Genetic Toxicology Studies of SALATRIM Structured Triacylglycerols. 1. Lack of Mutagenicity in the *Salmonella*/Microsome Reverse Mutation Assay

Johnnie R. Hayes*,† and Edward S. Riccio‡

RJR Nabisco, Bowman Gray Technical Center, Reynolds Boulevard, Winston-Salem, North Carolina 27102, and SRI International, 333 Ravenswood Avenue, Menlo Park, California 94025

SALATRIM triacylglycerols differ from other fats in their ratio of short-chain fatty acids to long-chain fatty acids and in that the predominant long-chain fatty acid is stearic acid. Caloric availability studies in rats indicate SALATRIM fats yield 4.5–6 kcal/g compared to 9.0 kcal/g to for corn oil. Structure/activity relationships predict SALATRIM would have no mutagenicity in the *Salmonella*/microsome reverse mutation assay (Ames assay). To test this hypothesis, the mutagenic potential of six SALATRIM fats, at doses as high a 1000 μg/plate, was determined using five *Salmonella* strains with and without metabolic activation. Concurrent positive control compounds produced the expected positive responses. Corn oil and solvent controls were negative. SALATRIM fats were without cytotoxic and mutagenic potential in the five tester strains with and without metabolic activation. The hypothesis that SALATRIM triacylglycerols are not mutagenic based upon structure/activity relationships was confirmed.

INTRODUCTION

SALATRIM is a family of structured triacylglycerols. They are typical fats, similar to fats that occur in the diet, but unique because of their distribution and content of short-chain fatty acids (SCFA) and the occurrence of stearic acid as the predominant long-chain fatty acid (LCFA). Members of the SALATRIM family are produced by interesterification among triacetin, tripropionin, tributyrin, and hydrogenated vegetable fats, such as hydrogenated canola oil, that contain high concentrations of stearic acid. Since SALATRIM fats are produced from dietary fats, they are composed of mixed triacylglycerols with a preponderance of acetic, butyric, propionic, and stearic acids esterified to the glycerol backbone. Small quantities of other fatty acids such as palmitic and oleic acid, among others, may occur as components of the triacylglycerols. Caloric availability studies in rats have shown that SALATRIM structured triacylglycerols yield 4.5–6 kcal/g compared to 9 kcal/g for corn oil (Finley et al., 1994). Members of the SALATRIM family have different chemical and physical properties based on the ratio of short- to long-chain fatty acids and the specific fatty acids esterified to the glycerol backbone.

In vitro genetic toxicology assays are used to determine the potential of a test material to produce mutations and genetic changes. If appropriate assays, careful methodology, and proper controls are used, genetic toxicology assays are useful tools to screen for potential biological activity. Currently, the human health relevance of these assays is not completely understood. The *Salmonella*/mammalian microsome reverse mutation assay, also known as the Ames assay, is a generally used short-term assay to determine the potential mutagenicity of a test material. This assay measures the ability of a chemical or chemical mixture to produce mutations in specific strains of the bacteria *Salmonella typhimurium*.

Review of the scientific literature and structure/activity relationships indicate no evidence that members of the SALATRIM family should produce either mutagenic activity or changes in DNA. Various studies have indicated that the triacylglycerols in edible oils have little or no potential for mutagenic activity under normal conditions (Hageman et al., 1990, 1991; Kensese et al., 1989; MacGregor et al., 1985; Taylor et al., 1983; Van Gastel et al., 1984). However, highly oxidized edible fats containing unsaturated fatty acids and fats subjected to high frying temperatures for prolonged periods may contain fatty acid hydroperoxides. These hydroperoxides may have slight mutagenic activity with specific *Salmonella* tester strains (Hageman et al., 1990, 1991; Kensese, 1989; MacGregor, 1985). The predominant LCFA in SALATRIM, stearic acid, is saturated, as are the SCFA. Therefore, SALATRIM fats should be less susceptible to peroxidation than fats containing high levels of unsaturated fatty acids. The members of the SALATRIM family tested in this study neither had high peroxide levels nor had they been abused at high temperatures. This would predict that SALATRIM triacylglycerols should not possess mutagenic activity. The purpose of the study was to confirm this hypothesis. This study also tests the hypothesis that neither the specific fatty acids esterified to glycerol nor the precursor fat used to produce SALATRIM fats alters the lack of genetic toxicity of SALATRIM.

MATERIALS AND METHODS

Reagents. Acetone was the carrier (solvent) for the members of the SALATRIM family and corn oil. Dimethyl sulfoxide (DMSO) was the carrier for the positive controls. Both carriers were obtained from Mallinckrodt, Paris, KY. The positive control chemicals were 9-aminoacridine hydrochloride (9-AA) (CAS 134-50-9) and 2-nitrofluorene (2-NF) (CAS 607-57-8) obtained from Aldrich Chemical Co., Milwaukee, WI, and 2-anthramine (2-AA) (CAS 613-13-8) and sodium azide (SA) (CAS 26628-22-8) obtained from Sigma Chemical Co., St. Louis, MO. The Aroclor 1254-induced rat liver S-9 preparation (from Sprague-Dawley rats) was obtained from Molecular Toxicology, Inc., Annapolis, MD. The *S. typhimurium* LT2 tester strains were TA98, TA100, TA1535, TA1537, and TA1538 obtained from Dr. Bruce Ames, University of California at Berkeley.

