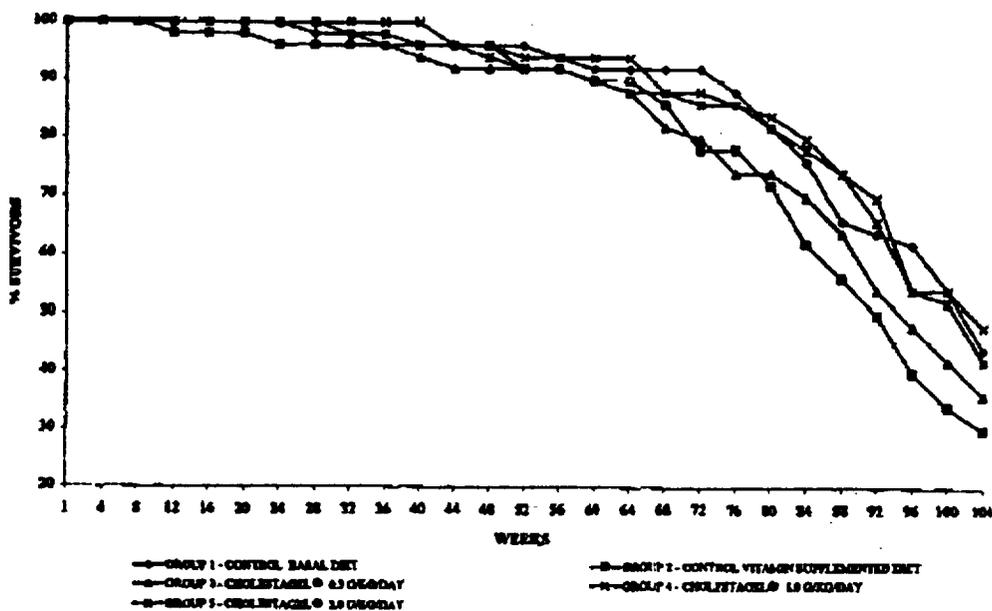


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FIGURE I

SURVIVAL - MALES
MAIN STUDY

PROJECT NO. 87966

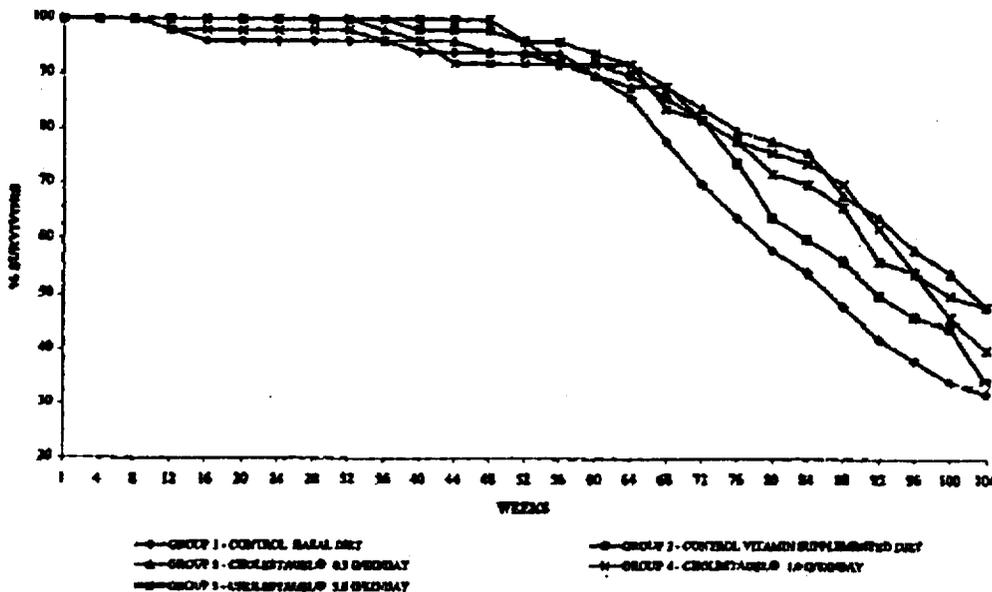


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FIGURE II

SURVIVAL - FEMALES
MAIN STUDY

PROJECT NO. 87966



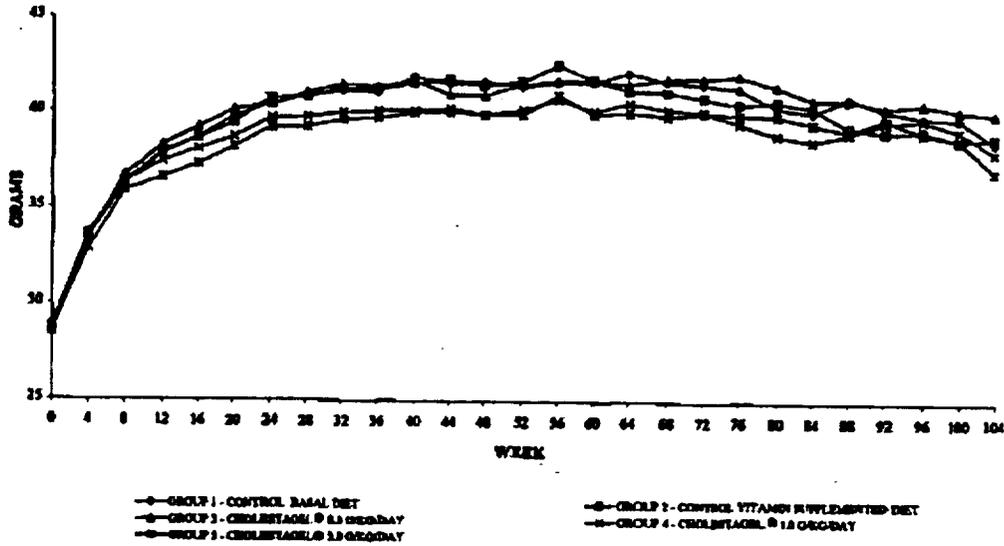
- 13 -

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FIGURE III

PROJECT NO. 87966

GROUP MEAN BODY WEIGHT
MAIN STUDY - MALES

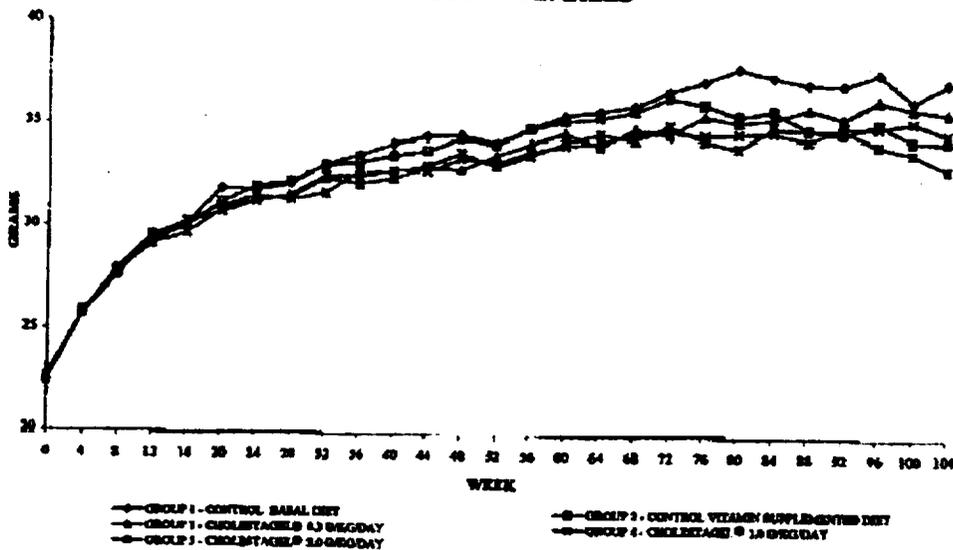


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FIGURE IV

PROJECT NO. 87966

GROUP MEAN BODY WEIGHT
MAIN STUDY - FEMALES



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APPENDIX 1

Sponsors Histopathology Tables

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TABLE NO.: 18
PROJECT NO.: 87966

INCIDENCE OF NEOPLASTIC LESIONS
CARCINOGENICITY GROUP - ALL ANIMALS

		SEX :		— M A L E —				
		DOSE GROUP :	DOSE GROUP :	1	2	3	4	5
		NO. OF ANIMALS IN DOSE GROUP :		50	50	50	50	50
GROUP 1	CONTROL BASAL DIET	GROUP 4	CHOLESTAGEL® 1.0 G/KG/DAY					
GROUP 2	CONTROL VITAMIN SUPPLEMENTED DIET	GROUP 5	CHOLESTAGEL® 3.0 G/KG/DAY					
GROUP 3	CHOLESTAGEL® 0.3 G/KG/DAY							
ADRENAL								
	-TOTAL EXAMINED			50	50	50	50	49
	-M-CORTICAL ADENOMA			1	0	0	1	0
BRAIN								
	-TOTAL EXAMINED			50	50	50	50	50
	-M-GLIOMA			1	0	0	0	0
FAT								
	-TOTAL EXAMINED			1	0	0	0	1
	-M-HEMANGIOMA			0	0	0	0	1
PANCREATIC ISLAND								
	-TOTAL EXAMINED			50	50	49	50	50
	-M-ADENOMA			2	5	2	2	4
	-M-ADENOCARCINOMA			1	0	0	0	0
TESTIS								
	-TOTAL EXAMINED			50	50	50	50	50
	-M-LEIOMYOMA			1	0	0	0	0
	-M-ADENOMA			0	0	0	0	1
KIDNEY								
	-TOTAL EXAMINED			40	50	50	50	50
	-M-TUBULAR CELL ADENOMA			0	0	0	1	0

