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Summary Report:

Pilot trial investigation of a proprietary liquid phytonutrient nutraceutical (Eniva VIBE Original Energy & Life™) and its impact on 8-hydroxydeoxyguanosine, a urinary biomarker of oxidative DNA damage

1. Introduction & Summary

8-hydroxydeoxyguanosine (8-OHdg) is a widely accepted and used urinary biomarker representing actual DNA damage in the body. As DNA contains the genetic material of human cells, alterations or damage to its integrity may lead to a disruption in normal cellular function and the initiation of pathologic processes, including uncontrolled cellular growth. Due to this, the investigation and identification of DNA protective substances which may decrease levels of this biomarker have been sought.

An elevation in urinary 8-OHdg has been documented in several serious health conditions, certain chemical exposures and is known to have a baseline level increase with aging. Excluding chemical exposure, the elevations in this biomarker have been mechanistically explained through free radical associated oxidative damage to DNA. When oxidative damage to DNA occurs, the damaged components of DNA are usually removed via repair enzymes. These sections of damaged DNA are then excreted by the body as nucleoside derivatives. 8-hydroxydeoxyguanosine is one such derivative that is excreted by the body in a relatively un-metabolized state. It represents a sensitive biomarker of oxidative DNA damage and potential repair.

This study undertook to evaluate the impact of a liquid phytonutrient dietary supplement (*Eniva VIBE Original Energy & Life™*) containing verified essential RDA nutrients, a concentrated blend of fruits, vegetables and aloe vera gel, as well as containing a proprietary green tea epigallocatechin gallate (EGCG) catechin complex, for its ability to impact the 8-OHdg biomarker through a speculated antioxidant mechanism. Potential renal and hepatic toxicity was also evaluated. A pilot trial involving 18 healthy adults was conducted. After a 14 day wash-out period free of any dietary supplementation, participants provided both urine and blood baseline samples. Participants were then given 60 ml of the test material. Additional urine and serum samples were collected at 4 and 24 hours post ingestion. Serum was analyzed for potential renal or hepatic toxicity via organ specific enzymes (testing performed by Lab One, MN). 8-OHdg analysis was performed (Brunswick Labs, MA) on urine sample series from 8 randomly selected individuals during the first phase of body-fluid analysis. A creatinine corrected methodology was used in calculating the 8-OHdg level after HPLC.

The results of the test material on urinary 8-OHdg levels are expressed most accurately as a percent change from baseline, representing mean 37% and 29% reductions at the 4 and 24 hour time points, respectively. While statistical significance was unable to be achieved due to low participant numbers, a correlation between test material ingestion and a decrease in 8-OHdg

urinary levels was observed through the 24 hour time point. Hepatic and renal biomarkers measured support a non-toxic nature of the test material.

As high value is placed on substances which may possess DNA protective properties, the results of this study warrant further investigation of the test material as a biologically active antioxidant formulation possessing potential DNA protective properties.

2. Experimental Design:

Method Synopsis:

A pilot trial involving 18 healthy adults was conducted. After a 14 day wash-out period free of any dietary supplementation, participants fasted after 21:30 on the fourteenth day and then had baseline biologic urine and serum samples initiated at 08:00 on Day 15. Participants received 2 ounces of the test material after baseline body fluids were obtained. Participants continued to fast until 13:00 on Day 15. Serial urine and serum samples were collected at 4 and 24 hrs post baseline labs. Fasting ceased at 13:00 on Day 15. Participants were instructed to continue their normal daily activities, but not to engage in any significant physical activity out of the ordinary. Adverse event questionnaires were provided and responses recorded.

1. Participants:

18 adult human volunteer participants were involved in the study. 10 females and 8 males. No individual had any known major health disorder. Female participants took a pregnancy test to ensure eligibility for the study. For additional characteristics, please see final manuscript.

2. **Test Material:** Eniva VIBE Original Energy & Life™ nutraceutical. Verified ingredient listing on file and provided in final manuscript.