Determination of SALATRIM Fatty Acid Composition. Members of the SALATRIM family were produced and provided by the Nabisco Foods Group, East Hanover, NJ. The members

* Author to whom correspondence should be addressed.
† RJR Nabisco.
‡ SRI International.

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Table 1. Experimental Design for the *Salmonella*/Mammalian Microsome Reverse Mutation Assays of SALATRIM Structured Triacylglycerols[a,b]

tester strain	metabolic activation	test fat high dose (μg/plate)	solvent control for test fat	positive control +S-9	positive control -S-9	solvent control for positive controls
TA98	±S-9	1000	acetone	2-AA	2-NF	DMSO
TA100	±S-9	1000	acetone	2-AA	SA	DMSO
TA1535	±S-9	1000	acetone	2-AA	SA	DMSO
TA1537	±S-9	1000	acetone	2-AA	9-AA	DMSO
TA1538	±S-9	1000	acetone	2-AA	2-NF	DMSO

[a] Abbreviations: 2-AA, 2-anthramine; 2-NF, 2-nitrofluorene; SA, sodium azide; 9-AA, 9-aminoacridine; DMSO, dimethyl sulfoxide; S-9, the 9000-g supernatant from homogenized rat livers treated with Aroclor 1254. [b] Concurrent stability controls were done with each study and included the test fat and the S-9 preparation and buffer, each done separately.

of the SALATRIM family of structured triacylglycerols investigated in this study were SALATRIM 4CA lot A006, SALATRIM 23CA lot A014, SALATRIM 32CA lot A015, SALATRIM 234CA lot A019, SALATRIM 234CS lot A018, and SALATRIM 23SO lot A020. Fatty acid composition of the SALATRIM fats was determined at EPL Bio-Analytical Services, Inc., Decatur, IL. Fatty acid compositon was obtained by saponification of the triacylglycerol mixture with methanolic sodium hydroxide followed by esterification with methanolic boron trifluoride. Methyl esters of the LCFA were quantitated by gas chromatography. SCFA were quantitated by direct gas chromatography of the saponified fat. Standard curves were constructed by bracketing the concentration level of the analyte with quantitation based upon peak height. Complete chemical characterization of these fats is presented elsewhere (Softly et al., 1994). The corn oil used as a control material was a commercial brand. The test fats were shipped frozen on solid carbon dioxide to SRI International, Menlo Park, CA, and maintained at −20 °C at the testing laboratory until use.

Study Design. The study design is presented in Table 1.

Experimental Procedures. The test fats were thawed at room temperature overnight and then heated to 55 °C until an apparent homogeneous mixture was obtained. Aliquots were weighed and frozen at −20 °C. For each assay, a weighed aliquot was thawed at room temperature and then heated to 50–55 °C. Acetone was added to produce concentrations that would deliver the fat at doses of 62.5, 125, 250, 500, 750, and 1000 μg/plate. The dosing solution was cooled to room temperature before addition to the preincubation mixture. SALATRIM 4CA lot A006 formed a fine suspension in all dosing solutions and was vortexed to assure homogeneity. No precipitate was noted on the plates after addition of the dosing solution. SALATRIM 23CA lot A014 was soluble at all of the dosing concentrations. However, a slight turbidity was observed at all doses after the dosing solution was added to the preincubation mixtures in the absence of metabolic activation. With metabolic activation, a slight increase in turbidity was noted at the 750 and 1000 μg/plate dose levels. SALATRIM 32CA lot A015, SALATRIM 234CA lot A019, and SALATRIM 234CS lot A018 behaved like SALATRIM 23CA lot A014 except an increase in turbidity could not be detected when metabolic activation was used because of the turbidity of the S-9 preparation. SALATRIM 23SO lot A020 behaved similarly to SALATRIM 23CA lot A014 except the slight increase in turbidity at 750 and 1000 μg/plate with metabolic activation could be detected in the 4% S-9 assay but not in the 10% S-9 assay. Also, after 35–45 min, a fine precipitate formed in the 1000 μg/plate dosing solution. This suspension was vortexed to ensure homogeneity before it was added to the assay with metabolic activation. These differences in behavior in the dosing and assay mixtures probably result from the different physical characteristics of individual members of the SALATRIM family. Corn oil produced a slight turbidity at all doses when added to the preincubation mixtures without metabolic activation. In the presence of S-9, no increase in turbidity could be detected above the background turbidity. Precipitation in the assay mixtures indicates that the doses were beyond the limits of solubility.