APPEARS THIS WAY
ON ORIGINAL

TABLE NO.: 18
PROJECT NO.: 87966

INCIDENCE OF NEOPLASTIC LESIONS
CARCINOGENICITY GROUP - ALL ANIMALS

GROUP 1 CONTROL BASAL DIET		GROUP 4 CHOLESTAGEL® 1.0 G/KG/DAY				
GROUP 2 CONTROL VITAMIN SUPPLEMENTED DIET		GROUP 5 CHOLESTAGEL® 3.0 G/KG/DAY				
GROUP 3 CHOLESTAGEL® 0.3 G/KG/DAY						
		SEX :				
		DOSE GROUP :				
NO. OF ANIMALS IN DOSE GROUP :		50	50	50	50	50
		M A L E				
		1	2	3	4	5
LIVER	-TOTAL EXAMINED	50	50	50	50	50
	-#-HEPATOCELLULAR ADENOMA	9	8	10	10	9
	-#-HEPATOCELLULAR CARCINOMA	1	3	0	2	5
	-#-HEMANGIOMA	1	3	2	2	1
	-#-HEMANGIOSARCOMA	0	1	4	3	2
	-#-CHOLANGIOMA	0	0	0	1	1
	-#-ADENOPLASTIC CARCINOMA	0	0	0	0	1
LUNG	-TOTAL EXAMINED	50	50	50	50	49
	-#-ALVEOLAR/BRONCHIOAL ADENOMA	5	9	8	14	11
	-#-ALVEOLAR/BRONCHIOAL CARCINOMA	0	1	7	5	7
LYMPH NODE	-TOTAL EXAMINED	12	14	22	21	19
	-#-LYMPHOSARCOMA	1	4	3	4	1
	-#-MYELOID LEUKEMIA	0	1	0	0	0
	-#-HISTIOCYTIC SARCOMA	3	0	2	0	1
	-#-METASTASIS, CARCINOMA OF UNKNOWN ORIGIN	0	0	0	0	1
PITUITARY	-TOTAL EXAMINED	50	50	50	50	49
	-#-CARCINOMA	0	0	1	0	0

- A208 -

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ON ORIGINAL

TABLE NO.: 1B
PROJECT NO.: 87966

INCIDENCE OF NEOPLASTIC LESIONS
CARCINOGENICITY GROUP - ALL ANIMALS

		SEX :				
		--- M A L E ---				
		1	2	3	4	5
		NO. OF ANIMALS IN DOSE GROUP :				
		50	50	50	50	50
PROSTATE	-TOTAL EXAMINED	50	50	50	50	50
	-00-ADENOMA	0	0	0	0	1
SALIVARY GLAND	-TOTAL EXAMINED	40	50	50	50	50
	-00-HEMANGIOMA	0	0	1	0	0
SKIN MISCELLANEOUS	-TOTAL EXAMINED	17	12	9	19	19
	-01-SQUAMOUS CELL CARCINOMA	0	0	0	1	0
	-02-SQUAMOUS CELL PAPILLOMA	1	0	0	0	0
SPLEEN	-TOTAL EXAMINED	50	50	50	50	50
	-01-HEMANGIOSARCOMA	0	2	0	1	0
	-02-HEMANGIOMA	0	1	2	1	0
STOMACH	-TOTAL EXAMINED	50	50	50	50	50
	-01-SQUAMOUS CELL CARCINOMA	0	0	1	0	0
SUBCUTANEOUS TISSUE	-TOTAL EXAMINED	8	10	9	5	9
	-01-HEMANGIOSARCOMA	0	0	0	1	0
	-02-FIBROSARCOMA	0	0	1	0	0

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ON ORIGINAL

TABLE NO.: 18
PROJECT NO.: 87966

INCIDENCE OF NEOPLASTIC LESIONS
CARCINOGENICITY GROUP - ALL ANIMALS

GROUP 1 CONTROL BASAL DIET		GROUP 4 CHOLESTABEL® 1.0 G/100g/DAY				
GROUP 2 CONTROL VITAMIN SUPPLEMENTED DIET		GROUP 5 CHOLESTABEL® 3.0 G/100g/DAY				
GROUP 3 CHOLESTABEL® 0.3 G/100g/DAY						
		SEX :				
		MALE				
		1	2	3	4	5
NO. OF ANIMALS IN DOSE GROUP :		50	50	50	50	50
TESTIS	-TOTAL EXAMINED	50	50	50	50	50
	-#-INTERSTITIAL CELL ADENOMA	0	2	0	0	1
	-#-PAPILLARY CYSTADENOMA, RETE TESTIS	0	0	1	0	0
THYROID	-TOTAL EXAMINED	50	50	50	50	50
	-#-FOLLICULAR CELL ADENOMA	0	0	0	2	0
	-#-FOLLICULAR CELL CARCINOMA	0	0	0	1	0
TONGUE	-TOTAL EXAMINED	50	50	50	50	50
	-#-SCUMOUS PAPILLOMA	0	0	0	1	0
TAIL	-TOTAL EXAMINED	0	1	0	0	1
	-#-FIBROMA	0	0	0	0	1

- NEOPLASM ## - BENIGN ### - MALIGNANT

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APPEARS THIS WAY
ON ORIGINAL

TABLE NO.: 18
PROJECT NO.: 87984

INCIDENCE OF NEOPLASTIC LESIONS
CARCINOGENICITY GROUP - ALL ANIMALS

GROUP 1 CONTROL BASAL DIET							GROUP 4 CHOLESTAGEL® 1.0 G/KG/DAY	
GROUP 2 CONTROL VITAMIN SUPPLEMENTED DIET							GROUP 5 CHOLESTAGEL® 3.0 G/KG/DAY	
GROUP 3 CHOLESTAGEL® 0.3 G/KG/DAY								
		SEX :		— FEMALE —				
		DOSE GROUP :		1	2	3	4	5
NO. OF ANIMALS IN DOSE GROUP :		50	50	50	50	50	50	50
ADRENAL	-TOTAL EXAMINED	50	50	50	50	50		
	-PHEOCHROMOCYTOMA	1	0	0	0	0		
	-PHENOCROMOCYTOMA	0	0	1	0	0		
	-ADRENAL CORTICAL CARCINOMA	0	0	0	1	0		
BONE MISCELLANEOUS	-TOTAL EXAMINED	0	1	2	0	1		
	-OSTEOMA	0	0	1	0	0		
BRAIN	-TOTAL EXAMINED	50	50	49	50	50		
	-GLIOMA	0	0	0	0	1		
PANCREAS	-TOTAL EXAMINED	50	50	50	50	50		
	-ADENOMA	1	0	2	0	3		
	-ADENOCARCINOMA	0	0	1	0	0		
LIVER	-TOTAL EXAMINED	50	50	50	50	50		
	-HEPATOCELLULAR ADENOMA	4	0	0	2	2		
	-HEPATOCELLULAR CARCINOMA	1	0	2	0	1		
	-HEMANGIOMA	0	0	1	0	1		
	-HEMANGIOCARCINOMA	1	1	1	0	0		
	-CHOLANGIOMA	0	0	0	0	1		

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ON ORIGINAL

TABLE NO.: 10
PROJECT NO.: 87966

INCIDENCE OF NEOPLASTIC LESIONS
CARCINOGENICITY GROUP - ALL ANIMALS

GROUP	DIET	NO. OF ANIMALS IN DOSE GROUP	SEX				
			— FEMALE —				
1	CONTROL BASAL DIET		1	2	3	4	5
2	CONTROL VITAMIN SUPPLEMENTED DIET		50	50	50	50	50
3	CHOLESTAGEL® 0.3 G/KG/DAY						
4	CHOLESTAGEL® 1.0 G/KG/DAY						
5	CHOLESTAGEL® 3.0 G/KG/DAY						
LUNG		-TOTAL EXAMINED	50	50	50	50	50
	-#0-ALVEOLAR/BRONCHIOLAR ADENOMA		6	7	8	4	5
	-#1-ALVEOLAR/BRONCHIOLAR CARCINOMA		1	4	2	1	3
LYMPH NODE		-TOTAL EXAMINED	21	25	19	22	12
	-#1-LYMPHOSARCOMA		4	0	7	0	5
	-#1-MYELOID LEUKEMIA		0	0	1	0	0
	-#1-HISTIOCYTIC SARCOMA		2	1	4	5	1
MAMMARY GLAND		-TOTAL EXAMINED	50	50	50	50	50
	-#1-ADENOCARCINOMA		2	1	1	0	1
	-#0-ADENOMA		0	0	1	0	0
MUSCLE SKELETAL		-TOTAL EXAMINED	50	50	50	48	50
	-#1-HEMANGIOSARCOMA		1	0	0	0	0
OVARY		-TOTAL EXAMINED	50	50	50	50	50
	-#1-ADENOCARCINOMA		1	1	0	0	0
	-#0-ADENOMA		0	1	1	1	0
	-#1-NEOVASCULAR LUTEOMA		0	0	1	0	0
	-#1-BENIGN LUTEOMA		0	0	0	0	1

