



Toxicological evaluation of oregano oil

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Abstract

Background: The present study evaluated the mutagenic and toxicological potential of a proprietary organic oregano/olive oil mix sold under the trade name Oreganano™. **Methodology:** The test article was investigated for its potential to induce gene mutations according to the plate incorporation and preincubation test by *Salmonella typhimurium* strains TA98, 100, 1535 and 1537 and tester strain *Escherichia coli* WP2uvrA at concentrations of 3.16, 10.0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate with and without metabolic activation. **Results:** Although toxic effects were noted in all tester strains, no biologically relevant increases in revertant colony numbers of any of the five tester strains were observed. Therefore, Oreganano™ did not cause gene mutations by base pair changes or frame shifts in the genome of the strains used and were considered to be non-mutagenic in the bacterial reverse mutation assay. In a 14-day feeding study of dietary levels of 0, 1.25, 2.5 and 5.0% in Sprague-Dawley rats, there were no adverse clinical, body weight, food consumption or macroscopic changes associated with the administration of Oreganano™. Body weight gain and food consumption was statistically reduced over the 14 days in both male and female animals; however, body weight and food efficiency was unaffected. **Conclusion:** There were no macroscopic findings attributable to test article administration. Therefore, the no-observed adverse-effect level (NOAEL) was 5.0% in the diet, the highest dose tested and Oreganano™ is considered safe and suitable for consumption.

Keywords: Toxicology, oregano, genotoxicity, mutagenic

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Introduction

The health benefits of certain herbal oil extracts has been claimed for centuries. Oil of oregano contains active ingredients that have been documented as effective against microbial agents such as bacteria, yeast, fungi and virus (**Sokmen et al., 2004**), including *Escherichia coli* O:157:H7, *Listeria monocytogenes*, *Salmonella typhimurium* and *Staphylococcus aureus* among others (**Elgayyar et al., 2001**), exerting its effects by destruction of microbial cell membranes (**Nostro et al., 2007**).

Further, antioxidant (**Martinez-Tome et al., 2001**), anti-inflammatory (**Ocana-Fuentes et al., 2010**), hepatoprotection and anti-tumorigenic properties have been attributed to this carvacrol- and thymol-containing seasoning agent (**Nostro et al., 2007**). The present study evaluates the safety of oregano oil under controlled conditions according to universally accepted toxicological guidelines. A commercially available oregano oil, Oreganano™, containing a proprietary mixture of natural *Origanum vulgare*, carvacrol (36 to 80%) and extra virgin olive oil (Factors Group, Coquitlam, BC, Canada) with a purity by certificate of analysis of 100% for the organic oregano oil (27.5 to 30 mg) and organic extra virgin olive oil (120 mg), respectively, has been newly tested for its safety both in a bacterial reverse mutation assay (Ames test) and a 14-day feeding study in Sprague-Dawley rats under Organization for Economic Co-operation and Development (OECD) guidelines. These studies, conducted at Bio-service Scientific Laboratories (BSL) GmbH in Planegg, Germany (Ames) and Eurofins Product Safety Labs (14-day study), were in compliance with OECD Principles of Good Laboratory Practices (ENV/MC/CHEM (98) 17 OECD, Paris, 1998). The studies were conducted in conformance with the OECD guidelines for Testing of Chemicals and Food Ingredients, Section 4, No. 471: Bacterial Reverse Mutation Test, and Part 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents (2008) and US FDA Toxicological Principles for the Safety Assessment of Food Ingredients, Redbook 2000, IV.C. 4 a. Short-Term Toxicity Studies with Rodents (2003). All work undertaken by the testing laboratory was in accordance with the most recent Guide for the Care and Use of Laboratory Animals (**National Research Council, 2011**), and according to AAALAC standards and accreditation.