3. Study Design:

This was an open label pilot trial. Participants made 2 trips to the study site, participating in the following protocol: **Days 1-14:** Participants refrained from consuming any dietary nutritional supplements, including the Eniva VIBE Product. **Day 14:** Subjects were asked to fast from 21:30 on Day 14 until 13:00 on Day 15. In addition, they were requested to collect their morning first urine on Day 15. **Day 15:** Subjects arrived at 07:45 with morning urine in hand. Subjects then had baseline vitals taken and a blood draw at approximately 08:00. Then, subjects were given 60 ml of the Eniva VIBE Original Energy & Life™ product. The subjects had repeat blood and urine samples 4 hrs from their administration of the test material. The blood was collected through peripheral veins. **Day 16:** The participants returned at 08:00 with a collection of their first morning urine. Vitals were taken and the participants then had another blood draw. Upon completion of the study, participants received fifty US dollars.

4. Body Fluid Collection:

- a. **Serum:** Serum was collected through peripheral veins by trained phlebotomists. Serum was placed into routine glass laboratory collection tubes specific for serum collection. Glass tubes and supplies were provided by LabOne (MN). Samples were placed into provider carriers and transported per protocol.
- b. **Urine:** Urine was collected by participants into sterile urine collection vials. Vials were labeled and placed into cool dark storage. Vials were later shipped, on dry ice, to Brunswick Labs (MA) for 8-OHdg analysis.

5. Body Fluid Identification:

The trial incorporated color coded labels to corresponding times of collection:

Plasma Label Color	Definition
White	1 st lab collection (08:00 Day 15)
Orange	2 nd lab collection (12:00 Day 15)
Blue	3 rd lab collection (08:00 Day 16)

URINE Label Color	Definition
White	Baseline urine (08:00 Day 15)
Pink	Post 4-6 hr of Vibe ingestion
Blue	08:00 Day 16

6. Body fluid analysis

a. **Serum:** Serum analysis for renal and hepatic biomarkers was performed at LabOne (MN) facilities per facility protocol. Renal biomarkers included serum creatinine (Cr) and blood urea nitrogen (BUN). Hepatic markers included aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (Alk-Phos).

b. **Urine:** 8-OHdg analysis was performed by Brunswick Labs (MA) on 8 randomly selected participant urine collection series (total 24 vials) during the first phase of body-fluid analysis. A creatinine corrected methodology was used in calculating the 8-OHdg level after performance of validated HPLC quantification methodology. Please see raw data files and final manuscript for methodology details.

7. **Data Analysis:** Standard statistical analysis was performed. Please see raw data files and final manuscript for equation details and methodologies. Variance of 8-OHdg testing methodology was +/- 5% of calculated value.

3. Results: Eniva VIBE Original Energy & Life™

1. Acute toxicity: None

a. **Hepatic profile:** Hepatic enzymes (AST, ALT, ALK-Phos) were established at baseline and then followed at 4 hrs and 24 hrs post ingestion of the test material. No significant change from baseline was present. Please see below Table 1.

Table 1. Hepatic values through 24 hrs ingestion of test material

Part. 1	AST	ALT	ALKP	Part. 10	AST	ALT	ALKP
baseline	24	38	66	baseline	16	14	66
04:00	29	37	64	04:00	27	14	66
24:00	22	34	63	24:00	14	13	63
Part. 2	AST	ALT	ALKP	Part. 11	AST	ALT	ALKP
baseline	14	11	77	baseline	25	21	97
04:00	17	8	78	04:00	25	20	96
24:00	13	6	75	24:00	23	20	96
Part. 3	AST	ALT	ALKP	Part. 12	AST	ALT	ALKP
Baseline	21	25	87	baseline	17	16	87
04:00	22	24	93	04:00	20	13	85
24:00	20	25	92	24:00	19	16	89
Part. 4	AST	ALT	ALKP	Part. 13	AST	ALT	ALKP
Baseline	15	6	57	Baseline	27	24	57
04:00	26	13	63	04:00	27	24	57
24:00	17	9	58	24:00	30	23	60
Part. 5	AST	ALT	ALKP	Part. 14	AST	ALT	ALKP
Baseline	16	9	73	Baseline	22	17	44
04:00	15	8	69	04:00	25	18	46
24:00	14	10	79	24:00	25	20	48
Part. 6	AST	ALT	ALKP	Part. 15	AST	ALT	ALKP
Baseline	16	15	51	Baseline	21	20	57
04:00	13	10	50	04:00	21	17	57
24:00	16	13	52	24:00	21	17	57
Part. 7	AST	ALT	ALKP	Part 16	AST	ALT	ALKP
Baseline	22	19	122	Baseline	16	14	73
04:00	22	21	128	04:00	18	8	63
24:00	none	none	none	24:00	15	10	70
Part. 8	AST	ALT	ALKP	Part. 17	AST	ALT	ALKP
Baseline	20	14	94	Baseline	19	15	33
04:00	18	11	86	04:00	21	15	30
24:00	21	13	88	24:00	23	16	32
Part. 9	AST	ALT	ALKP	Part. 18	AST	ALT	ALKP
Baseline	16	14	66	Baseline	19	6	63
04:00	27	14	66	04:00	21	13	72
24:00	14	13	63	24:00	18	9	71