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TABLE NO.: 18
PROJECT NO.: 87966

INCIDENCE OF NEOPLASTIC LESIONS
CARCINOGENICITY GROUP - ALL ANIMALS

GROUP 1 CONTROL BASAL DIET		GROUP 4 CHOLESTAGEL® 1.0 G/100g/DAY				
GROUP 2 CONTROL VITAMIN SUPPLEMENTED DIET		GROUP 5 CHOLESTAGEL® 3.0 G/100g/DAY				
GROUP 3 CHOLESTAGEL® 0.3 G/100g/DAY						
		SEX :				
		BOSE GROUP :				
NO. OF ANIMALS IN BOSE GROUP :		1	2	3	4	5
		50	50	50	50	50
PITUITARY						
	-TOTAL EXAMINED	48	50	49	50	49
	-M-ADENOMA, PARS DISTALIS	1	2	2	1	1
	-M-ADENOMA, PARS INTERMEDIA	1	0	0	1	0
SKIN MISCELLANEOUS						
	-TOTAL EXAMINED	6	10	10	10	10
	-M-BASAL CELL CARCINOMA	0	1	0	0	0
	-M-BENIGN KERATOCANTHOMA	0	1	0	0	0
	-M-SQUAMOUS CELL PAPILLOMA	0	0	1	0	0
SPLEEN						
	-TOTAL EXAMINED	50	50	50	50	50
	-M-HEMANGIOSARCOMA	0	2	1	0	1
SUBCUTANEOUS TISSUE						
	-TOTAL EXAMINED	11	12	6	13	0
	-M-FIBROSARCOMA	1	0	0	2	0
	-M-HYROMA	0	1	0	0	0
	-M-BASAL CELL CARCINOMA	1	0	0	0	0
THYROID						
	-TOTAL EXAMINED	50	50	50	50	50
	-M-FOLLICULAR CELL ADENOMA	1	0	0	0	1

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ON ORIGINAL

TABLE NO.: 18
PROJECT NO.: 87966

INCIDENCE OF NEOPLASTIC LESIONS
CARCINOGENICITY GROUP - ALL ANIMALS

GROUP 1	CONTROL BASAL DIET	GROUP 4	CHOLESTAGEL® 1.0 G/RS/DAY			
GROUP 2	CONTROL VITAMIN SUPPLEMENTED DIET	GROUP 5	CHOLESTAGEL® 3.0 G/RS/DAY			
GROUP 3	CHOLESTAGEL® 0.3 G/RS/DAY					
		SEX :				
		DOSE GROUP :				
		NO. OF ANIMALS IN DOSE GROUP :				
		F E M A L E				
		1	2	3	4	5
		50	50	50	50	50
UTERUS	-TOTAL EXAMINED	50	50	50	50	50
-M-ENOMETRIAL STROMAL SARCOMA		2	1	1	3	1
-M-ADENOCARCINOMA		1	0	0	0	1
-M-LEIOMYOSARCOMA		0	1	2	1	0
-B-BENIGN ENOMETRIAL STROMAL POLYP		2	1	3	3	2
-B-LEIOMYOMA		2	1	3	1	2
-M-HEMANGIOSARCOMA		0	0	1	2	0
-B-FIBROMA		1	0	0	1	0
-B-HEMANGIOMA		0	1	0	0	0
-B-ADENOMA		0	0	0	0	1
VAGINA	-TOTAL EXAMINED	48	50	50	50	50
-M-LEIOMYOSARCOMA		1	0	0	0	0
-M-SQUAMOUS CELL CARCINOMA		0	1	0	0	0
† -NEOPLASH ‡B-BENIGN †M-MALIGNANT						

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ADDENDUM TO REVIEW OF CARCINOGENICITY STUDIES

ADDITIONAL EVALUATION OF TUMOR FINDINGS IN RAT STUDY

Historical control data for a number of tumors were submitted by the Sponsor (Submission Date: March 30, 2000) as requested by the Reviewer. In addition, for the rat study, an additional trend test with only data from control group 2 was carried out, as well as a number of pairwise comparisons. The results were taken into consideration in the following evaluation of tumorigenicity data in the rat. Note that the carcinogenicity studies were done with _____ SD rats, and the historical control values were from studies with _____ SD rats.

Pancreatic acinar cell adenoma in male rats

Incidence	0-0-0-2-3
N _{examined}	60-60-45-40-60
Incidence % (per n _{examined})	0%-0%-0%-5%-5%
Incidence % (per n=60)	0%-0%-0%-3.3%-5%
Historical control incidence %	0%-2.1%-0.8%-0%-0%
Historical control range	0%-2.1% (5 studies, _____ rats)
Results trend test:	
Trend test with both controls:	p=0.002 (significant, p< cut off value for rare and common tumor, 0.025 and 0.005)
Trend test with control 2 only:	p=0.007 (significant, p< cut off value for rare tumor, 0.025)

The incidences in the mid and high dose groups were outside the historical control range for _____ SD rats, and the finding was statistically significant according to a trend test with both controls and a trend test with control 2 only. There was also a slight increase in the incidence of acinar cell hyperplasia in mid and high dose males.

Conclusion: In the opinion of this Reviewer the finding of pancreatic acinar cell adenoma in male rats is significant, and needs to be mentioned in the label.

Thyroid C-cell (calcitonin-producing cell) adenoma in male rats

Incidence	8-7-7-5-13
N _{examine}	60-60-49-41-60
Incidence % (per n _{examined})	13%-12%-14%-12%-22%
Incidence % (per n=60)	13%-12%-12%-8.3%-22%
Historical control range	3.3%-11.4% (5 studies, _____ rats)
Results trend test:	
Trend test with both controls:	p=0.115
Trend test with control 2 only:	p=0.061
Pairwise test (7 vs. 13)	p=0.22

The incidences (per n_{examined}) were outside the historical control range for _____ SD rats in all dose groups. The finding was statistically not significant. There was a slight increase in the incidence of thyroid C-cell hyperplasia in mid and high dose males.

Conclusion: This Reviewer feels that the biological significance of the finding of thyroid C-cell adenoma in male rats is not clear.

Thyroid C-cell adenoma in female rats

Incidence:	20-11-4-5-19
N _{examined}	60-60-28-23-60

Incidence % (per n _{examined})	33%-18%-14%-22%-32%
Incidence % (per n=60)	33%-18%-6.7%-8.3%-32%
Historical control range	1.7%-7.9% (5 studies, _____ rats)
Results trend test:	
Trend test with both controls:	p=0.148
Trend test with control 2 only:	p=0.003 (significant, p< cut off value for common tumor, 0.005)
Pairwise test (11 vs. 19)	not significant

The incidences (per n_{examined}) were outside the historical control range for _____ SD rats in all dose groups. The finding was not statistically significant according to a trend test with both control groups included. However, it was statistically significant when only control group 2 was included. There was no increase in the incidence of thyroid C-cell hyperplasia in the females.

Conclusion: This Reviewer feels that the data on thyroid C-cell adenoma in males and females taken together indicate a signal of possible thyroid C-cell tumorigenicity. Mention of the statistically significant finding vs. control group 2 for female rats in the label is recommended.

Pancreatic islet cell carcinoma in female rats

Incidence:	0-0-0-3-1
N _{examined}	60-60-28-21-60
Incidence % (per n _{examined})	0%-0%-0%-14%-1.7%
Incidence % (per n=60)	0%-0%-0%-5%-1.7%
Historical control incidence %	1.4%-5.7%-4.2%-1.7%-0%

Since this issue was raised at the executive CAC meeting (March 21, 2000) the Reviewer called the Sponsor on March 31, 2000, to ask if it was possible to analyze the remaining pancreatic tissues from mid and low dose female rats to obtain a full data set for pancreatic islet cell carcinoma. In response to this the Sponsor sent a Fax communication from the testing laboratory (Date April 4, 2000) stating that the pancreatic islet cell carcinomas that were diagnosed histopathologically were evident at gross necropsy, and that it is very unlikely that a carcinoma like this would not be evident macroscopically. Thus, we could assume that the total incidence in the groups of 60 animals is 0-0-0-3-1, i.e., 0-0-0-5%-1.7%. This would mean that the incidence in the MD group does not exceed the historical control range.

Upon request of the Reviewer the Statistics Reviewer (Dr. M. Ng) performed a pairwise test (Fisher's Exact Test) of the data from the mid dose group versus the concurrent controls. This test indicated that the finding was statistically significant (p=0.035) when compared to the two concurrent control groups combined. In this test the incidence of tumors was taken to be per 60 animals per group.

Conclusion: Although the increased incidence of pancreatic islet cell carcinoma in mid dose female rats was statistically significant according to a pairwise test, this Reviewer feels that the biological significance of this finding is not clear, since it was seen in the mid dose group and did not exceed historical control incidence values.