The *Salmonella*/microsome reverse mutation assay has been described in detail by Ames et al. (1975), modified by Yahagi et al. (1975) and revised by Maron and Ames (1983). Because the preincubation assay is of equal or greater sensitivity than the plate incorporation assay (Maron and Ames, 1983; Yahagi and Ames, 1977), it was used in this studies. Basically, 0.50 mL of either the metabolic activation mixture (+S-9) or buffer (−S-9) was added to a test tube in a 37 °C heating block. This was followed by 0.05 mL of the specific tester strain (~10^8 bacteria) followed by the appropriate amount of test fat in acetone. The mixture was allowed to incubate with agitation for 20 min. Then, 2 mL of 0.6% agar containing 0.6% NaCl, 0.05 mM biotin and 0.05 mM histidine was added, stirred, and poured onto plates containing 25 mL of minimal glucose agar. The plates were incubated for 48 h at 37 °C. Revertant colonies per plate were determined by automated counting.

A preliminary assay was conducted with strain TA100 with and without metabolic activation to determine an appropriate dose range and to determine potential cytotoxicity. After the range-finding study, the fats were assayed twice using the five tester strains, three plates per dose level, with and without metabolic activation. During the first assay, a metabolic activation system using 4% S-9 was used. If the test fat was negative in the assay, the second assay used a metabolic activation system with 10% S-9. The dose range used in these studies was 0, 62.5, 125, 250, 500, 750, and 1000 μg/plate. The results of the second assay using 10% S-9 are reported here because no differences were detected between the assays at 4% and 10% S-9.

Analysis of Results. The data are presented as the mean and standard deviation for histidine revertants per plate for each dose level. A fat was considered a mutagen if it induced a reproducible dose-related increase in the mean number of revertants in one or more strains. The increase would have to occur for at least three consecutive dose levels and, at its highest point, be at least twice the mean solvent control value.

RESULTS

Fatty Acid Composition of SALATRIM Fats. The SCFA and stearic acid composition of the various SALATRIM fats used in this study is presented in Table 2. The vast majority of the triacylglycerols in these fats contain at least one stearic acid molecule esterified to glycerol. SALATRIM 4CA lot A006 was produced from tributyrin and hydrogenated canola oil, resulting in most of the triacylglycerols having one or more butyric acid molecules. SALATRIM 23CA lot A014 was produced from triacetin, tripropionin, and hydrogenated canola oil, with triacetin being the predominant source of the SCFA. This results in most of the triacylglycerols having acetic acid esterified to glycerol. SALATRIM 32CA lot A015 was produced from triacetin, tripropionin, and hydrogenated canola oil, with tripropionin predominating. This results in most of the triacylglycerols having propionic acid esterified to glycerol. SALATRIM 234CA lot A019 was produced from triacetin, tripropionin, tributyrin, and hydrogenated canola oil. This results in a relatively equal and random distribution of acetic, propionic, and butyric acids among the SCFA esterified to glycerol. SALATRIM 234CS lot A018 was produced from triacetin, tributyrin, tripropionin, and hydrogenated cottonseed oil, resulting in a slightly different distribution of LCFA. Stearic acid is still the predominant LCFA in SALATRIM 234CS lot

Table 2. SALATRIM Fats Assayed for Potential Mutagenicity in the Ames Assay and Their Short-Chain Fatty Acid and Stearic Acid Contents[a]

SALATRIM fat	interesterification reactants	wt % SCFA[b]			wt % of stearic acid
		acetic	propionic	butyric	
4CA lot A006	tributyrin, hydrogenated canola oil	<0.1	<0.1	21.0	58.0
23CA lot A014	triacetin, tripropionin, hydrogenated canola oil	21.1	2.6	<0.4	57.0
32CA lot A015	triacetin, tripropionin, hydrogenated canola oil	1.7	21.0	<0.4	51.6
234CA lot A019	triacetin, tripropionin, tributyrin, hydrogenated canola oil	8.2	9.1	11.7	46.8
234CS lot A018	triacetin, tripropionin, tributyrin, hydrogenated cottonseed oil	6.8	7.8	10.7	39.8
23SO lot A020	triacetin, tripropionin, hydrogenated soy oil	21.3	2.8	<0.2	53.1

[a] Data represent the mean of triplicate assays. [b] SCFA, short-chain fatty acid.

Table 3. Ames Assay for Corn Oil Using Five *Salmonella* Strains with and without Metabolic Activation

dose (μg/plate)	revertants per plate[a]									
	TA98		TA100		TA1535		TA1537		TA1538	
	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9
0	23 ± 3	40 ± 4	142 ± 6	155 ± 5	15 ± 3	18 ± 3	6 ± 1	8 ± 5	30 ± 2	33 ± 6
62.5	25 ± 4	34 ± 4	157 ± 10	166 ± 7	16 ± 1	15 ± 2	9 ± 6	9 ± 1	23 ± 3	26 ± 6
125	28 ± 2	33 ± 6	143 ± 23	150 ± 12	19 ± 5	11 ± 3	7 ± 1	9 ± 3	27 ± 6	27 ± 1
250	24 ± 4	33 ± 6	148 ± 12	138 ± 21	17 ± 3	17 ± 2	10 ± 3	6 ± 1	20 ± 11	33 ± 8
500	23 ± 7	33 ± 11	151 ± 6	148 ± 10	18 ± 7	14 ± 4	8 ± 4	10 ± 7	26 ± 8	32 ± 5
750	25 ± 5	34 ± 7	149 ± 9	145 ± 13	14 ± 4	15 ± 5	7 ± 2	9 ± 3	19 ± 5	34 ± 2
1000	22 ± 5	37 ± 7	148 ± 20	145 ± 11	18 ± 8	16 ± 7	10 ± 2	7 ± 2	24 ± 6	40 ± 18

[a] Data represent the mean ± standard deviation for triplicate plates.