b. **Kidney profile:** Renal function was assessed at baseline, and then at 4 and 24 hours post test-material ingestion. This was done through blood urea nitrogen (BUN) and creatinine (Cr) levels. No acute significant changes from baselines were observed during the trial.

Figure 1. Renal Assessment: BUN value through 24 hrs post ingestion of test material.

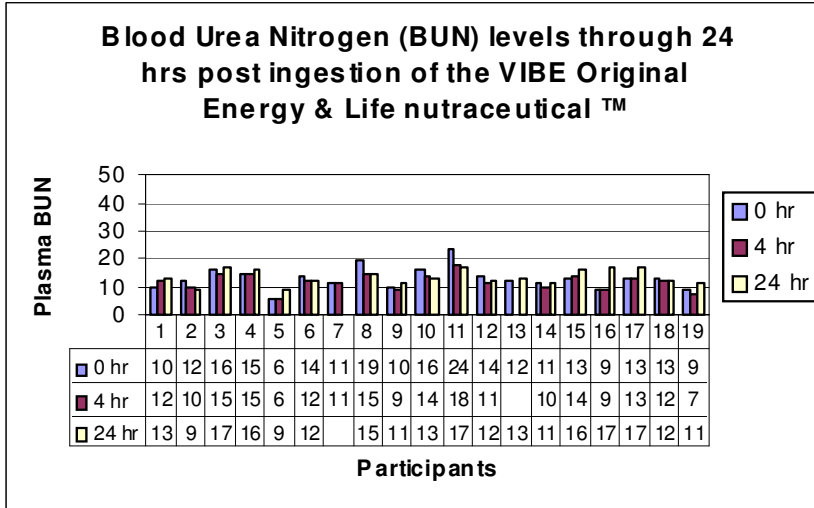
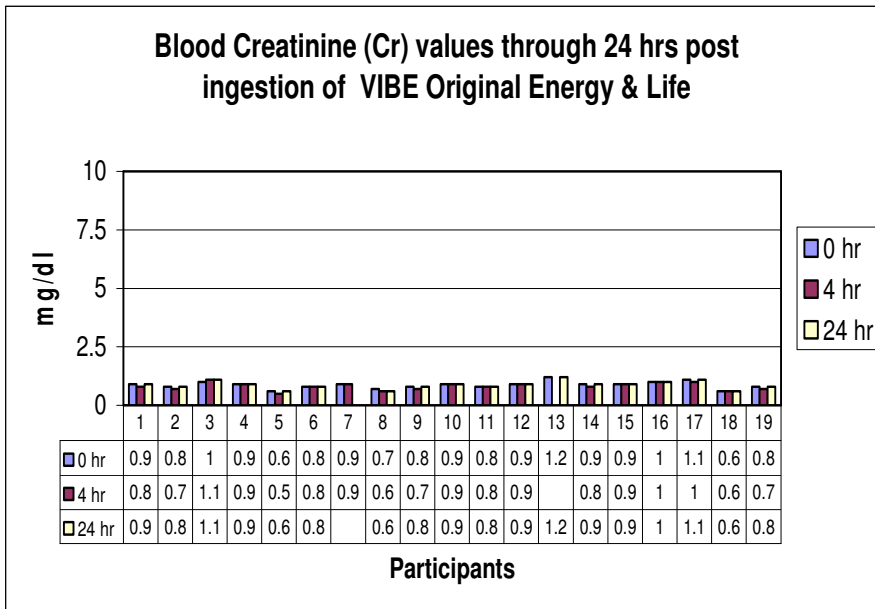


Figure 2. Renal Assessment: Cr value through 24 hrs post ingestion of test material



2. Oxidative stress and DNA induced damage

a. **8-hydroxydeoxyguanosine:** Sterile urine from eight participant urine sample series (three measured time points) were selected at random from the test population to assess for urinary 8-OHdg impact. After 8-OHdg data was provided (Table 1), means were calculated at baseline, 4 hours and 24 hrs. In order to account for the variation in starting 8-OHdg levels, an analysis was performed in which the dependent variable was a *change* in the creatinine adjusted 8-OHdg from baseline through the time collection points. Please see figures below.