Combined organ schwannoma in male and female rats

Incidence males:	1-0-0-2-0
Results trend test:	p=0.559 (not significant; data from control group 2 used only)
Incidence females:	2-3-4-0-4
Results trend test:	p=0.502 (not significant; data from control group 2 used only)

Conclusion: No biologically or statistically significant increases in schwannomas.

SUMMARY

Rat study

- Statistically significant dose-tumor positive linear trend in incidence of benign pancreatic acinar cell adenoma in male rats ($p=0.002$). Together with the finding of increased incidence of pancreatic acinar cell hyperplasia in mid and high dose males this suggests a significant effect of colesevelam hydrochloride, or any of its degradants, on pancreatic tumorigenesis.
- Statistically significant dose-tumor positive linear trend in incidence of benign thyroid C-cell adenoma in female rats, when analysis was done using data from concurrent control group 2 only ($p=0.003$). Together with the increased incidence of thyroid C-cell hyperplasia and C-cell adenoma in males this suggests a significant effect of colesevelam hydrochloride, or any of its degradants, on thyroid C-cell tumorigenesis.
- Statistically significant increase in incidence of pancreatic islet cell carcinoma in the mid dose female rats, according to pairwise analysis versus combined control groups ($p=0.035$). Since the finding was in the mid dose group its significance is unclear.

Mouse Study

No significant tumor findings. Note, however, that toxicity in the mouse carcinogenicity study dose groups was minimal, and that the doses used were therefore not optimal.

**APPEARS THIS WAY
ON ORIGINAL**

**CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC) REPORT
AND
FDA-CDER RODENT CARCINOGENICITY DATABASE FACTSHEET**

P/T REVIEWER(s):	Gemma Kuijpers
DATE:	March 21, 2000
NDA:	21,141
DIVISION:	Division of Metabolic and Endocrine drug Products (HFD-510)
DRUG NAME:	Colesevelam Hydrochloride
DRUG CODE#:	GT31-104HB
SPONSOR:	Geltex Pharmaceuticals, Inc.
LABORATORY:	<hr/>
CARCINOGENICITY STUDY REPORT DATES:	June 30, 1999
THERAPEUTIC CATEGORY:	Bile Acid Sequestrant
PHARMACOLOGICAL CLASSIFICATION:	Polymer
GENOTOXICITY/CLASTOGENICITY OF PARENT COMPOUND:	Ames test (extract): Negative In vitro CHO cell clastogenicity test (extract): Positive with metabolic activation Mouse micronucleus test: Negative
GENOTOXICITY/CLASTOGENICITY OF FOUR DEGRADANTS:	Ames tests: Negative In vitro CHO cell clastogenicity tests: Positive for Decylamine HCl and Aminoethyltrimethyl ammonium chloride HCl without metabolic activation, negative for other test compounds and conditions

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ON ORIGINAL**

RAT CARCINOGENICITY STUDY (Study GT-02-TX-25)

RAT STUDY DURATION:	104 weeks
STUDY STARTING DATE:	November 1996
STUDY ENDING DATE:	November 1998
RAT STRAIN:	HSD:Sprague-Dawley
DOSING ROUTE:	Oral (diet), daily
DOSING COMMENTS:	None
NUMBER OF RATS:	
- Control 1:	60 (Control diet)
- Control 2:	60 (Vitamin-supplemented diet)
- Low Dose (LD):	60 (Vitamin-supplemented diet)
- Middle Dose (MD):	60 (Vitamin-supplemented diet)
- High Dose (HD):	60 (Vitamin-supplemented diet)
RAT DOSE LEVELS (g/kg/day):	
- Low Dose:	0.4 g/kg/day
- Middle Dose:	1.2 g/kg/day
- High Dose:	2.4 g/kg/day
BASIS FOR DOSES SELECTED:	% of test article in diet/Human dose multiples
PRIOR FDA DOSE CONCURRENCE:	No
RAT CARCINOGENICITY:	Positive (males)
RAT TUMOR FINDINGS (significant findings):	Males: Pancreas acinar cell adenoma Incidence (Control 1-Control 2-LD-MD-HD): 0-0-0-2-3 Trend analysis: P = 0.002 (statistically significant)
RAT STUDY COMMENTS:	Decreased mortality in HD males

**APPEARS THIS WAY
ON ORIGINAL**

MOUSE CARCINOGENICITY STUDY (Study GT-02-TX-24)

MOUSE STUDY DURATION: 104 weeks
STUDY STARTING DATE: November 1996
STUDY ENDING DATE: November 1998
MOUSE STRAIN: Crl:CD^R-1) (ICR)BR
ROUTE: Oral (diet)
DOSING COMMENTS: None

NUMBER OF MICE:
- Control 1: 50 (Control diet)
- Control 2: 50 (Vitamin-supplemented diet)
- Low Dose (LD): 50 (Vitamin-supplemented diet)
- Middle Dose (MD): 50 (Vitamin-supplemented diet)
- High Dose (HD): 50 (Vitamin-supplemented diet)

MOUSE DOSE LEVELS (g/kg/day):
- Low Dose: 0.3 g/kg/day
- Middle Dose: 1.0 g/kg/day
- High Dose: 3.0 g/kg/day

BASIS FOR DOSES SELECTED: Diet level/Human dose multiple

PRIOR FDA DOSE CONCURRENCE: No

MOUSE CARCINOGENICITY: Negative

MOUSE TUMOR FINDINGS (significant findings): No significant dose-related increases in tumor incidence at any site

MOUSE STUDY COMMENTS: None

**APPEARS THIS WAY
ON ORIGINAL**

**Executive CAC
March 21, 2000**

Committee: Joseph DeGeorge, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-901, Member
Glenna Fitzgerald, Ph.D., HFD-120, Alternate Member
Ronald Steigerwalt, Ph.D., Team Leader
Gemma Kuijpers, Ph.D., Presenting Reviewer

Author of Draft: Gemma Kuijpers

The following information reflects a brief summary of the Committee discussion and its conclusions. Detailed study information can be found in the individual reviews.

NDA # 21,141
Drug Name: Colesevelam Hydrochloride (Welchol)
Category: Bile acid sequestrant
Sponsor: Geltex Pharmaceuticals, Inc., MA

1. Rat Carcinogenicity Study

104-week study

Doses: 0, 0, 0.4, 1.2, 2.4 g/kg/day

Discussed were mortality data, body weight data, dose levels and tumor findings. Histopathology examination was carried out of all animals in groups 1 and 2 (controls) and group 5 (high dose group), and of preterminally sacrificed or dead animals in groups 3 and 4 (low and mid dose groups). All macroscopic abnormalities and all organs/tissues were evaluated by histopathological examination.

Colesevelam was associated with an increase in survival in high dose male rats, and a slight decrease in body weight in mid dose and high dose male rats. In the male rats, survival was 18% and 32% in control groups 1 and 2, respectively, and 58% in the high dose group. There was a statistically significant linear trend in survival distribution among the dose groups in the male rat. In the mid and high dose groups, body weight was 97% and 93%, respectively, of control group 2 in male rats, at 104 weeks. There was no effect on body weight in female rats.

Test article concentrations in the diet of the high dose groups reached 5% for males in week 46-47 and 4% for females in week 62 of the study. 80% and 90% of this diet percentage level was reached for males in weeks 10 and 20, and for females in weeks 6 and 14.

There was a significant dose-tumor positive linear trend for benign pancreatic acinar cell adenoma in male rats ($p=0.002$). In males, there was an increased incidence of thyroid C-cell adenoma as compared to control groups 1 and 2, and in females there was an increased incidence of thyroid C-cell adenoma as compared to control group 2 but not control group 1. There was also an increased incidence of pancreatic acinar cell hyperplasia and thyroid C-cell hyperplasia in mid and high dose males, both as compared to control 1 as well as control 2. Thyroid C-cell hyperplasia was not increased in females.

The Committee considered the dose levels adequate as the dietary concentrations had reached at least 85% of the maximum by study week 20. The question was raised whether the statistical analysis of tumor incidence was carried out with the data for the two control groups pooled, and it was suggested to do the analysis with only the data from control group 2. The Committee was concerned about the pancreatic findings (tumors, nodules and hyperplasia) in the males, and the thyroid C-cell adenoma in both sexes. The Committee was further concerned about a possible increased incidence of combined organ schwannomas, and an increased incidence of pancreatic islet cell carcinoma in the mid dose females (0/60 in control groups, and 3 out of 21 in mid dose group). It was suggested to ask the Sponsor to analyze the remaining low and mid dose animals. A question was also raised about the nature of the non-neoplastic lung granulomas with increased incidence in high dose males. The Committee also noted that the compound and/or some of its degradants had positive reactions in the CHO chromosomal aberration assay and asserted to not neglect these findings since they have not been shown to be irreproducible.

2. Mouse Carcinogenicity Study

104-week study

Doses: 0, 0, 0.3, 1.0, 3.0 mg/kg/day

Discussed were mortality data, body weight data, dose levels and tumor findings. Histopathology examination was carried out of all animals in all groups and of and all organs/tissues.

