Toxicology Reports 5 (2018) 196–206

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Mutagenicity and genotoxicity of ClearTaste

B.K. Soni*, J.P. Langan

MycoTechnology, Inc., 3155 N Chambers Rd, Suite E, Aurora, CO, 80011, United States

ARTICLE INFO

Keywords:
Mutagenicity
Genotoxicity
Ames
Micronucleus
ClearTaste

ABSTRACT

The present study investigates whether ClearTaste is mutagenic/genotoxic by employing it as a test article in bacterial reverse mutation (Ames test) and in vitro human peripheral blood lymphocyte micronucleus assays conducted by a Good Laboratory Practice certified third party as parameterized by the United States Food and Drug Administration. ClearTaste is a taste modulator derived from the filtrate of submerged Cordyceps sinensis and is typically processed into a powder. It functions as a bitter, sour, astringency, metallic and lingering aftertaste mitigator/blocker. The Ames test includes revertant colony counts almost exclusively less than 100/plate and significantly fewer ClearTaste counts as opposed to known mutagen counts. The micronucleus assay reported cytotoxicity exclusively < 25% for doses up to 2,000 μg/L with Cytokinesis Block Proliferation Indices less than water and statistically significant differences between micronucelated cells post dosing compared to cyclophosphamide and vinblastine controls. The conclusion of these data is that ClearTaste is neither muta- nor carcinogenic.

1. Introduction

The commercialization of any novel ingredient/foodstuff is requisitely accompanied by safety tests. The present journal article discusses bacterial reverse mutation (Ames test) and *in vitro* human peripheral blood lymphocyte (HPBL) micronucleus assays utilizing ClearTaste, a novel taste modulating powder made through the culturing of *Cordyceps sinensis*, as a test article. ClearTaste was discovered at MycoTechnology, Inc. in July 2014.

Taste modulation has been the subject of much interest over the decades in part due to the discipline's important economic implications in driving consumer preference. While the perception and modulation of all five conventional tastes have been intensely investigated and better understood over the last 2–3 decades, food science has taken particularly extensive measures to identify novel bitter blockers, an effort perhaps only matched by the investigation of sweetness intensifiers [1–8]. ClearTaste is unique as a bitter blocker being that it is derived through the culturing of a fungus. When used at proper concentrations (typically < 50 ppm) ClearTaste can also mitigate sour, metallic and lingering off tastes. ClearTaste's functionality makes it highly alluring to the food and flavor industry, heightening the pertinence of this journal article.

The purpose of reverse mutation and micronucleus assays are, respectively, to investigate the extent to which a test article is mutagenic or genotoxic/induces chromosome instability. Reverse mutation assays analyze frameshift and basepair substitution mutations in *Salmonella typherium* and *Eschericia coli*. Micronucleus assays monitor the extent that micronuclei, small cytoplasmic membrane bodies carrying pieces of or an entire chromosome due to a malfunctioning anaphase, form when exposed to a test article. Known mutagens and micronuclei inducers are used as control articles in each test, respectively. These tests determine an important aspect of food safety and are essential to informing potential consumers about the nature of novel food. Some physicochemical properties and the proximate analysis of ClearTaste are shown in Tables 1 and 2.

2. Materials and methods

2.1. Statement of GLP validation

The bacterial reverse mutation and *in vitro* HBPL micronucleus assays were conducted by a third party according to Good Laboratory Practice as parameterized by the United States Food and Drug Administration. Detailed methods for the execution of these procedures and be found in the List of References, with certain references discussing the bacterial reverse mutation assay [9–11] and others discussing the micronucleus assay [12–14].

2.2. Bacterial reverse mutation assay

2.2.1. Test system

The tester strains used were the *Salmonella typhimurium* histidine

* Corresponding author.
E-mail addresses: bsoni@mycotechcorp.com (B.K. Soni), jim@mycotechcorp.com (J.P. Langan).

https://doi.org/10.1016/j.toxrep.2017.12.015
Received 8 May 2017; Received in revised form 21 June 2017; Accepted 13 December 2017
Available online 12 January 2018

Table 1
Physicochemical Properties of ClearTaste.

Solubility	~99.5% soluble at up to 6% ClearTaste m/v
Density	0.5 g/L
pH[a]	4.3
Melting Point	193–205 °C
Ignitability	Not ignitable

[a] Done according to EPA method SW9045C.

Table 2
ClearTaste Proximate Composition.