Table 2: 8-OHdg values at baseline, 4 hrs and 24 hrs

Participant	Baseline 8-OHdg	4 hrs 8-OHdg	24 hrs 8-OHdg
Particip A	0.63	0.23	0.63
Particip B	0.41	0.24	0.36
Particip C	0.76	1.04	0.18
Particip D	0.7	0.13	0.35
Particip D	0.14	0.09	0.13
Particip E	4.91	1.91	3.4
Particip F	0.27	0.33	0.11
Particip G	0.22	0.06	0.22

Figure 3. Mean 8-OHdg values at baseline and after test material supplementation
(test variance 5%)

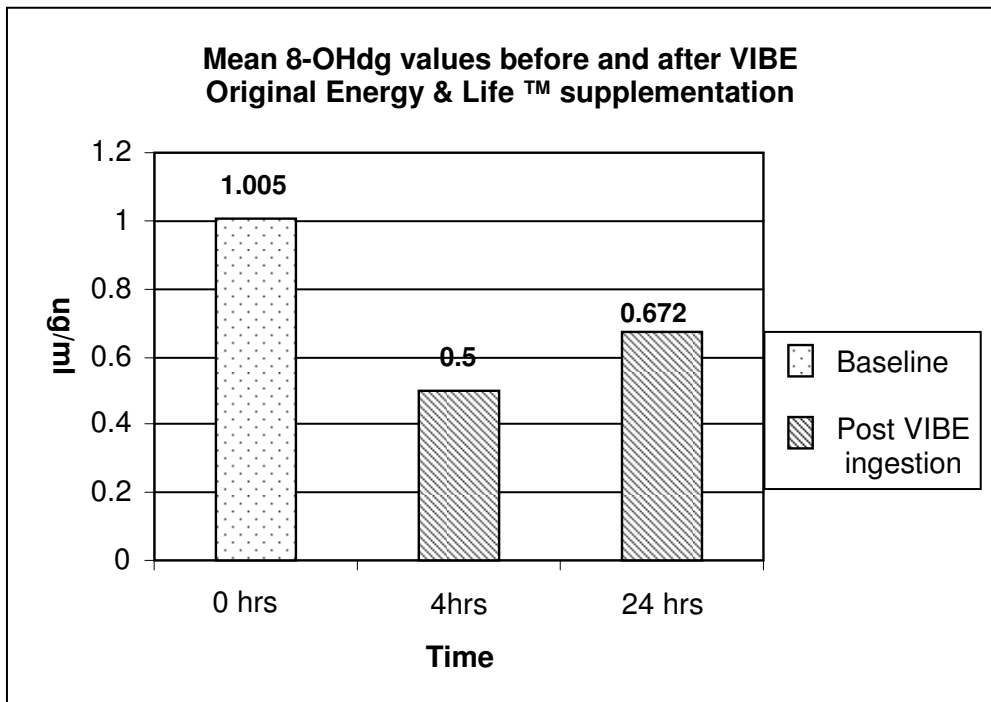


Figure 4. Percent of baseline 8-OHdg levels versus time post test material ingestion
(test variance 5%).