Colesevelam had no significant effect on mortality. Body weight was decreased in the high dose males and females at the end of the study. Another drug-related adverse effect appeared to be a decrease in the serum vitamin E levels in mid and high dose animals.

Test article concentrations in the diet of the high dose groups reached 2.1% for males in week 24 and 1.7% for females in week 23 of the study. 90% of this diet percentage level was reached for males in week 7, and for females in week 18.

There were no significant increases in the incidence of any tumor type in male or female mice.

The Committee judged that on the basis of the trends in body weight the doses used in this study were adequate. There was no concern about the tumorigenicity of the test compound in mice.

Conclusions:

Rat study: In a 104-week rat carcinogenicity study with colesevelam hydrochloride there was a statistically significant increase in the incidence of pancreatic acinar cell adenoma in male rats. There also appeared to be an increased incidence of thyroid C-cell tumor incidence in male and female animals. Additional statistical analysis was recommended to come to a more comprehensive conclusion on the thyroid tumor findings.

Mouse study: In a 104-week mouse carcinogenicity study with colesevelam hydrochloride there appeared to be no effects on tumor incidence in any organ in either male or female mice.

Joseph DeGeorge, Ph.D.
Chair, Executive CAC

cc:\

/Division File, HFD-510
/G. Kuijpers, HFD-510
/R. Steigerwalt, HFD-510
/R. Hedin, HFD-510
/A. Seifried, HFD-024

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GENETIC TOXICOLOGY

The genotoxicity of colesevelam hydrochloride has been assessed in a battery of three assays, a bacterial reverse mutation assay, an in vitro mammalian cytogenetics assay, and an in vivo mouse micronucleus assay. In addition, Sponsor has evaluated the genotoxic potential of 4 degradants, decylamine HCl, didecylamine HCl, decylamino-6-hexyltrimethyl ammonium chloride hydrochloride (decylaminoquat), and aminohexyltrimethylammonium chloride hydrochloride (aminoquat). These degradants appear at levels approaching —% in the drug substance and product during long-term stability studies. The source of these degradants are the mono- and dialkylated amines in the drug substance.

Table 5.3-26: Genotoxicity Studies

SECTION NUMBER	STUDY TITLE	SPECIES	TREATMENT DURATION	DOSE	GLP
5.3.5.1	Micronucleus Cytogenetic Assay in Mice (Study No. GT-02-TX-26)	Mouse	2 Days	5000 mg/kg	Yes
5.3.5.2	Bacterial reverse mutation assay conducted with a test article extract (Study No. GT-02-TX-29)	--	--	100 µL/mL	Yes
5.3.5.3	Chromosomal Aberration in the Chinese Hamster Ovary (CHO) Cells conducted with a Test Article Extract (Study No. GT-02-TX-31)	--	--	100 µL/mL	Yes
5.3.5.4	Bacterial reverse Mutation Assay with Decylamine HCL (Study No. GT-0069-TX-1)	--	--	5000 µg/plate	Yes
5.3.5.5	Bacterial reverse Mutation Assay with Aminohexyltrimethyl ammonium chloride hydrochloride (Study No. GT-0070-TX-1)	--	--	5000 µg/plate	Yes
5.3.5.6	Bacterial reverse Mutation Assay with Didecylamine HCL (Study No. GT-0073-TX-1)	--	--	100 to 333 µg/plate	Yes
5.3.5.7	Bacterial reverse Mutation Assay with Decylamino-6-hexyltrimethyl ammonium chloride hydrochloride (Study No. GT-0071-TX-1)	--	--	5000 µg/plate	Yes
5.3.5.8	<i>In vitro</i> Mammalian Chromosome Aberration Test with Decylamine HCL HCL (Study No. GT-0069-TX-2)	--	--	15 to 30 µg/mL	Yes
5.3.5.9	<i>In vitro</i> Mammalian Chromosome Aberration Test with Aminohexyltrimethyl ammonium chloride hydrochloride (Study No. GT-0070-TX-2)	--	--	5000 µg/mL	Yes
5.3.5.10	<i>In vitro</i> Mammalian Chromosome Aberration Test with Didecylamine HCL (Study No. GT-0073-TX-2)	--	--	4 to 12 µg/mL	Yes
5.3.5.11	<i>In vitro</i> Mammalian Chromosome Aberration Test with Decylamino-6-hexyltrimethyl ammonium chloride hydrochloride (Study No. GT-0071-TX-2)	--	--	400 to 4500 µg/mL	Yes

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BACTERIAL REVERSE MUTATION ASSAY CONDUCTED WITH A TEST ARTICLE EXTRACT

Study Title: Bacterial reverse mutation assay conducted with a test article (colesevelam hydrochloride) extract
Study No: GT-02-TX-29
Study Type: Mutation assay
Volume #, Page #: Electronic submission
Conducting Laboratory:
Study Initiation Date: August 07, 1998
Study Completion Date: May 4, 1999
GLP Compliance: Yes
QA- Reports: Yes (x) No ()
Drug Lot Number: TLMC004-1839
Study Endpoint: *In vitro* mutagenesis

METHODOLOGY

Strains: *Salmonella typhimurium* TA1535, TA1537, TA98 and TA100, *Escherichia coli* WP2uvrA pKM101
Negative Controls: 0.1 N HCl (extraction blank)
Positive Controls:

Positive Controls

S9 Activation	Strains	Positive control	Concentration (ug/plate)
-	TA 98	2-Nitrofluorene	1.0
-	TA100	Sodium Azide	1.0
-	TA 1537	9-Aminoacridine	75.0
-	TA 1535	Sodium Azide	1.0
-	WP2uvrA	MMS*	1000
+	TA 98	2-Aminoanthracene	1.0
+	TA 100	2-Aminoanthracene	1.0
+	TA1537	2-Aminoanthracene	1.0
+	TA 1535	2-Aminoanthracene	1.0
+	WP2uvrA	2-Aminoanthracene	10.0

*MMS = methyl methanesulfonate

Preparation of extract: Two (2) g of test article was extracted with 40 ml 0.1 N HCl for three days at 50°C, and extract was decanted and stored at room temperature. 0.1 N HCl extraction blank was prepared without test article addition.

Doses used in assay: A minimum of five dose levels of test article extract with appropriate vehicle (extraction blank) and positive controls were plated with the tester strains, in absence and presence of S9 mix. The maximum dose level was 100 ul of undiluted test article extract per plate. All dose levels and controls were plated in triplicate.

Metabolic activation system: Aroclor 1254-induced rat liver S9 mix.

Assay method: Plate incorporation method (Ames et al, 1975)

Plating method: 0.5 ml S9 or Sham mix, 100 ul of tester strain and 100 ul of extraction blank or test article extract, were added to 1 ml of top agar. Mixture was overlaid onto a surface of 25 ml bottom agar.

Positive controls were 50 ul aliquots. Plates were incubated for 48-72h at 37°C. The experiment was done in duplicate (B1, B2)?

ANALYSIS:

Scoring method:

Mean and standard deviation of the number of revertants per plate were counted automatically or by hand and calculated. Test article extract toxicity (background lawn) and precipitate were evaluated and scored relative to the vehicle control plate according to a coding system.

Cytotoxic endpoints:

Condition of bacterial background lawn

Genetic toxicity endpoints:

Number of revertant colonies per plate

Statistical methods:

N/A

Criteria for Positive Results:

A positive response is defined as a dose-related increase in the mean revertants per plate of at least one tester strain with a minimum of two increasing concentrations of test article extract. For TA1535 and TA1537 the increase at the peak of the dose response must be equal to or greater than three (3) times the mean vehicle control value. For TA98, TA100 and WP2uvrA the increase at the peak of the dose response must be equal to or greater than two (2) times the mean vehicle control value.

Criteria for Valid Test:

Mean number of spontaneous revertants in the vehicle controls should be within the characteristic value range of : TA98, 10-50; TA100, 80-240; TA1535, 5-45; TA1537, 3-21; WP2uvrA, 10-60. Mean of each positive control should be at least three-fold the vehicle control value. A minimum of 3 non-toxic dose levels are required to evaluate assay data. A dose level is toxic if either there is a >50% reduction in the mean number of revertants as compared to control, or there is a reduction in the background lawn.

RESULTS:

Table 11 summarizes the results. Data are from Experiment B2.

