy volunteers to demonstrate any toxicity for the three test compounds namely Cellfood, NCODE and Switch supplied by Oxygen For Life.

In this type of direct toxicity assay, cells in culture are used. Different cell types can be used for this assay and include isolated blood cells, primary cell cultures, immortalized or cancer cell lines. Each of these cell types show a distinct susceptibility to the test compounds with the isolated cells and primary cell cultures usually showing greater sensitivity to toxic compounds as they have been removed from their natural environment.

The lymphocytes of normal healthy blood can survive for about two weeks under ideal culture conditions before they start showing signs of senescence and dying off. The cells can be stimulated to grow for a longer time by adding a mitogenic compound that stimulates the secretion of growth maintaining mediators. The most common mitogen used for this stimulation is phytohaemagglutinin (PHA). Lymphocytes are an easily accessible and good source of susceptible cells for toxicity testing and are routinely used in many laboratories as an initial *in vitro* test to indicate the safety of new compounds being tested for possible toxic effects. If no signs of toxicity are seen in a lymphocyte survival/ proliferation assay it does not necessarily mean that there will not be toxicity in other *in vitro* or *in vivo* tests, but it does eliminate the possibility that the compound is highly toxic to lymphocytes and probably also many other cultured cell lines.

The lymphocyte survival/ proliferation assay was used in this study to show that the compounds do not show high toxicity.

Materials and methods:

The assays were repeated three times using blood from different healthy volunteers.
Fresh blood was drawn by venipuncture into K_3EDTA blood collection tubes from healthy volunteers. The mononuclear cells were separated from the other blood cells by centrifugation on a step density gradient using Histopaque-1077 (Sigma Diagnostics, St Louis, MO, USA) as described by Zafiropoulos *et al.*, (1997). The mononuclear cells were isolated under sterile conditions and washed with culture medium, counted, re-suspended and diluted to a concentration of 2×10^6 cells/ml in complete RPMI 1640 cell culture medium (supplemented with 1% glutamine, penicillin and streptomycin at 100 µg/ml and 10 % complement inactivated foetal calf serum).

The mixed mononuclear lymphocytes were incubated in a round bottom 96 well microtitre culture plates containing 100µl cell suspension, 80µl complete RPMI and 20µl of the test compounds: Cellfood, NCODE or Switch to make six replicates each in a range of dilutions (1:1, 1:10, 1:100, 1:1000, and 1:10000) in the wells.

The culture plates were incubated for 1 hour under sterile conditions (37 °C in an atmosphere of 5% CO_2) and then one half of the wells were stimulated by addition of PHA (Murex Biotech Ltd., Dartford, England) dissolved in complete RPMI to a final concentration of 5 µg/ml. The cells were then further incubated for 3 days at 37°C under a 5% CO_2 atmosphere.

The lymphocyte survival/proliferation was measured by the colorimetric tetrazolium dye cell enumeration method of Mosmann (1983) using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (Sigma Diagnostics, St Louis, MO, USA).

Plates were read on a Ceres UV 900 micro-ELISA plate reader using a test wavelength of 570 nm and a reference wavelength of 620 nm. The readings of the controls (cells not exposed to the test compounds) were normalized and the resting and PHA stimulated lymphocyte readings for each concentration of the test compounds were compared on a basis of percent of control.

Result and Discussion:

Initial tests using the three test substances (Cellfood, NCODE and Switch) at the concentration supplied resulted in total cell death. After adjusting the pH and retesting, the same toxic effect was seen and therefore the testing was repeated using different concentrations made by serial 1:10 dilutions. Under these conditions the tested compounds exhibited no toxic effect on lymphocytes at dilutions of 1:100, 1:1000 and 1:10000 in neither the resting nor the PHA stimulated lymphocytes (Figures 1 to 3). An obvious decrease in lymphocyte numbers could still be observed in both the resting and the PHA stimulated lymphocytes at dilutions of 1:10 and 1:1 for all three of the test compounds. This could be due to the high concentration of salts from the buffer required to adjust the pH to neutral.

