# Evaluation of the possible genotoxic effects of a carbohydrate derived fulvic acid (CHD-FA) using the micronucleus assay and Ames test

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## INTRODUCTION

CHD-FA is a safe and heavy metal free carbohydrate derived fulvic acid. For registration purposes, pharmaceuticals require a comprehensive assessment of their genotoxic potential.

A standard battery of tests usually includes a (i) bacterial reverse mutation test (Ames test) and (ii) evaluation of chromosomal damage using the micronucleus (MN) assay.

Micronuclei are expressed in dividing cells that either contain chromosome breaks lacking centromeres (acentric fragments) and/or whole chromosomes that are unable to travel to the spindle poles during mitosis.

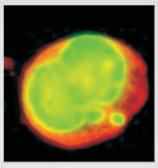

Figure 1: *Micronuclei in a binucleated T-lymphocyte*

## RESULTS

(i) FA was not mutagenic at the concentrations tested to any of the bacterial strains used in the Ames test when compared to the spontaneous revertant counts. FA was cytotoxic to all strains and even though bacterial colonies could be counted for TA 100 (with and without S-9) and TA 97a (with S-9), the background lawn was not consistent with a normal lawn (broken up or moth eaten). As a result the revertant colony count was lower than the spontaneous background count (underlying cytotoxic effect).

At 2 x FA, cytotoxicity was still detected against TA 98 (with and without S-9) and TA 102 (without S9). A reduction in the revertant count was also noticed with TA 97a due to an underlying cytotoxic effect in the absence of S-9. In the presence of S-9 some cytotoxicity was detected with TA100.

No toxicity was detected from 5x and higher dilutions. It is also noted that bacterial colony counts were lower when S9 was absent compared to the presence of S9 for all strains and dilutions tested.

(ii) Both LMW and raw CHD-FA compared closely to the negative control with respect to the number or lack of micronuclei observed (Table 1).

## METHODS

(i) The Ames test was used to test for the genotoxic potential of CHD-FA at the highest non toxic concentration. This test was done using susceptible bacterial strains of . Diagnostic mutagens were included for each strain to test for the revertant potential.

(ii) CHD-FA was buffered to a pH of 5.5 for the MN assay. In this test raw CHD-FA was compared to a LMW product thereof. The concentrations tested were calculated according to the degree of toxicity to lymphocytes. Cells were collected from healthy non-smoking volunteers, fixed and stained with acridine orange and scored using a fluorescence microscope.

Table 1: Summary of Micronuclei with raw and LMW fulvic acid.

| FA Concentration | Total Cells | 0MN | 1MN | 2MN | >2MN |
|---|---|---|---|---|---|
| First Culture | | | | | |
| Positive Control | 2804 | 680 | 264 | 50 | 6 |
| Negative Control | 3556 | 832 | 140 | 20 | 8 |
| Raw 150 µg/ml | 4048 | 796 | 164 | 32 | 8 |
| LMW 150 µg/ml | 2880 | 944 | 52 | 8 | 0 |
| Second Culture | | | | | |
| Positive Control | 3240 | 816 | 144 | 32 | 8 |
| Negative Control | 3000 | 960 | 36 | 4 | 0 |
| Raw 150 µg/ml | 2992 | 944 | 40 | 12 | 4 |
| LMW 150 µg/ml | 2880 | 944 | 40 | 12 | 4 |

## CONCLUSION:

This study indicated no genotoxicity above background for both LMW (determined by the Ames test and the MN assay) and raw (in the case of the MN assay only) CHD-FA.