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**Salmonella/E. coli Mutagenicity Assay
Summary of Results**

Table 11

Test Article Id : Colesevelam hydrochloride
 Study Number : G98AX80.502203 Experiment No : B2
 Extraction Medium : 0.1 N HCl

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µL)	TA98		TA100		TA1535		TA1537		WP2 uvrA	
0.0	22 ±	3	159 ±	13	10 ±	6	5 ±	3	13 ±	3
3.3	20 ±	6	182 ±	7	11 ±	4	6 ±	3	17 ±	7
10	28 ±	6	196 ±	9	9 ±	1	5 ±	1	10 ±	2
33	25 ±	9	181 ±	17	9 ±	3	7 ±	3	14 ±	4
50	21 ±	4	171 ±	7	9 ±	6	5 ±	1	12 ±	4
100	14 ±	3	185 ±	11	8 ±	3	6 ±	3	15 ±	4
Pos	304 ±	18	485 ±	5	255 ±	15	409 ±	103	152 ±	4

Liver Microsomes: Rat liver S9

Dose (µL)	TA98		TA100		TA1535		TA1537		WP2 uvrA	
0.0	24 ±	5	185 ±	27	9 ±	4	4 ±	1	13 ±	4
3.3	26 ±	8	196 ±	7	13 ±	3	5 ±	2	14 ±	9
10	29 ±	9	182 ±	6	11 ±	4	5 ±	3	10 ±	1
33	25 ±	6	199 ±	5	11 ±	5	6 ±	2	15 ±	6
50	26 ±	4	158 ±	23	12 ±	3	6 ±	1	16 ±	4
100	17 ±	7	202 ±	35	12 ±	6	3 ±	1	13 ±	7
Pos	264 ±	38	565 ±	40	103 ±	14	46 ±	13	41 ±	33

0.0 - Vehicle plating aliquot of 100 µL

Pos - Positive Control concentrations as specified in Materials and Methods section

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Study Outcome:

1. Data shown are from Experiment B2. In Experiment B1, an undefined error precluded evaluation of the results.
2. According to the criteria of evaluation of the background lawn and number of revertants per plate no toxicity was observed at any dose level for any of the strains.
3. Precipitate was observed for all tester strains at concentrations of ≥ 3.3 ul per plate. Therefore, all plates were counted manually.
4. In Experiment B2 no positive responses were observed with any of the tester strains in the absence or presence of S9 metabolic activation.

Study Validity:

The study was valid:

1. The mean number of spontaneous revertants in the vehicle controls were within the characteristic value ranges specified in the methods.
2. The mean number of revertants for each positive control was at least three-fold the vehicle control value.
3. A minimum of 3 non-toxic dose levels was available for all tester strains and conditions to evaluate the assay data.

SUMMARY:

Under the conditions of the assay, colesevelam hydrochloride was negative in the Ames bacterial reverse mutation assay with and without metabolic activation.

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BACTERIAL REVERSE MUTATION ASSAY WITH DECYLAMINE HYDROCHLORIDE

Study Title: Bacterial reverse mutation assay
Study No: GT-0069-TX-1
Study Type: Mutation assay
Volume #, Page #: Vol. 1.18 (Appendix 5-22)
Conducting Laboratory: _____
Study Initiation Date: February 19, 1999
Study Completion Date: June 7, 1999
GLP Compliance: Yes
QA- Reports: Yes (x) No ()
Drug Lot Number: P-1039-174A7
Study Endpoint: *In vitro* mutagenesis

METHODOLOGY

Strains: *Salmonella typhimurium* TA1535, TA1537, TA98 and TA100,
Escherichia coli WP2uvrA pKM101
Negative Controls: Distilled water
Positive Controls:

Positive Controls			
S9 Activation	Strains	Positive control	Concentration (ug/plate)
-	TA 98	2-Nitrofluorene	1.0
-	TA100	Sodium Azide	1.0
-	TA 1537	9-Aminoacridine	75.0
-	TA 1535	Sodium Azide	1.0
-	WP2uvrA	MMS*	1000
+	TA 98	2-Aminoanthracene	1.0
+	TA 100	2-Aminoanthracene	1.0
+	TA1537	2-Aminoanthracene	1.0
+	TA 1535	2-Aminoanthracene	1.0
+	WP2uvrA	2-Aminoanthracene	10.0

*MMS = methyl methanesulfonate

Preparation of test article: Test article (decylamine HCl) was dissolved in water
Doses used in assays: In a preliminary toxicity test ten (10) dose levels of the test article were plated (1 plate/dose) with/out S9 activation in order to establish the dose range for the main assay.
 In the main assay a minimum of five dose levels of test article with appropriate vehicle and positive controls were plated with the tester strains, in absence and presence of S9 mix. The maximum dose level was established from the preliminary toxicity test (5000 ug per plate). All dose levels and controls were plated in triplicate.

Metabolic activation system: Aroclor 1254-induced male rat liver S9 mix.
Assay method: Plate incorporation method (Ames et al, 1975)
Plating method: 0.5 ml S9 or Sham mix, 100 ul of tester strain and 50 ul of vehicle or test article, were added to 1 ml of top agar. Mixture was overlaid onto a surface of 25 ml bottom agar. Positive controls were 50 ul aliquots. Plates were incubated for 48-72h at 37°C. The experiment was done in duplicate (B1, B2)?

ANALYSIS:

Scoring method:

Mean and standard deviation of the number of revertants per plate were counted automatically or by hand and calculated. Test article extract toxicity (background lawn) and precipitate were evaluated and scored relative to the vehicle control plate according to a coding system.

Cytotoxic endpoints:

Condition of bacterial background lawn

Genetic toxicity endpoints:

Number of revertant colonies per plate

Statistical methods:

N/A

Criteria for Positive Results:

A positive response is defined as a dose-related increase in the mean revertants per plate of at least one tester strain with a minimum of two increasing concentrations of test article extract. For TA1535 and TA1537 the increase at the peak of the dose response must be equal to or greater than three (3) times the mean vehicle control value. For TA98, TA100 and WP2uvrA the increase at the peak of the dose response must be equal to or greater than two (2) times the mean vehicle control value.

Criteria for Valid Test:

Mean number of spontaneous revertants in the vehicle controls should be within the characteristic value range of : TA98, 10-50; TA100, 80-240; TA1535, 5-45; TA1537, 3-21; WP2uvrA, 10-60. Mean of each positive control should be at least three-fold the vehicle control value. A minimum of 3 non-toxic dose levels are required to evaluate assay data. A dose level is toxic if either there is a >50% reduction in the mean number of revertants as compared to control, or there is a reduction in the background lawn.

RESULTS:

Results of preliminary toxicity test showed toxicity at doses ≥ 667 to ≥ 3333 ug per plate. Precipitation was not observed. The maximum dose plated in the main assay was 5000 ug per plate.

Table 17 summarizes the results of the main assay. The data are partly from Experiment B1 and partly from Experiment B2.

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**Salmonella/E. coli Mutagenicity Assay
Summary of Results**

Table 17

Test Article Id : Decylamine HCL
 Study Number : AA12SS.502.BTL Experiment Nos : B1/B2

Average Revertants Per Plate \pm Standard Deviation

Liver Microsomes: None

Dose (μ g)	TA98	TA100	TA1535	TA1537	WP2 uvrA
0.0	19 \pm 4	236 \pm 5	10 \pm 3	6 \pm 3	15 \pm 3
7.5	13 \pm 2	225 \pm 2	11 \pm 4	4 \pm 3	
25	19 \pm 4	212 \pm 19	16 \pm 4	3 \pm 2	
75	12 \pm 1	234 \pm 12	13 \pm 3	5 \pm 3	20 \pm 5
200	13 \pm 6	217 \pm 30	16 \pm 3	5 \pm 1	14 \pm 5
600	3 \pm 1	33 \pm 16	6 \pm 3	2 \pm 3	12 \pm 4
1800	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	1 \pm 1
5000					0 \pm 0
Pos	490 \pm 35	872 \pm 34	713 \pm 63	663 \pm 6	198 \pm 12

Liver Microsomes: Rat liver S9

Dose (μ g)	TA98	TA100	TA1535	TA1537	WP2 uvrA ^a
0.0	25 \pm 7	224 \pm 32	16 \pm 3	8 \pm 5	19 \pm 8
7.5	22 \pm 8	227 \pm 20	15 \pm 8	8 \pm 5	18 \pm 7
25	21 \pm 2	225 \pm 8	14 \pm 1	9 \pm 3	11 \pm 4
75	19 \pm 2	243 \pm 10	12 \pm 5	11 \pm 0	16 \pm 8
200	19 \pm 3	215 \pm 13	10 \pm 1	8 \pm 2	15 \pm 5
600	3 \pm 2	147 \pm 29	9 \pm 3	3 \pm 3	11 \pm 1
1800	0 \pm 0	0 \pm 0	0 \pm 1	0 \pm 0	6 \pm 5
5000					0 \pm 0
Pos	412 \pm 35	675 \pm 50	86 \pm 8	59 \pm 15	543 \pm 62

0.0 = Vehicle plating aliquot of 30 μ l
 Pos = Positive Control concentrations as specified in Materials and Methods section.
 a = Data from Experiment B2

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Study Outcome:

1. In Experiment B1 there was excessive toxicity with tester strain WP2uvrA. Therefore, data for this strain are from Experiment B2.
2. According to the predefined criteria toxicity was observed at doses ≥ 600 ug per plate for all Salmonella tester strains and at ≥ 1800 ug per plate for the E. coli WP2uvrA strain.
3. No positive responses were observed with any of the tester strains in the absence or presence of S9 metabolic activation.

Study Validity:

The study was valid:

1. The mean number of spontaneous revertants in the vehicle controls were within the characteristic value ranges specified in the methods.
2. The mean number of revertants for each positive control was at least three-fold the vehicle control value.
3. A minimum of 3 non-toxic dose levels was available for all tester strains and conditions to evaluate the assay data.

SUMMARY:

Under the conditions of the assay, decylamine hydrochloride was negative in the Ames bacterial reverse mutation assay with and without metabolic activation.