Property	Concentration (%)
Moisture (vacuum oven)	1.8
Protein	1.3
Fat (acid hydrolysis)	0.7
Ash	2.6
Carbohydrates (by difference)	93.6

All values not done by difference conducted according to AOAC methods at Certified Labs, Inc.

auxotrophs TA98, TA100, TA1535 and TA1537 as described by Ames et al. [9] and *Escherichia coli* WP2 *uvr*A as described by Green and Muriel [10].

Tester strains TA98 and TA1537 are reverted from histidine dependence (auxotrophy) to histidine independence (prototrophy) by frameshift mutagens. Tester strain TA1535 is reverted by mutagens that cause basepair substitutions. Tester strain TA100 is reverted by mutagens that cause both frameshift and basepair substitution mutations. Specificity of the reversion mechanism in *E. coli* is sensitive to basepair substitution mutations rather than frameshift mutations. *Salmonella* tester strains were derived from Dr. Bruce Ames' cultures; *E. coli* tester strains were from the National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland. Historical data for the test system is provided in Table 3. Historical data are more important in micronucleus assays for determining outcomes of the assay but are included herein for the Ames assay for those interested.

2.2.2. Preparation of tester strain

Overnight cultures were prepared by inoculating from the appropriate frozen permanent stock into a vessel containing 30–50 mL of culture medium. To assure that cultures were harvested in late log phase, the length of incubation was controlled and monitored. Following inoculation, each flask was placed in a shaker/incubator programmed to begin shaking at 125–175 rpm and incubating at 37 ± 2 °C for approximately 12 h before the anticipated time of harvest. Each culture was monitored spectrophotometrically for turbidity and was harvested at a percent transmittance yielding a titer of greater than or equal to 0.3×10^9 cells/mL. The actual titers were determined by viable count assays on agar plates.

Table 4
Historical Negative and Positive Control Values for Non-S9 Activated Micronucleus Assay, 2013–2015.

Micronucleated Binucleated Cells (%)	Negative Control		Positive Control[a]	
	4 h	24 h	4 h	24 h
Mean	0.36	0.39	3.77	1.76
Standard Deviation	0.23	0.31	1.66	0.86
95% Control Limits	0.00–0.82	0.00–1.01	0.46–7.08	0.04–3.48
Range[b]	0.05–1.43	0.10–2.00	1.00–10.10	0.50–5.70

[a] Positive control for non-activated 4 h studies is Mitomycin C, Positive control for activated 24 hour study is Vinblastine.
[b] Range is from minimum to maximum.

Table 5
Historical Negative and Positive Control Values for S9 Activated Micronucleus Assay, 2013–2015.

	Micronucleated Binucleated Cells (%)	
	Negative Control	Positive Control[a]
Mean	0.33	1.51
Standard Deviation	0.23	0.50
95% Control Limits	0.00–0.78	0.50–2.51
Range[b]	0.10–1.50	0.40–3.30

[a] Positive control for S9 activated studies is cyclophosphamide.
[b] Range is from minimum to maximum.

Table 6
Reverse Mutation Assay Tester Strain Titer Results.

Experiment	Tester Strain				
	TA98	TA100	TA1535	TA1537	WP2 uvrA
	Titer Value ($x10^9$ cells/mL)				
Mutagenicity Assay	11.5	11.1	8.7	11.2	12.4
Confirmatory Mutagenicity Assay	3.0	4.0	2.4	6.5	2.6

2.2.3. Exogenous metabolic activation

Aroclor™ 1254-induced rat liver S9 was used as the metabolic

Table 3
Historical Negative and Positive Control Values for Reverse Mutation Assay, 2014.

Strain	Control	Activation: None					Activation: Rat Liver				
		Mean	SD	Min	Max	95% CL[a]	Mean	SD	Min	Max	95% CL*
TA98	Neg	16	5	5	42	6–26	24	7	5	53	10–38
	Pos	232	258	57	2691		400	165	109	1382	
TA100	Neg	94	14	66	152	66–122	102	18	63	164	66–138
	Pos	681	176	213	1767		681	259	186	2793	
TA1535	Neg	11	4	2	31	3–19	13	5	2	36	3–23
	Pos	586	226	16	2509		117	99	23	1060	
TA1537	Neg	7	3	1	19	1–13	9	4	1	23	1–17
	Pos	411	355	32	2921		72	52	10	562	
WP2 uvrA	Neg	25	7	7	62	11–39	28	8	10	55	12–44
	Pos	376	123	99	1026		302	102	91	687	

[a] 95% CL = mean ± 2 SD (but not less than zero).