Experimental Methods

Bacterial Reverse Mutation Assay

The test item Oreganano™ was investigated for its potential to induce gene mutations according to the plate incorporation test (Experiment I) and the preincubation test (Experiment II) (**Ames et al., 1973; Maron and Ames, 1983**) using *S.*

typhimurium strains TA 98, TA 100, TA 1535, TA 1537 and tester strain E. coli WP2 uvrA with and without metabolic activation in triplicate in the following concentrations: Experiment I: 10.0, 31.6, 100, 316, 1000 2500 and 5000 µg/plate. Experiment II: 3.16, 10.0, 31.6, 100, 316, 1000, 2500, and 5000 µg/plate. Controls (positive, sodium azide, 4-nitro-o-phenylene-diamine, methyl methane sulfonate, 2-aminoanthracene and negative, distilled water) were tested for validity of the assay. Data were evaluated for cytotoxicity (diminution of the background lawn or a reduction in the number of revertants), and mutagenicity (mutation factor = mean revertant value of test article/ mean revertants of control).

Fourteen Days Dietary Toxicity Study

A 14-day dietary toxicity study was conducted in CRL SpragueDawley CD® IGS rats to determine the potential of Oreganano™ to produce toxicity. Forty healthy rats (20 males and 20 females) were selected for the test and equally distributed into four groups (5 males and 5 females per group). Dietary levels of 1.25, 2.5 and 5.0% of Oreganano™, as well as a basal diet control (0%), were selected for the test. The test and control diets were presented to their respective groups on day 0 of the study. Additional diet was provided as needed throughout the study to insure ad libitum feeding. The animals were observed daily for viability, signs of gross toxicity and behavioral changes and on days 0, 7 and 14 for a battery of detailed observations. Body weights were recorded during the acclimation period including prior to test product introduction (day 0), and on days 3, 7, 11, and 14 prior to terminal sacrifice. Individual food consumption was also recorded to coincide with body weight measurements. Gross necropsies were performed on all animals. Male and female rats were evaluated separately. Mean and standard deviations were calculated for all body weight, mean daily body weight gain, mean daily food consumption and mean daily food efficiency. Data within groups was evaluated for homogeneity of variances and normality by Bartlett's test (Bartlett, 1937), analysis of variance (ANOVA), (Dunnett, 1964; 1980) in Provantis™ version 8.4.2.0, Tables and Statistics version 8.4.2.0, In stem LSS, Staffordshire UK; INSTAT Biostatistics, Graph Pad Software, San Diego, CA.

Results

Bacterial Reverse Mutation Test

The test item Oreganano™ was investigated for its potential to induce gene mutations according to the plate incorporation test (Experiment I) and the preincubation test (Experiment II) using S. typhimurium strains TA 98, TA 100, TA 1535, TA 1537 and tester strain E. coli WP2 uvrA.

In two independent experiments, several concentrations of the test item were used. Each assay was conducted with and without metabolic activation. The concentrations, including the controls, were tested in triplicate. The following concentrations of the test item were prepared and used in the experiments: Experiment I: 10.0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate. Experiment II: 3.16, 10.0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate. No precipitation of the test item was observed in any tester strain used in Experiments I and II (with and without metabolic activation). Toxic effects of the test item were noted in all tester strains used in Experiments I and II: In Experiment I (**Table 1**), toxic effects of the test item were observed at concentrations of 1000 µg/plate and higher (without metabolic activation) and at concentrations of 2500 µg/plate and higher (with metabolic activation), depending on the particular tester strain. In Experiment II (**Table 2**), toxic effects of the test item were noted at concentrations of 316 µg/plate and higher (without metabolic activation) and at concentrations of 1000 µg/plate and higher (with metabolic activation), depending on the particular tester strain. Despite the toxic effects of the test product to the bacterial strains (which may be indicative of anti-microbial activity), no biologically relevant increases in revertant colony numbers of any of the five tester strains were observed following treatment with Oreganano™ at any concentration level, neither in the presence nor absence of metabolic activation in Experiments I and II as per the criteria for guideline validity. The reference mutagens induced a distinct increase of revertant colonies indicating the validity of the experiments.