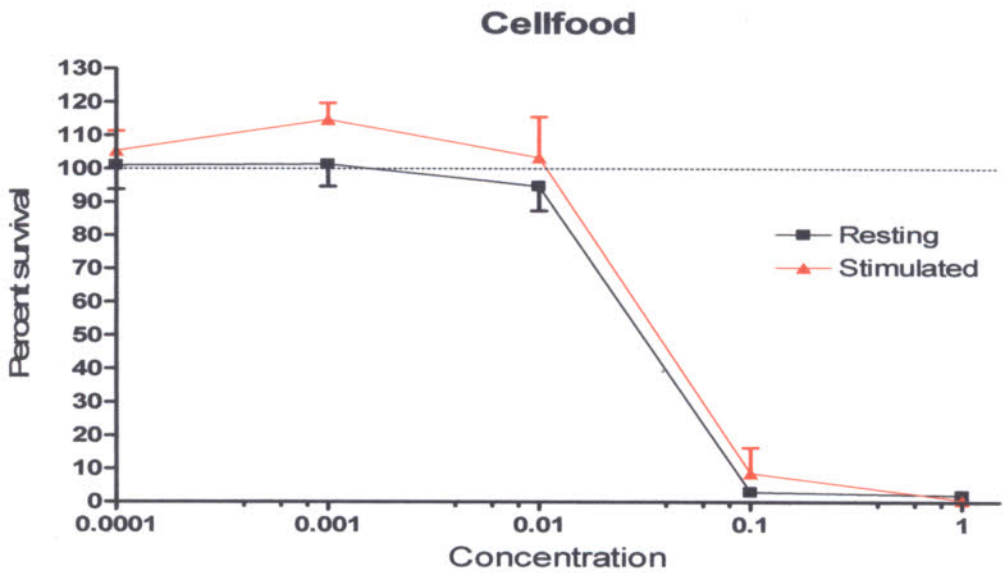


Figure 1: Lymphocyte survival/ proliferation of resting and PHA stimulated cells at different concentrations of Cellfood. Note that the standard error bars only extend to one side for clarity. No significance could be demonstrated. n = 6 per concentration.

The results (Figure 1) indicate that Cellfood had no toxicity effect on either resting cells or PHA stimulated cells at dilutions of 1:100, 1:1000 and 1:10000. The 1:1000 dilution showed a minor but insignificant stimulatory effect on the PHA stimulated cells.

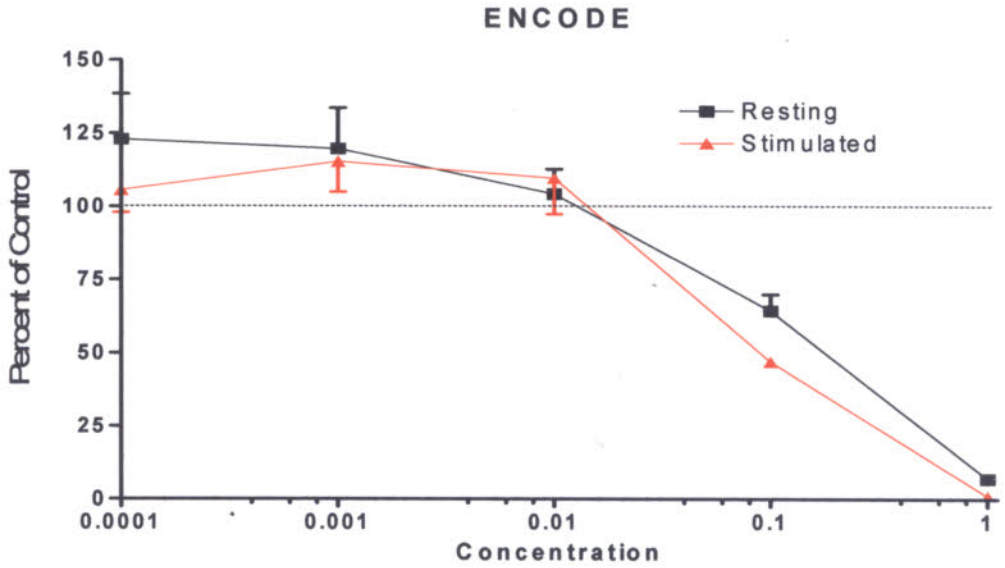


Figure 2: Lymphocyte survival/ proliferation of resting and PHA stimulated cells at different concentrations of NCODE. Note that the standard error bars only extend to one side for clarity. No significant difference could be demonstrated. n = 6 per concentration.