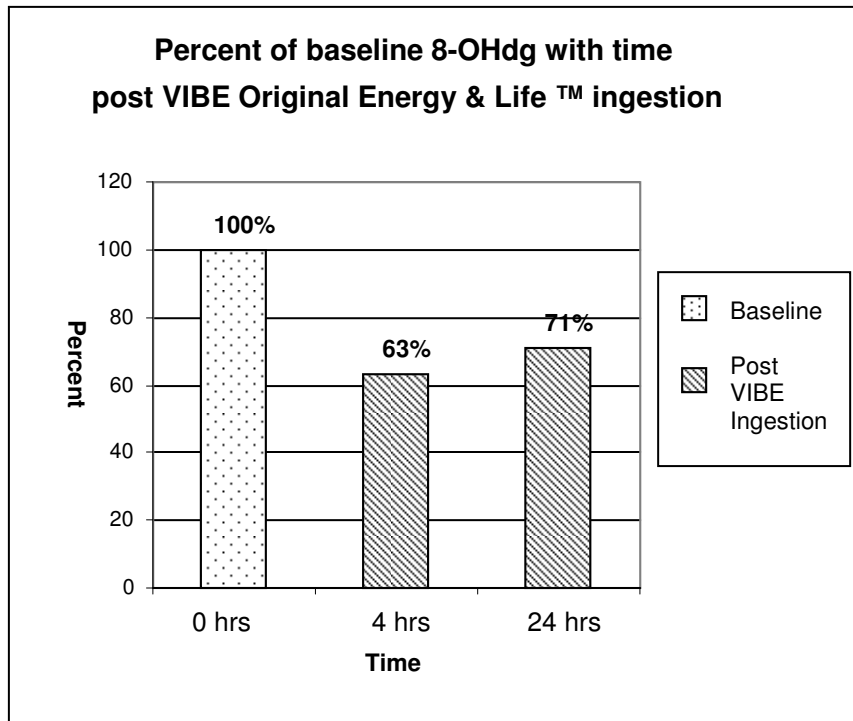
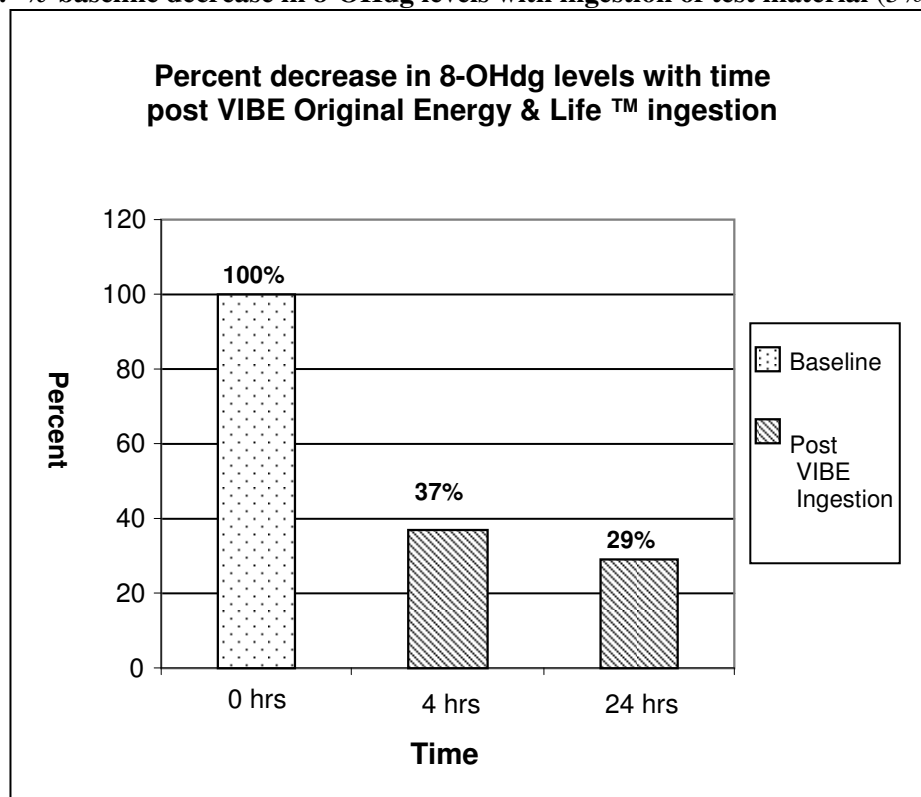


Figure 5. % baseline decrease in 8-OHdg levels with ingestion of test material (5% variance)



4. Conclusions: Eniva VIBE Original Energy & Life™

1. Renal Function:

The test material did not demonstrate acute toxicity to kidney function as measured through BUN and Cr values.

2. Hepatic Function:

The test material did not demonstrate acute toxicity to hepatic function as measured through AST, ALT and ALK-P hepatic enzymes.