Table 4. Ames Assay for SALATRIM 4CA Lot A006 Using Five *Salmonella* Strains with and without Metabolic Activation

dose (μg/plate)	revertants per plate[a]									
	TA98		TA100		TA1535		TA1537		TA1538	
	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9
0	22 ± 2	26 ± 8	124 ± 16	135 ± 16	9 ± 2	13 ± 3	7 ± 2	9 ± 7	20 ± 7	22 ± 4
62.5	16 ± 3	27 ± 2	130 ± 4	150 ± 3	7 ± 3	12 ± 4	9 ± 4	14 ± 1	16 ± 1	19 ± 7
125	16 ± 3	29 ± 11	123 ± 17	148 ± 10	10 ± 2	9 ± 1	6 ± 2	5 ± 2	14 ± 2	22 ± 3
250	18 ± 3	24 ± 6	143 ± 10	150 ± 12	9 ± 2	10 ± 4	6 ± 2	8 ± 3	17 ± 1	23 ± 6
500	22 ± 6	26 ± 5	126 ± 12	148 ± 24	11 ± 3	11 ± 4	8 ± 1	6 ± 2	12 ± 5	20 ± 5
750	23 ± 4	27 ± 6	139 ± 8	157 ± 13	10 ± 2	10 ± 4	6 ± 6	9 ± 4	19 ± 8	23 ± 8
1000	17 ± 4	21 ± 5	127 ± 12	144 ± 18	10 ± 2	11 ± 5	5 ± 3	10 ± 6	12 ± 4	20 ± 5

[a] Data represent the mean ± standard deviation for triplicate plates.

A018. SALATRIM 23SO lot A020 was produced from triacetin, tripropionin, and hydrogenated soy oil, with triacetin predominating. Therefore, SALATRIM 23SO lot A020 was similar to SALATRIM 23CA lot A014, except it was made with hydrogenated soy oil instead of hydrogenated canola oil.

Concurrent Positive Controls. To ensure the proper sensitivity of the assay, concurrent positive controls were used. Under the conditions of the assay, strain TA98 was shown to be sensitive to the mutagenicity of 2-AA with metabolic activation and to 2-NF without metabolic activation. TA100 demonstrated positive responses to 2-AA with metabolic activation and to SA without metabolic activation. Strain TA1535 was positive with 2-AA with metabolic activation and with SA without metabolic activation. Strain TA1537 was sensitive to the mutagenicity of 2-AA with metabolic activation and to 9-AA without metabolic activation. TA1538 demonstrated positive responses to 2-AA with metabolic activation and to 2-NF without metabolic activation. These data validate the known strain sensitivity to mutagens requiring metabolic activation and to direct-acting mutagens under the conditions of the assays.

Assay with Corn Oil. Data from the assay of corn oil using five tester strains of *Salmonella* with and without metabolic activation are shown in Table 3. Over a dose range of 0–1000 μg/plate, no tester strain showed an increase in revertants per plate in a dose-related manner. There were no dose-related trends in any of the data. None of the data meet the requirements for a positive response. There was no evidence of bacterial cytotoxicity at any dose.

Assay with SALATRIM 4CA Lot A006. Table 4 presents the data from the assay with SALATRIM 4CA lot A006 in five *Salmonella* tester strains with and without metabolic activation. As was the case with corn oil, no strain produced results that meet the criteria for a positive response. There were no dose-dependent trends in any of the data. Indeed, there was not a biologically significant difference between the solvent control and any dose level for any tester strain. No indication of bacterial cytotoxicity was evident at any dose.

Assay with SALATRIM 23CA Lot A014. Results with SALATRIM 23CA lot A014 are presented in Table 5. As with the high-butyrate SALATRIM 4CA lot A006, this high-acetate SALATRIM did not produce evidence of potential mutagenicity. There was also no evidence of cytotoxicity in the assay.

Assay with SALATRIM 32CA Lot A015. SALATRIM 32CA lot A015 differs from SALATRIM 23CA lot A014 in that it has a high proportion of propionate as opposed to acetate. Table 6 presents the data for SALATRIM 32CA lot A015. There was no evidence of potential mutagenicity for this member of the SALATRIM family. No evidence of cytotoxicity was noted in the assay.