Fourteen Days Dietary Study

In a 14-day ad libitum feeding study, Oreganano™, as received and in the diet, was considered stable and to be both homogeneously distributed in the diets and at the targeted concentrations throughout the study. Diet preparations and neat test substance were not analyzed as part of this subacute dietary study and preparations were mixed as is, from the manufacturer, for both test and control diets. There were no test substance-related or other mortalities. There were no adverse clinical observations associated with Oreganano™ product. Mean body weights for male and female rats at 1.25, 2.5, and 5.0% were considered comparable with control values throughout the study (**Figure 1**). Decreases from control were found at 5.0% in male body weight from days 3 to 11 and in female body weight on day 3. Body weight returned to comparable-to-control levels by the end of the study in both males and females. Mean daily body weight gain for male and female rats at 1.25, 2.5, and 5.0% was generally comparable with control values with the exception of decreases in male and female body weight gain at 5.0% for days 0 to 3 and overall (days 0 to 14) in males and females, and at 1.25% from days 3 to 7 in males.

Table 1. Results of a plate-incorporation test (Experiment I) of Oreganano™ on *S. typhimurium*/E. coli in the presence (+) and absence (-) of S9 mixture.

Test article	Dose level (µg/plate)	S9 mix	Revertant colony counts (mean) ^a									
			TA98	Mutation factor	TA100	Mutation factor	TA 1535	Mutation factor	TA 1537	Mutation factor	WP2 <i>uvrA</i>	Mutation factor
Oreganano™	0 ^b		22	1.6	105	1.1	5	0.8	9	1.5	51	1.2
	10.0		25	1.8	94	1.0	3	0.5	9	1.6	47	1.1
	31.6		24	1.7	102	1.1	5	0.8	6	1.1	42	1.0
	100		23	1.7	92	1.0	4	0.6	7	1.1	39	0.9
	316		18	1.3	94	1.0	4	0.7	6	1.0	45	1.0
	1000		15	1.1	85	0.9	2	0.3	2	0.3	44	1.0
	2500	-	14	1.0	40	0.4	0	0.0	1	0.2	33	0.8
	5000		8	0.6	0	0.0	0	0.0	0	0.0	23	0.5
	10				975	10.3	202	30.3	95	15.8		
	1µl										529	12.1
4-NOPD ^c	10/40		432	30.9								
DMSO ^d	0		14	1.0	94	1.0	7	1.0	6	1.0	44	1.0
Oreganano™	0 ^b		26	1.2	103	1.1	5	0.6	7	1.3	59	1.0
	10.0		29	1.3	104	1.1	7	0.9	9	1.6	49	0.8
	31.6		28	1.3	110	1.1	8	1.0	5	1.0	43	0.8
	100		29	1.3	107	1.1	4	0.5	6	1.1	53	0.9
	316	+	25	1.2	117	1.2	6	0.8	7	1.4	54	0.9
	1000		30	1.4	112	1.1	5	0.7	10	1.8	60	1.0
	2500		21	1.0	80	0.8	2	0.3	2	0.4	41	0.7
	5000		18	0.8	0	0.0	0	0.0	2	0.4	39	0.7
	2.5/10		1595	73.6	2516	25.8	75	9.3	118	22.2	175	3.0
	DMSO ^d	0		22	1.0	97	1.0	8	1.0	5	1.0	57

aMean of replicate (3) plates. **b**Negative (solvent control): distilled water; **c**Positive control agents: NaN₃ = sodium azide; 4-NOPD = 4-nitro-o-phenylene-diamine; 2-AA = 2-aminoanthracene; **MMS** = methyl methane sulfonate. **d**Solvent control: DMSO = dimethyl sulfoxide.

Table 2. Results of a preincubation test (Experiment II) of Oreganano™ on *S. typhimurium*/E. coli in the presence (+) and absence (-) of S9 mixture.