3. 8-OHdg urinary levels:

a. There was a correlation identified between test material ingestion and a reduction in urinary 8-OHdg levels through the 24 hour time period which was greater than the variance of the test method. However, this correlation was not statistically significant due to low numbers of participants enrolled.

b. When calculated as percent change from baseline, the material tested provided a 37% and 29% reduction at the 4 and 24 hour time points, respectively. This represented 63% and 71% of starting baseline 8-OHdg values, respectively.

c. The material tested appears to possess biologically active antioxidants and potential DNA protective properties.

5. Discussion:

Free radical induced oxidative stress is a recognized mechanism of DNA damage in the human body. As DNA contains the genetic material of human cells, alterations or damage to its integrity may lead to a disruption in normal cellular function and the initiation of pathologic processes, including uncontrolled cellular growth. 8-hydroxydeoxyguanosine (8-OHdg) is a widely accepted and used urinary biomarker representing actual DNA damage in the body.

An elevation in urinary 8-OHdg has been documented in several serious health conditions, certain chemical exposures, and is known to have baseline level increase with aging. Excluding chemical exposure, the elevations in this biomarker have been mechanistically explained through free radical associated oxidative damage to DNA. When oxidative damage to DNA occurs, the damaged components of DNA are usually removed via repair enzymes and these sections of damaged DNA are then excreted by the body as nucleoside derivatives. 8-hydroxydeoxyguanosine is one such derivative that is excreted by the body in a relatively un-metabolized state. It represents a sensitive biomarker of oxidative damage and potential repair of DNA. As well, it has been suggested 8-OHdg provides insight into the overall oxidative stress state of the body as it relates to damaging free radicals.

However, it should be noted that antioxidant and free radical balance is a fine homeostatic process. Free radicals are essential components of a healthy functioning human organism. They are critical for certain defense mechanisms, as well as likely play a role in the signaling of appropriate self-induced cellular death (apoptosis). It is when this delicate balance between body antioxidant resources and free radical level generation and propagation is disrupted that cellular havoc may ensue, including DNA damage. Deoxyribonucleic acids are particularly susceptible to free radical damage due to their electron dense nature within the double helix.

In examining the results regarding the Eniva VIBE Original Energy & Life™ nutraceutical, this investigation demonstrated a correlation between ingestion of the test material and an associated decrease in the 8-OHdg marker. While not statistically significant due to low participant number, the decreases were well beyond the variance of the methodology used. This result suggests both biologically active antioxidant activity *in vivo* and potential DNA protective properties for the test material, not always being one in the same. When evaluating the test material's formulation, the suggested antioxidant activity is likely related to the broad spectrum phytonutrient formulation which includes fruit, vegetable, aloe vera gel and green tea components. The green tea complex likely is a strong contributor. The formulation also contains verified essential RDA nutrients, some of which are known antioxidants. When evaluating the literature, very few studies have been performed assessing liquid dietary supplements with significant RDA essential nutrient levels in combination with a broad phytonutrient matrix for DNA protective properties via the 8-OHdg assay.

Some limitations of this present study should be noted. Due to the fact this was a pilot trial and only a portion of the enrolled patients had an analysis performed in relation to the test material's impact on 8-OHdg levels, the study was not powered for statistical significance. However, a correlation was able to be identified, as previously discussed. There have also been concerns raised about the validity of methods and labs offering the 8-OHdg assay. This assay requires a high level of technical skill and is able to be performed through various methodologies (ELISA, HPLC, GC-MS). Fortunately, with creatinine standardization, there appears to be only mild variations between labs in their reported results. However, to address this potential problem, we selected Brunswick Labs, an experienced antioxidant research laboratory which regularly performs this assay with skilled personnel.

This study represents promise as to the ability of the Eniva VIBE Original Energy & Life™ nutraceutical to beneficially impact a marker of actual *in vivo* DNA damage. The results presented here correlate with a potential DNA protective mechanism, likely related to known antioxidant properties of certain phytonutrients within the test material's formulation, including a proprietary green tea catechin complex. The investigation demonstrated a correlation between ingestion of the test material and an associated decrease in the 8-OHdg marker. As high value is placed on substances which may possess *in vivo* antioxidant activity and DNA protective properties, additional research is warranted regarding this specific nutraceutical.

Acknowledgements:

We would like to thank Brunswick Labs for their assistance in testing.

Additions & Copyrights:

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