Assay with SALATRIM 234CA Lot A019. This member of the SALATRIM family contains acetate, butyrate, and propionate as the SCFA. Data from the assay of SALATRIM 234CA lot A019 are presented in

Table 5. Ames Assay for SALATRIM 23CA Lot A014 Using Five *Salmonella* Strains with and without Metabolic Activation

dose (μg/plate)	revertants per plate[a]									
	TA98		TA100		TA1535		TA1537		TA1538	
	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9
0	24 ± 8	23 ± 3	128 ± 3	153 ± 22	13 ± 2	15 ± 1	10 ± 5	10 ± 5	18 ± 9	21 ± 2
62.5	21 ± 5	30 ± 6	142 ± 13	172 ± 11	10 ± 3	15 ± 4	7 ± 3	9 ± 6	20 ± 6	19 ± 7
125	17 ± 4	20 ± 4	119 ± 9	174 ± 13	15 ± 4	14 ± 1	12 ± 6	8 ± 1	16 ± 2	12 ± 3
250	17 ± 4	24 ± 4	129 ± 18	171 ± 8	10 ± 3	12 ± 1	14 ± 3	8 ± 1	13 ± 3	18 ± 2
500	21 ± 7	26 ± 2	130 ± 18	157 ± 10	12 ± 3	15 ± 1	6 ± 0	9 ± 0	16 ± 3	20 ± 6
750	19 ± 9	27 ± 3	123 ± 7	172 ± 18	10 ± 2	12 ± 3	6 ± 1	14 ± 2	13 ± 2	18 ± 7
1000	17 ± 4	19 ± 7	116 ± 18	174 ± 4	10 ± 2	14 ± 1	6 ± 1	11 ± 4	16 ± 3	20 ± 7

[a] Data represent the mean ± standard deviation for triplicate plates.

Table 6. Ames Assay for SALATRIM 32CA Lot A015 Using Five *Salmonella* Strains with and without Metabolic Activation

dose (μg/plate)	revertants per plate[a]									
	TA98		TA100		TA1535		TA1537		TA1538	
	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9
0	21 ± 3	28 ± 1	156 ± 24	162 ± 15	20 ± 6	19 ± 4	13 ± 5	9 ± 4	25 ± 5	25 ± 5
62.5	21 ± 8	20 ± 6	171 ± 20	164 ± 2	22 ± 6	17 ± 3	14 ± 10	12 ± 4	18 ± 5	23 ± 5
125	15 ± 2	31 ± 7	166 ± 8	166 ± 21	17 ± 4	19 ± 5	11 ± 7	11 ± 3	17 ± 4	25 ± 3
250	22 ± 7	31 ± 6	164 ± 21	176 ± 7	22 ± 4	21 ± 6	8 ± 4	8 ± 4	23 ± 6	20 ± 8
500	16 ± 4	25 ± 6	161 ± 5	175 ± 12	16 ± 4	12 ± 6	8 ± 1	14 ± 1	16 ± 1	18 ± 2
750	20 ± 5	33 ± 4	148 ± 12	159 ± 13	21 ± 8	16 ± 4	11 ± 5	12 ± 4	17 ± 4	21 ± 6
1000	21 ± 6	24 ± 4	144 ± 12	166 ± 18	17 ± 4	13 ± 2	8 ± 4	9 ± 5	22 ± 3	24 ± 3

[a] Data represent the mean ± standard deviation for triplicate plates.

Table 7. Ames Assay for SALATRIM 234CA Lot A019 Using Five *Salmonella* Strains with and without Metabolic Activation

dose (μg/plate)	revertants per plate[a]									
	TA98		TA100		TA1535		TA1537		TA1538	
	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9
0	19 ± 7	22 ± 2	165 ± 11	146 ± 12	10 ± 2	14 ± 2	7 ± 0	8 ± 2	20 ± 6	20 ± 6
62.5	17 ± 3	20 ± 8	163 ± 22	149 ± 27	10 ± 2	13 ± 1	7 ± 2	10 ± 3	17 ± 4	15 ± 0
125	18 ± 7	29 ± 2	150 ± 1	131 ± 16	11 ± 2	10 ± 2	8 ± 1	10 ± 4	14 ± 1	18 ± 3
250	16 ± 4	21 ± 3	167 ± 14	162 ± 6	11 ± 2	14 ± 1	11 ± 2	7 ± 2	18 ± 3	15 ± 1
500	18 ± 2	24 ± 7	169 ± 16	160 ± 7	10 ± 2	15 ± 5	8 ± 2	9 ± 3	17 ± 6	21 ± 6
750	21 ± 7	28 ± 4	154 ± 15	164 ± 6	11 ± 3	12 ± 3	6 ± 1	8 ± 3	15 ± 2	16 ± 3
1000	17 ± 3	22 ± 4	154 ± 12	175 ± 14	11 ± 3	10 ± 3	10 ± 5	12 ± 3	18 ± 11	21 ± 4

[a] Data represent the mean ± standard deviation for triplicate plates.