A small increase in lymphocyte proliferation can be observed for both the resting and PHA stimulated cells at dilutions of 1:100, 1:1000 and 1:10000, with a significant toxic

effect on both the resting or PHA stimulated cells at concentrations of 1:10 and 1:1 (Figure 2).

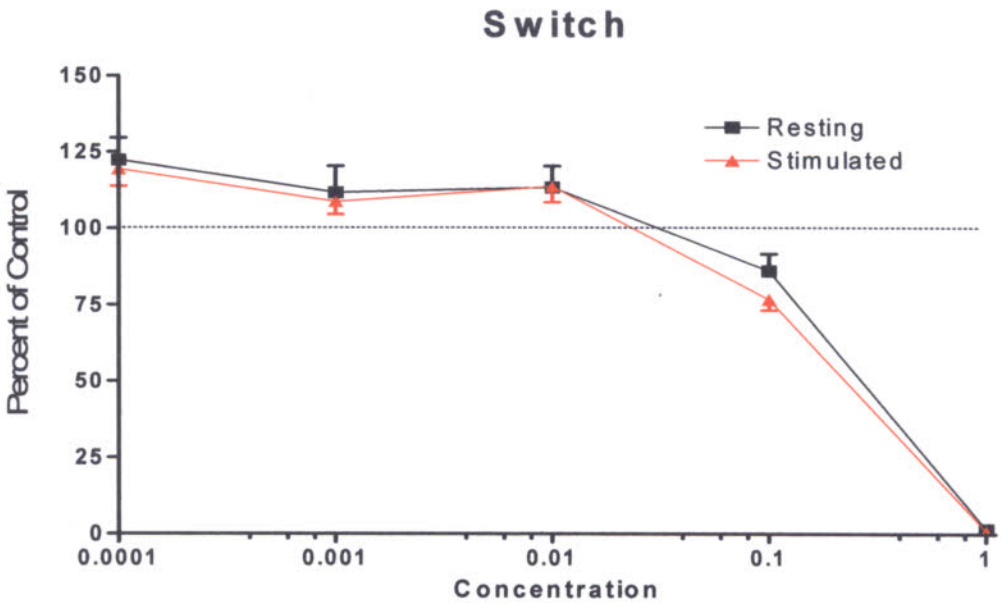


Figure 3: Lymphocyte survival/ proliferation of resting and PHA stimulated cells at different concentrations of Switch. Note that the standard error bars only extend to one side for clarity. No significance could be demonstrated. n = 6 per concentration.

A small increase in lymphocyte proliferation can be observed for both the resting and PHA stimulated cells at concentrations of 1:100, 1:1000 and 1:10000, with a significant toxic effect on both the resting and PHA stimulated cells at dilutions of 1:10 and 1:1 (Figure 3).

These results would indicate that all three compounds have minimal to no toxicity at higher dilutions (1:100, 1:1000 and 1:1000). Exposure to concentrations of 1:100 would not be achieved physiologically during normal supplementation with Cellfood, NCODE or Switch if the recommended dosing regimen is followed. The toxic effect seen at higher concentrations (less diluted test samples) could be due to their low pH that must be neutralised with a fairly concentrated physiologically acceptable buffer (phosphate buffer).

Despite the slight stimulatory trend seen from the graphs for the lower concentrations, no statistical significance could be found for Cellfood, NCODE or Switch treated cells verses control cells for either the resting or the PHA stimulated lymphocytes using the Students t-test statistical analysis in Prism Graphpad® statistics software package.

The slight stimulation occurring at the lower concentrations (more diluted samples) indicates the possibility that the three compounds tested (Cellfood, NCODE and Switch) may have immuno-stimulatory effects, but this would need to be tested by different methods that can assess particular immune responses. No claim in this regard can be

made without specific immunological tests being done on each test compound to confirm any immuno-modulating effects.

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

Yours faithfully

AD Cromarty

Dr A.D. Cromarty