Test article	Dose level (µg/plate)	S9 mix	Revertant colony counts (mean) ^a									
			TA 98	Mutation factor	TA 100	Mutation factor	TA 1535	Mutation factor	TA 1537	Mutation factor	WP2 <i>uvrA</i>	Mutation factor
Oreganano™	0 ^b		28	1.1	90	1.2	10	1.0	6	1.0	47	1.1
	3.16		30	1.1	80	1.1	7	0.6	7	1.0	39	0.9
	10.0		19	0.7	69	0.9	14	1.4	7	1.1	36	0.9
	31.6		21	0.8	78	1.0	9	0.9	5	0.7	43	1.0
	100		24	0.9	78	1.0	12	1.1	9	1.3	39	0.9
	316		24	0.9	63	0.9	8	0.8	4	0.6	34	0.8
	1000		15	0.6	26	0.3	12	1.2	2	0.3	27	0.6
	2500	-	11	0.4	0	0.0	6	0.6	0	0.0	0	0.0
	5000		4	0.1	0	0.0	0	0.0	2	0.4	0	0.0
					1015	13.7	1093	105.7				
NaN ₃ ^c	10											
MMS	1µL									548	12.9	
4-NOPD ^e	10/40		722	27.4				121	18.1			
DMSO ^d	0		26	1.0	74	1.0	10	1.0	7	1.0	42	1.0
Oreganano™	0 ^b		29	0.9	82	1.1	8	1.5	9	1.2	47	1.2
	3.16		32	1.0	87	1.2	8	1.0	8	1.0	32	0.8
	10.0		29	0.9	93	1.3	7	1.0	8	1.1	38	0.9
	31.6		30	1.0	92	1.2	8	1.1	4	0.5	43	1.1
	100	+	33	1.1	99	1.3	7	0.9	7	1.0	48	1.2
	316		26	0.8	91	1.2	10	1.3	10	1.3	61	1.5
	1000		25	0.8	83	1.1	12	1.6	4	0.6	47	1.2
	2500		1	0.0	0	0.0	4	0.5	0	0.0	0	0.0
	5000		0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
				1387	44.7	1028	13.8	73	14.6	86	11.7	194
2AA ^c	2.5/10											
DMSO ^d	0		31	1.0	75	1.0	8	1.0	7	1.0	41	1.0

^aMean of replicate (3) plates. ^bNegative (solvent) control: Distilled water. ^cPositive control agents: NaN₃ = sodium azide; 4-NOPD = 4-nitro-o-phenylene-diamine; 2-AA = 2-aminoanthracene; MMS = methyl methane sulfonate. ^dSolvent control: DMSO = dimethyl sulfoxide.