Table 8. Ames Assay for SALATRIM 234CS Lot A018 Using Five *Salmonella* Strains with and without Metabolic Activation

dose (μg/plate)	revertants per plate[a]									
	TA98		TA100		TA1535		TA1537		TA1538	
	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9
0	23 ± 3	24 ± 3	166 ± 7	174 ± 12	13 ± 6	16 ± 3	10 ± 7	8 ± 1	23 ± 6	28 ± 9
62.5	25 ± 5	32 ± 5	160 ± 27	164 ± 4	12 ± 6	17 ± 2	10 ± 2	6 ± 2	14 ± 3	21 ± 10
125	22 ± 3	24 ± 3	144 ± 24	159 ± 18	9 ± 1	16 ± 3	4 ± 2	9 ± 3	17 ± 2	21 ± 7
250	23 ± 2	23 ± 2	163 ± 9	168 ± 12	15 ± 4	13 ± 2	10 ± 5	9 ± 3	18 ± 4	21 ± 6
500	19 ± 6	33 ± 8	162 ± 18	186 ± 11	13 ± 5	13 ± 3	7 ± 2	6 ± 2	15 ± 1	23 ± 5
750	22 ± 6	26 ± 1	177 ± 10	191 ± 12	15 ± 3	14 ± 1	7 ± 2	12 ± 4	16 ± 3	22 ± 3
1000	21 ± 6	27 ± 3	158 ± 6	183 ± 13	16 ± 3	13 ± 4	9 ± 4	11 ± 3	20 ± 4	24 ± 7

[a] Data represent the mean ± standard deviation for triplicate plates.

Table 7. Again, there was no evidence of mutagenicity or cytotoxicity associated with this SALATRIM.

Assay with SALATRIM 234CS Lot A018. SALATRIM 234CS lot A018 is similar to SALATRIM 234CA lot A019 except hydrogenated cottonseed oil was used as the precursor fat rather than canola oil. Therefore, although both SALATRIM 234CA lot A019 and SALATRIM 234CS lot A018 have a preponderance of stearic acid, the other LCFA will vary according to the fatty acid composition of the precursor fat. Neither mutagenic potential nor cytotoxicity was evident from the data. The data in Table 8 indicate that change in the precursor fat did not alter the lack of mutagenicity or cytotoxicity of this fat.

Assay with SALATRIM 23SO Lot A020. SALATRIM 23SO lot A020 differs from SALATRIM 23CA lot A014 only in that it is produced from hydrogenated soy oil instead of hydrogenated canola oil. Again, the choice of precursor oil did not alter the results of the assay, as seen in Table 9. No evidence of either potential mutagenicity or cytotoxicity was noted.

DISCUSSION

Several members of the SALATRIM family of structured triacylglycerols were tested in the *Salmonella*/mammalian microsome reverse mutation assay (Ames assay). The data from the assays indicate that SALATRIM triacylglycerols have no potential for mutagenic activity under the conditions of the assay when tested both with and without metabolic activation. Also, no indication of bacterial cytotoxicity was evident under any condition of the assays.

Table 9. Ames Assay for SALATRIM 23SO Lot A020 Using Five *Salmonella* Strains with and without Metabolic Activation

dose (μg/plate)	revertants per plate[a]									
	TA98		TA100		TA1535		TA1537		TA1538	
	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9
0	23 ± 3	23 ± 6	149 ± 16	161 ± 10	13 ± 3	17 ± 2	8 ± 4	15 ± 2	18 ± 6	26 ± 2
62.5	19 ± 9	21 ± 5	167 ± 7	164 ± 23	20 ± 5	15 ± 6	5 ± 2	12 ± 3	19 ± 2	29 ± 11
125	22 ± 6	26 ± 5	168 ± 6	171 ± 13	14 ± 2	12 ± 4	8 ± 4	9 ± 6	18 ± 2	19 ± 6
250	18 ± 3	23 ± 6	164 ± 12	177 ± 4	16 ± 3	11 ± 5	8 ± 3	8 ± 5	15 ± 2	26 ± 11
500	21 ± 3	21 ± 2	162 ± 5	183 ± 26	12 ± 7	11 ± 3	9 ± 3	9 ± 3	14 ± 3	21 ± 5
750	25 ± 5	24 ± 3	169 ± 19	179 ± 13	13 ± 1	12 ± 4	6 ± 3	10 ± 3	13 ± 5	19 ± 2
1000	19 ± 5	27 ± 2	151 ± 4	190 ± 17	15 ± 5	15 ± 1	7 ± 2	8 ± 3	20 ± 5	19 ± 5

[a] Data represent the mean ± standard deviation for triplicate plates.

The dose range in this study was 0–1000 μg/plate and included six doses and a solvent control. A minimum of three to five adequately spaced doses of test material should be evaluated in short-term genotoxicity assays. If the test material is either cytotoxic or relatively insoluble, the selection of exposure concentrations is straightforward with either cytotoxicity or solubility limiting the dose range. Although the SALATRIM triacylglycerols showed no evidence of cytotoxicity in these studies, they generally showed evidence of insolubility at least at the high-dose concentration (1000 μg/plate). Therefore, 1000 μg/plate is the limit dose for these fats in this assay.