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BACTERIAL REVERSE MUTATION ASSAY WITH DIDECYLAMINE HYDROCHLORIDE

Study Title: Bacterial reverse mutation assay
Study No: GT-0073-TX-1
Study Type: Mutation assay
Volume #, Page #: Vol. 1.18 (Appendix 5-24)
Conducting Laboratory: _____
Study Initiation Date: April 01, 1999
Study Completion Date: June 22, 1999
GLP Compliance: Yes
QA- Reports: Yes (x) No ()
Drug Lot Number: — 154-171
Study Endpoint: *In vitro* mutagenesis

METHODOLOGY

Strains: *Salmonella typhimurium* TA1535, TA1537, TA98 and TA100,
Escherichia coli WP2uvrA pKM101
Negative Controls: Ethanol (vehicle)
Positive Controls:

Positive Controls

S9 Activation	Strains	Positive control	Concentration (ug/plate)
-	TA 98	2-Nitrofluorene	1.0
-	TA100	Sodium Azide	1.0
-	TA 1537	9-Aminoacridine	75.0
-	TA 1535	Sodium Azide	1.0
-	WP2uvrA	MMS*	1000
+	TA 98	2-Aminoanthracene	1.0
+	TA 100	2-Aminoanthracene	1.0
+	TA1537	2-Aminoanthracene	1.0
+	TA 1535	2-Aminoanthracene	1.0
+	WP2uvrA	2-Aminoanthracene	10.0

*MMS = methyl methanesulfonate

Preparation of test article: Test article (didecylamine HCl) was dissolved in ethanol to a maximum stock concentration of 400 mg/ml
Doses used in assays: In a preliminary toxicity test ten (10) dose levels of the test article were plated (1 plate/dose) with/out S9 activation in order to establish the dose range for the main assay.
 In the main assay a minimum of five dose levels of test article with appropriate vehicle and positive controls were plated with the tester strains, in absence and presence of S9 mix. The maximum dose level was established from the preliminary toxicity test (100 ug per plate for all strains with/out S9, and 333 ug for WP2uvrA with S9). All dose levels and controls were plated in triplicate.
Metabolic activation system: Aroclor 1254-induced male rat liver S9 mix.
Assay method: Plate incorporation method (Ames et al, 1975)
Plating method: 0.5 ml S9 or Sham mix, 100 ul of tester strain and 50 ul of vehicle or test article, were added to 1 ml of top agar. Mixture was overlaid onto a surface of 25 ml bottom agar. Positive

controls were 50 ul aliquots. Plates were incubated for 48-72h at 37°C. The experiment was done in duplicate (B1, B2)?

ANALYSIS:

Scoring method:

Mean and standard deviation of the number of revertants per plate were counted automatically or by hand and calculated. Test article extract toxicity (background lawn) and precipitate were evaluated and scored relative to the vehicle control plate according to a coding system.

Cytotoxic endpoints:

Condition of bacterial background lawn

Genetic toxicity endpoints:

Number of revertant colonies per plate

Statistical methods:

N/A

Criteria for Positive Results:

A positive response is defined as a dose-related increase in the mean revertants per plate of at least one tester strain with a minimum of two increasing concentrations of test article extract. For TA1535 and TA1537 the increase at the peak of the dose response must be equal to or greater than three (3) times the mean vehicle control value. For TA98, TA100 and WP2uvrA the increase at the peak of the dose response must be equal to or greater than two (2) times the mean vehicle control value.

Criteria for Valid Test:

Mean number of spontaneous revertants in the vehicle controls should be within the characteristic value range of : TA98, 10-50; TA100, 80-240; TA1535, 5-45; TA1537, 3-21; WP2uvrA, 10-60. Mean of each positive control should be at least three-fold the vehicle control value. A minimum of 3 non-toxic dose levels are required to evaluate assay data. A dose level is toxic if either there is a >50% reduction in the mean number of revertants as compared to control, or there is a reduction in the background lawn.

RESULTS:

Results of preliminary toxicity test showed toxicity at ≥ 33 or ≥ 67 ug per plate in the absence of S9, and at ≥ 67 or ≥ 100 ug per plate in the presence of S9. Precipitation was generally observed at ≥ 33 or ≥ 67 ug per plate. Based on the toxicity findings, the maximum doses plated in the main assay were 33 ug per plate with WP2uvrA in the presence of S9, and 100 ug per plate with all the other tester strains and conditions.

Table 16 summarizes the results of the main assay. The data are partly from Experiment B1 and partly from Experiment B2.

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**Salmonella/E. coli Mutagenicity Assay
Summary of Results**

Table 16

Test Article Id : Didecylamine
Study Number : AA14PA.502.BTL Experiment Nos : B1/B2

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg)	TA98	TA100	TA1535	TA1537	WP2 <i>uvrA</i>
0.0	15 ± 2	124 ± 7	7 ± 3	6 ± 2	10 ± 0
0.33	20 ± 3	140 ± 14	10 ± 3	8 ± 1	13 ± 3
1.0	12 ± 4	120 ± 12	10 ± 1	8 ± 2	15 ± 4
3.3	18 ± 4	134 ± 2	9 ± 1	6 ± 1	14 ± 2
10	18 ± 6	125 ± 12	12 ± 2	9 ± 1	11 ± 4
33	16 ± 3	128 ± 17	8 ± 2	4 ± 1	17 ± 4
100	1 ± 1	21 ± 4	1 ± 1	0 ± 0	4 ± 1
Pos	441 ± 44	699 ± 74	527 ± 9	1922 ± 116	177 ± 24

Liver Microsomes: Rat liver S9

Dose (µg)	TA98	TA100	TA1535	TA1537	TA1537 ^a	WP2 <i>uvrA</i>
0.0	23 ± 2	127 ± 12	15 ± 3	4 ± 1	12 ± 4	13 ± 1
0.33	19 ± 4	123 ± 15	19 ± 5	8 ± 4		10 ± 1
1.0	28 ± 5	135 ± 21	11 ± 3	7 ± 3	15 ± 4	16 ± 2
3.3	24 ± 7	141 ± 10	10 ± 2	8 ± 4	12 ± 5	11 ± 3
10	29 ± 4	148 ± 8	13 ± 4	6 ± 2	12 ± 6	17 ± 7
33	24 ± 8	150 ± 17	14 ± 3	10 ± 5	14 ± 7	13 ± 4
50					20 ± 8	
75					10 ± 6	
100	30 ± 8	119 ± 8	8 ± 4	6 ± 2	5 ± 2	13 ± 2
150					3 ± 3	
500						0 ± 0
Pos	384 ± 137	573 ± 72	58 ± 11	40 ± 9	71 ± 13	321 ± 67

0.0 = Vehicle plating aliquot of 50 µL

Pos = Positive Control concentrations as specified in Materials and Methods section.

^a = Data from Experiment B2

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Study Outcome:

1. In Experiment B1 a maximally 2.5-fold non-dose responsive increase in the number of revertants was observed with tester strain TA1537 in the presence of S9 activation. Therefore, Experiment B2 was performed in which nine doses of test article were tested up to 150 ug per plate. In this experiment no positive response was observed with this strain in the presence of S9 activation.
2. According to the predefined criteria toxicity was observed at doses ≥ 33 or ≥ 100 ug per plate.
3. Precipitate was observed for all tester strains at concentrations of 100 ug per plate in the absence of S9 activation.
4. According to the predefined criteria, no positive responses were observed with any of the tester strains in the absence or presence of S9 metabolic activation.

Study Validity:

The study was valid:

4. The mean number of spontaneous revertants in the vehicle controls were within the characteristic value ranges specified in the methods.
5. The mean number of revertants for each positive control was at least three-fold the vehicle control value.
6. A minimum of 3 non-toxic dose levels was available for all tester strains and conditions to evaluate the assay data.

SUMMARY:

Under the conditions of the assay, didecylamine HCl was negative in the Ames bacterial reverse mutation assay with and without metabolic activation.

**APPEARS THIS WAY
ON ORIGINAL**

BACTERIAL REVERSE MUTATION ASSAY WITH AMINOHEXYLTRIMETHYL AMMONIUM CHLORIDE HYDROCHLORIDE

Study Title: Bacterial reverse mutation assay
Study No: GT-0070-TX-1
Study Type: Mutation assay
Volume #, Page #: Vol. 1.18 (Appendix 5-23)
Conducting Laboratory: _____
Study Initiation Date: February 19, 1999
Study Completion Date: June 7, 1999
GLP Compliance: Yes
QA- Reports: Yes (x) No ()
Drug Lot Number: 0171-251
Study Endpoint: *In vitro* mutagenesis

METHODOLOGY

Strains: *Salmonella typhimurium* TA1535, TA1537, TA98 and TA100, *Escherichia coli* WP2uvrA pKM101
Negative Controls: Distilled water
Positive Controls:

Positive Controls

S9 Activation	Strains	Positive control	Concentration (ug/plate)
-	TA 98	2-Nitrofluorene	1.0
-	TA100	Sodium Azide	1.0
-	TA 1537	9-Aminoacridine	75.0
-	TA 1535	Sodium Azide	1.0
-	WP2uvrA	MMS*	1000
+	TA 98	2-Aminoanthracene	1.0
+	TA 100	2-Aminoanthracene	1.0
+	TA1537	2-Aminoanthracene	1.0
+	TA 1535	2-Aminoanthracene	1.0
+	WP2uvrA	2-Aminoanthracene	10.0

*MMS = methyl methanesulfonate

Preparation of test article: Test article (aminohexyltrimethyl ammonium chloride hydrochloride) was dissolved in water

Doses used in assays: In a preliminary toxicity test ten (10) dose levels of the test article were plated (1 plate/dose) with/out S9 activation in order to establish the dose range for the main assay. In the main assay a minimum of five dose levels of test article with appropriate vehicle and positive controls were plated with the tester strains, in absence and presence of S9 mix. The maximum dose level was established from the preliminary toxicity test (5000 ug per plate). All dose levels and controls were plated in triplicate.