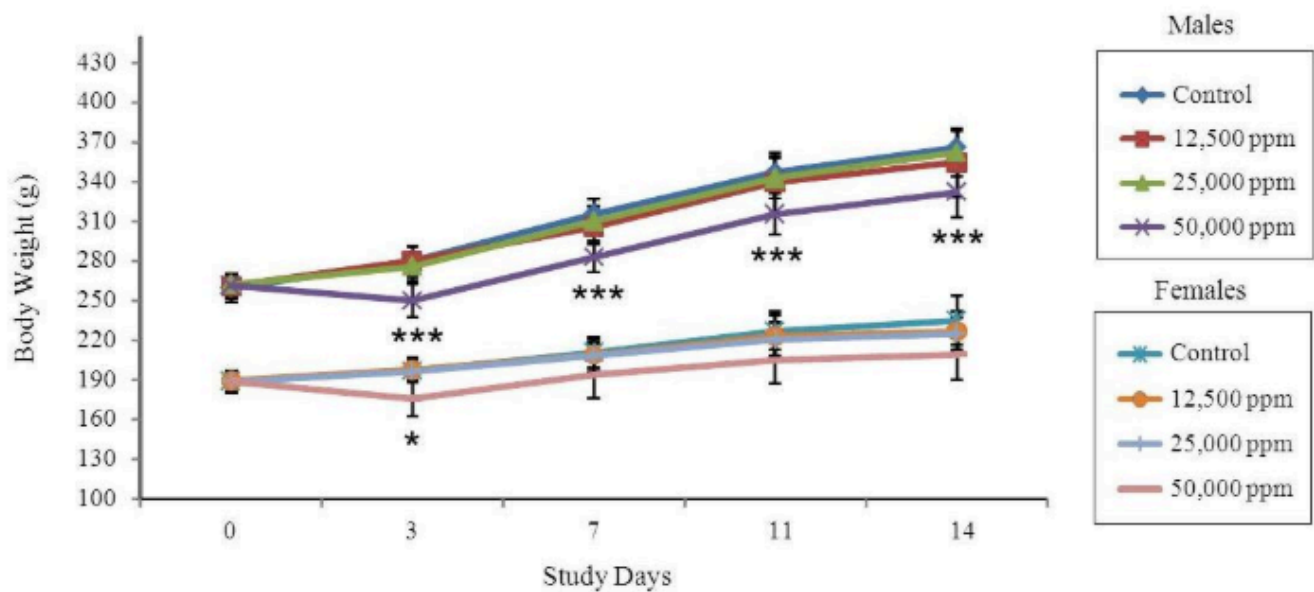


Figure 1. Mean body weight

Mean daily food consumption for male and female rats at 1.25, 2.5 and 5.0% were generally comparable with control values throughout the study (**Figure 2**) with reductions at 2.5% (days 0 to 3) and 5.0% (days 0 to 7 and overall, 0 to 14) in males, and at 1.25% (days 11 to 14), 2.5% (days 0 to 3, 11 to 14 and 0 to 14) and at 5.0% (days 0 to 3, 11 to 14 and 0 to 14) in females. Overall (days 0 to 14) and mean food efficiency for male and female rats at 1.25, 2.5, and 5.0% were generally comparable with control values with the exception of reductions at days 0 to 3 in males and females, and increases in females on days 3 to 7 in females all at the 5.0% dietary level. Although significant changes from control in body weight gain and food consumption at 5% in males and females persisted overall (days 0 to 14), these decreases were considered the non-adverse residual result of losses extending from the beginning of the study, as animals of both genders recovered much of their loss as the study progressed. There were no macroscopic observations at necropsy associated with the ad libitum dietary intake of Oreganano™ at the levels tested. The mean overall (days 0 to 14) daily intake of Oreganano™ in male rats fed dietary concentrations of 1.25, 2.5, and 5.0% was 0, 1137.9, 2219.2, and 4092.0 mg/kg/day, respectively. For the same dietary concentrations, the mean overall daily intake of Oreganano™ in female rats was 0, 1123.7, 2134.6, and 4041.8 mg/kg/day, respectively. Therefore, the animals were considered to have received the targeted exposures with a no-adverse-effect level of 5.0% in the diet. Clinically, the recommended daily dose of Oreganano™ is approximately 0.5 mg/kg (30 mg per 60 kg human), making the highest dose tested in the present study over 8130 times suggested human intake.