The data in this paper are completely predictable considering the current knowledge of the mutagenic potential of triacylglycerols. Members of the SALATRIM family have a typical triacylglycerol structure. Their uniqueness comes from the ratio of SCFA to LCFA esterified to the glycerol backbone. A structure/activity review of SALATRIM reveals no evidence of a potential to produce mutagenesis either in its unmetabolized state or in its predicted metabolized state. It can be predicted that changes in the SCFA components of the triacylglycerols and changes in the ratio of the SCFA to LCFA would not alter the mutagenic potential of the triacylglycerol. Data from this study confirm that prediction.

Several of the chemical moieties comprising SALATRIM and other triacylglycerols have been tested for genotoxic potential. For example, glycerol has been tested in a genetic toxicology battery that included the Ames assay, sister chromatid exchange assay, chromosomal aberration assay, HPRT gene mutation assay, and unscheduled DNA synthesis assay in isolated hepatocytes (Doolittle et al., 1988). This study indicated that glycerol was without mutagenic and clastogenic potential. Other studies have also indicated no evidence of genotoxic potential for glycerol (Cotruvo et al., 1977; Shimuzu et al., 1985; Ishidate et al., 1984). Propionic acid has been tested for genotoxicity in a battery consisting of the *Escherichia coli* DNA repair assay, the SOS chromotest, the Ames assay, sister chromatid exchanges, and the *in vivo* Chinese hamster micronucleus assay (Basler et al., 1987). All of the assays were negative, with the apparent exception of the *E. coli* DNA repair assay. The authors interpreted their data as a demonstration that propionic acid is not mutagenic. The negative Ames assay for propionic acid confirms the results obtained in other laboratories (Litton Bionetics, 1976; Ishidate et al., 1984). Sodium propionate did not produce chromosomal aberrations in Chinese hamster lung cells, whereas calcium propionate caused a very slight increase in aberrations, in contrast to the studies of Basler et al. (1987) using propionic acid.

Acetic acid and its sodium salt were not mutagenic in the Ames assay with and without metabolic activation (Cotruvo et al., 1977; Ishidate et al., 1984; Litton Bionetics, 1975; McCann et al., 1975; McMahon et al., 1979) or in *E. coli* (McCarroll et al., 1981) or in *Saccharomyces cerevisiae* (Cotruvo et al., 1977; Litton Bionetics, 1975). No chromosomal damage was noted in Chinese hamster ovary cells with and without metabolic activation (Ishidate et al., 1984), although chromosomal damage attributed to low pH was noted by Brusick (1986). Some evidence of genotoxicity was noted by Stumm-Tegethoff (1969) in *Drosophilia melanogaster* exposed to either 0.1% acetic acid vapor or 0.1% dietary acetic acid.

There appear to be no data in the scientific literature that indicate mutagenicity is associated with edible oils unless these oils have significantly oxidized or have been subjected to high heat for prolonged time periods. Van Gastel et al. (1984) determined the potential mutagenicity of refined peanut oil, hydrogenated vegetable oil, refined coconut oil, refined mustard oil, safflower oil, and unrefined peanut oil in the Ames assay before and after the oil was heated at 180 °C for a total of 24 h. Dimethyl sulfoxide extracts of these oils were negative in Ames tester strains TA98 and TA100, with and without metabolic activation. The data reported here indicate that corn oil, tested under the assay conditions described in this paper, presents no evidence of potential mutagenicity, in agreement with Van Gastel et al. (1984). Hageman et al. (1991) found coconut oil and a vegetable oil were not mutagenic. Taylor et al. (1983) noted that deep-fat frying of foods does not produce mutagens, unless common frying methods are replaced with more severe conditions.

This study was performed to test the hypothesis that members of the SALATRIM family would have no potential for mutagenicity as measured by the Ames assay. It also tested the hypothesis that varying the chemical nature of the precursor fat and the SCFA would not alter the lack of mutagenic potential of members of the SALATRIM family.

The lack of mutagenic potential for members of the SALATRIM family of structured triacylglycerols confirms the hypothesis that SALATRIM should have no mutagenic potential. The lack of mutagenicity is completely predictable on the basis of literature reviews and structure/activity relationships. This study also confirms that varying the SCFA between acetic, propionic, and butyric acids does not alter the mutagenic potential of SALATRIM. Furthermore, it also confirms that changing the precursor oil does not alter the lack of mutagenicity, even though the LCFA distribution may vary among the precursor oils.

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LITERATURE CITED

Ames, B. N.; McCann, J.; Yamasaki, E. Methods for Detecting Carcinogens and Mutagens with the *Salmonella*/Mammalian Microsome Mutagenicity Test. *Mutat. Res.* **1975**, *31*, 347–364.

Basler, A.; Von der Hude, W.; Scheutwinkel, M. Screening of the Food Additive Propionic Acid for Genotoxic Properties. *Food Chem. Toxicol.* **1987**, *25*, 287–290.

Brusick, D. Genotoxic Effects in Cultured Mammalian Cells Produced by Low pH Treatment Conditions and Increased Ion Concentrations. *Environ. Mutagen.* **1986**, *8*, 879–886.

Cotruvo, J. A.; Simmon, V. F.; Spanggord, R. J. Investigation of Mutagenic Effects of Products of Ozonation Reactions in Water. *Ann. N.Y. Acad. Sci.* **1977**, *298*, 124–140.