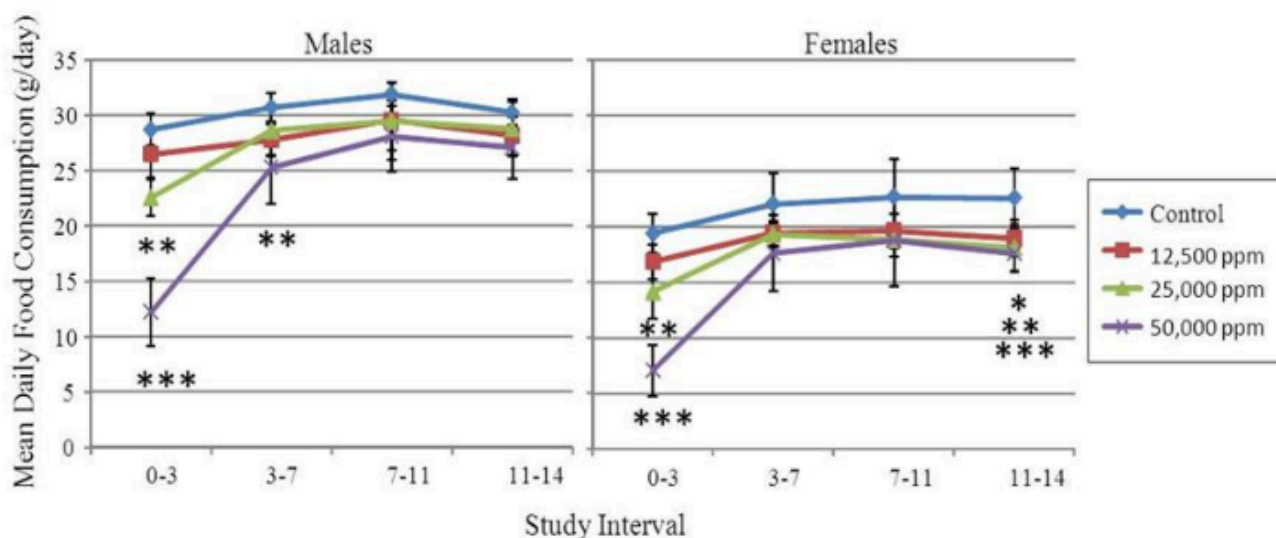


Figure 2. Mean food consumption.

Conclusion

The present study examines the potential for oregano oil, long an herbal remedy, to produce toxicity when administered in the diet to young adult rats. The decreases in body weight (males), body weight gain and food consumption are attributed to initial effects at the introduction of the test article possibly owing to the initial acclimation and/or pungent odor. As such, the administration of the test substance at the highest dose appeared to notably reduce food consumption without lasting adverse effect to the animals as indicated by the maintenance of body weight and slowed reductions in body weight gain as the study progressed. Numerous studies have reported on the *in vitro* and *in vivo* evidence for the effects of flavonoids, specifically, luteolin, (Lopez-Lazaro, 2009) to which the botanical oregano (*O. vulgare*) belongs. Biological effects consist of antioxidant, anti-inflammatory, antimicrobial, anticancer, antiallergy and cardiovascular protective activities for which there is evidence both preclinically and clinically (Lopez-Lazaro, 2009). Although, the true influence of oregano oil on human health requires long-term, controlled clinical studies, recent studies in mammals and humans report on the beneficial effects of lipid profiles (Ozdemir et al., 2008), angiogenic (Loboda et al, 2005), and antioxidant (El-Ashmawy et al., 2005) activities and wound healing (Al-Howiriny et al., 2009; Ragi et al., 2011) among others (Dundar et al., 2008; Force et al., 2000).

In the present study, the dietary administration of Oregano™, a plant food concentrate, was well tolerated by rats up to a concentration of 5.0% in the diet.

Dietary supplements over this level have the potential to adversely influence nutritional intake (**Borzelleca, 1992**). Based on the experimental conditions of this mutagenicity and 14 days test and the toxicological endpoints evaluated, these results indicate that Oreganano™ did not cause gene mutations by base pair changes or frame shifts in the genome of the tester strains used. Therefore, Oreganano™ is considered to be non-mutagenic in the bacterial reverse mutation (Ames) assay. Neither did the subacute dietary administration of Oreganano™ result in any adverse toxicological effects. Therefore, the use of appropriate levels of Oreganano™ is considered safe. A study of longer duration is appropriate to confirm and extend these results.

Credit Authorship Contribution Statement

All authors equally contributed to Conceptualization, Methodology, Formal Analysis, Investigation, Writing and Visualization, under supervision of the corresponding author.

Acknowledgement

The Oreganano™ product used in the present study is a commercial grade product obtained from the producer (Factors Group of Nutritional Companies Inc, BC, Canada). Oreganano™ is a registered trademark of the Factors Group (BC, Canada). Simon Wood received financial support from Factors Group of Nutritional Companies, Inc. (Canada), owned by R. Gahler which retains an interest in Oreganano™.

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Conflict of Interest

The authors declare that there was no conflict of interest from preparation to publication of this manuscript.

Ethics Approval

The studies were conducted in conformance with the OECD guidelines for Testing of Chemicals and Food Ingredients, Section 4, No. 471: Bacterial Reverse Mutation Test, and Part 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents (2008) and US FDA Toxicological Principles for the Safety Assessment of Food Ingredients, Redbook 2000, IV.C. 4 a. Short-Term Toxicity Studies with Rodents (2003). All work undertaken by the testing laboratory was in accordance with the most recent Guide for the Care and Use of Laboratory Animals (National Research Council, 2011), and according to AAALAC standards and accreditation.

Participant Consent

This study did not require any human participation for consent.

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