Doolittle, D. J.; Lee, D. A.; Lee, C. K. The Genotoxic Activity of Glycerol in an *In Vitro* Test Battery. *Food Chem. Toxicol.* **1988**, *26*, 631–635.

Finley, J. W.; Klemann, L. P.; Leveille, G. A.; Otterburn, M. S.; Walchak, C. G. Caloric Availability of SALATRIM in Rats and Humans. *J. Agric. Food Chem.* **1994**, one of several papers in this issue.

Hageman, G.; Hermans, R.; Ten Hoor, F.; Kleinjans, J. Mutagenicity of Deep-Frying Fat, and Evaluation of Urine Mutagenicity After Consumption of Fried Potatoes. *Food Chem. Toxicol.* **1990**, *28*, 75–80.

Hageman, G.; Verhagen, H.; Schutte, B.; Kleinjans, J. Biological Effects of Short-Term Feeding to Rats of Repeatedly Used Deep-Frying in Relation to Fat Mutagen Content. *Food Chem. Toxicol.* **1991**, *29*, 689–698.

Ishidate, M., Jr.; Sofuni, T.; Yoshikawa, K.; Hayashi, M.; Hohmi, T.; Sawada, M.; Matsuoka, A. Primary Mutagenicity Screening of Food Additives Currently Used in Japan. *Food Chem. Toxicol.* **1984**, *22*, 623–636.

Kensese, S. M.; Teng, J. I.; Smith, L. L. Mutagenic Lipid Peroxides from Edible Oils. *Teratog. Carcinog. Mutagen.* **1989**, *9*, 133–145.

Litton Bionetics. "Mutagenic Evaluation of Compounds, Contract FDA 75-3 Sodium Acetate 3-H_2O, NF Granular"; U.S. Department of Commerce, NTIS No. PB-254 514/35T; National Technical Information Service: Springfield, VA, 1975.

Litton Bionetics. "Mutagenic Evaluation of Compounds, Contract FDA 75-62 Propionic Acid"; U.S. Department of Commerce, NTIS No. PB-266-879; National Technical Information Service: Springfield, VA, 1976.

MacGregor, J. T.; Wilson, R. E.; Neff, W. E.; Frankel, E. N. Mutagenicity Test of Lipid Oxidation Products in *Salmonella typhimurium*: Monohydroperoxides and Secondary Oxidation Products of Methyl Linolenate and Methyl Linoleate. *Food Chem. Toxicol.* **1985**, *23*, 1041–1047.

Maron, D. M.; Ames, B. N. Revised Methods for the *Salmonella* Mutagenicity Test. *Mutat. Res.* **1983**, *121*, 173–215.

McCann, J.; Choi, E.; Yamasaki, E.; Ames, B. N. Detection of Carcinogens as Mutagens in the *Salmonella*/Microsome Test: Assay of 300 Chemicals. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 5135–5139.

McCarroll, N. E.; Piper, C. E.; Keech, B. H. An *E. coli* Microsuspension Assay for the Detection of DNA Damage Induced by Direct Acting Agents and Promutagens. *Environ. Mutagen.* **1981**, *3*, 429–444.

McMahon, R. E.; Cline, J. C.; Thompson, C. Z. Assay of 855 Test Chemicals in Ten Tester Strains Using a New Modification of the Ames Test for Bacterial Mutagens. *Cancer Res.* **1979**, *39*, 682–693.

Shimuzu, H.; Suzuka, Y.; Takemura, N.; Goto, S.; Matsushita, H. The Results of Microbial Mutation Test for Forty-Three Industrial Chemicals. *Jpn. J. Health* **1985**, *27*, 400–419.

Softly, B.; Huang, A.; Finley, J.; Templeman, G.; Otterburn, M. Comparison of the Composition of the SALATRIM Family of Edible Fats. *J. Agric. Food Chem.* **1994**, one of several papers in this issue.

Stumm-Tegethoff, B. F. A. Formaldehyde-Induced Mutations in *Drosophila melanogaster* in Dependence of the Presence of Acids. *Theor. Appl. Genet.* **1969**, *39*, 330–334.

Taylor, S. L.; Berg, C. M.; Shoptaugh, N. H.; Traisman, E. Mutagen Formation in Deep-Fat Fried Foods as a Function of Frying Conditions. *J. Am. Oil Chem. Soc.* **1983**, *60*, 576–580.

Van Gastel, A.; Mathur, R.; Roy, V. V.; Rukmini, C. Ames Mutagenicity Test of Repeatedly Heated Edible Oils. *Food Chem. Toxicol.* **1984**, *22*, 403–405.

Yahagi, T. M.; Ames, B. N. Mutagenicities of N-nitrosamines on *Salmonella*. *Mutat. Res.* **1977**, *48*, 121–130.

Yahagi, T.; Degawa, M.; Seino, Y.; Matsushima, J.; Nagao, M.; Sugimura, T.; Itashimoto, Y. Mutagenicity of Carcinogenic Azo Dyes and Derivatives. *Cancer Lett.* **1975**, *1*, 91–97.

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