Metabolic activation system: Aroclor 1254-induced male rat liver S9 mix.

Assay method: Plate incorporation method (Ames et al, 1975)

Plating method: 0.5 ml S9 or Sham mix, 100 ul of tester strain and 50 ul of vehicle or test article, were added to 1 ml of top agar. Mixture was overlaid onto a surface of 25 ml bottom agar. Positive

controls were 50 ul aliquots. Plates were incubated for 48-72h at 37°C. The experiment was done in duplicate (B1, B2)?

ANALYSIS:

Scoring method:

Mean and standard deviation of the number of revertants per plate were counted automatically or by hand and calculated. Test article extract toxicity (background lawn) and precipitate were evaluated and scored relative to the vehicle control plate according to a coding system.

Cytotoxic endpoints:

Condition of bacterial background lawn.

Genetic toxicity endpoints:

Number of revertant colonies per plate

Statistical methods:

N/A

Criteria for Positive Results:

A positive response is defined as a dose-related increase in the mean revertants per plate of at least one tester strain with a minimum of two increasing concentrations of test article extract. For TA1535 and TA1537 the increase at the peak of the dose response must be equal to or greater than three (3) times the mean vehicle control value. For TA98, TA100 and WP2uvrA the increase at the peak of the dose response must be equal to or greater than two (2) times the mean vehicle control value.

Criteria for Valid Test:

Mean number of spontaneous revertants in the vehicle controls should be within the characteristic value range of : TA98, 10-50; TA100, 80-240; TA1535, 5-45; TA1537, 3-21; WP2uvrA, 10-60. Mean of each positive control should be at least three-fold the vehicle control value. A minimum of 3 non-toxic dose levels are required to evaluate assay data. A dose level is toxic if either there is a >50% reduction in the mean number of revertants as compared to control, or there is a reduction in the background lawn.

RESULTS:

Results of preliminary toxicity test showed no toxicity. Precipitation was not observed. The maximum dose plated in the main assay was 5000 ug per plate.

Table 16 summarizes the results of the main assay. The data are partly from Experiment B1 and partly from Experiment B2.

**APPEARS THIS WAY
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**Salmonella/E. coli Mutagenicity Assay
Summary of Results**

Table 16

Test Article Id : Aminohexyltrimethyl ammonium chloride hydrochloride
Study Number : AAL2SR.502.BTL Experiment Nos : B1/B2

Average Revertants Per Plate \pm Standard Deviation

Liver Microsomes: None

Dose (μ g)	TA98	TA100	TA1535	TA1537	WP2 uvrA
0.0	15 \pm 1	203 \pm 12	8 \pm 3	5 \pm 3	13 \pm 4
100	15 \pm 2	193 \pm 22	7 \pm 1	6 \pm 2	19 \pm 4
333	18 \pm 3	193 \pm 2	9 \pm 3	7 \pm 2	23 \pm 1
1000	18 \pm 2	180 \pm 8	11 \pm 4	8 \pm 2	22 \pm 5
3333	15 \pm 2	183 \pm 26	11 \pm 3	4 \pm 1	21 \pm 4
5000	15 \pm 2	179 \pm 21	12 \pm 3	8 \pm 4	22 \pm 9
Pos	415 \pm 80	821 \pm 27	633 \pm 53	788 \pm 319	193 \pm 20

Liver Microsomes: Rat liver S9

Dose (μ g)	TA98	TA100 ^a	TA1535	TA1537	WP2 uvrA
0.0	20 \pm 3	190 \pm 28	15 \pm 4	12 \pm 3	21 \pm 9
100	18 \pm 4	172 \pm 30	14 \pm 3	8 \pm 3	22 \pm 6
333	20 \pm 2	186 \pm 19	14 \pm 5	10 \pm 3	21 \pm 2
1000	28 \pm 6	192 \pm 17	11 \pm 3	9 \pm 3	18 \pm 4
3333	22 \pm 2	193 \pm 13	19 \pm 2	8 \pm 2	19 \pm 4
5000	20 \pm 11	202 \pm 20	15 \pm 3	9 \pm 2	20 \pm 1
Pos	368 \pm 67	605 \pm 31	59 \pm 13	43 \pm 10	507 \pm 73

0.0 = Vehicle plating aliquot of 50 μ l.
Pos = Positive Control concentrations as specified in Materials and Methods section.
^a = Data from Experiment B2

**APPEARS THIS WAY
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Study Outcome:

4. In Experiment B1 there was an unacceptable positive control value for the experiment with tester strain TA100 with metabolic activation. Therefore, data for this strain in the presence of S9 mix are from Experiment B2.
5. According to the predefined criteria no toxicity was observed at any dose level for any strain.
6. No positive responses were observed with any of the tester strains in the absence or presence of S9 metabolic activation.

Study Validity:

The study was valid:

7. The mean number of spontaneous revertants in the vehicle controls were within the characteristic value ranges specified in the methods.
8. The mean number of revertants for each positive control was at least three-fold the vehicle control value.
9. A minimum of 3 non-toxic dose levels was available for all tester strains and conditions to evaluate the assay data.

SUMMARY:

Under the conditions of the assay, aminoethyltrimethyl ammonium chloride hydrochloride was negative in the Ames bacterial reverse mutation assay with and without metabolic activation.

**APPEARS THIS WAY
ON ORIGINAL**

BACTERIAL REVERSE MUTATION ASSAY WITH 6-DECYLAMINOHEXYLTRIMETHYL AMMONIUM CHLORIDE HYDROCHLORIDE

Study Title: Bacterial reverse mutation assay
Study No: GT-0071-TX-1
Study Type: Mutation assay
Volume #, Page #: Vol. 1.18 (Appendix 5-25)
Conducting Laboratory: _____
Study Initiation Date: April 01, 1999
Study Completion Date: June 9, 1999
GLP Compliance: Yes
QA- Reports: Yes (x) No ()
Drug Lot Number: 198-129
Study Endpoint: *In vitro* mutagenesis

METHODOLOGY

Strains: *Salmonella typhimurium* TA1535, TA1537, TA98 and TA100, *Escherichia coli* WP2uvrA pKM101
Negative Controls: Distilled water
Positive Controls:

Positive Controls

S9 Activation	Strains	Positive control	Concentration (ug/plate)
-	TA 98	2-Nitrofluorene	1.0
-	TA100	Sodium Azide	1.0
-	TA 1537	9-Aminoacridine	75.0
-	TA 1535	Sodium Azide	1.0
-	WP2uvrA	MMS*	1000
+	TA 98	2-Aminoanthracene	1.0
+	TA 100	2-Aminoanthracene	1.0
+	TA1537	2-Aminoanthracene	1.0
+	TA 1535	2-Aminoanthracene	1.0
+	WP2uvrA	2-Aminoanthracene	10.0

*MMS = methyl methanesulfonate

Preparation of test article: Test article (6-decylaminohexyltrimethyl ammonium chloride hydrochloride) was dissolved in water
Doses used in assays: In a preliminary toxicity test ten (10) dose levels of the test article were plated (1 plate/dose) with/out S9 activation in order to establish the dose range for the main assay. In the main assay a minimum of five dose levels of test article with appropriate vehicle and positive controls were plated with the tester strains, in absence and presence of S9 mix. The maximum dose level was established from the preliminary toxicity test (5000 ug per plate). All dose levels and controls were plated in triplicate.
Metabolic activation system: Aroclor 1254-induced male rat liver S9 mix.
Assay method: Plate incorporation method (Ames et al, 1975)
Plating method: 0.5 ml S9 or Sham mix, 100 ul of tester strain and 50 ul of vehicle or test article, were added to 1 ml of top agar. Mixture was overlaid onto a surface of 25 ml bottom agar. Positive

controls were 50 ul aliquots. Plates were incubated for 48-72h at 37°C. The experiment was done in duplicate (B1, B2)?

ANALYSIS:

Scoring method:

Mean and standard deviation of the number of revertants per plate were counted automatically or by hand and calculated. Test article extract toxicity (background lawn) and precipitate were evaluated and scored relative to the vehicle control plate according to a coding system.

Cytotoxic endpoints:

Condition of bacterial background lawn.

Genetic toxicity endpoints:

Number of revertant colonies per plate

Statistical methods:

N/A

Criteria for Positive Results:

A positive response is defined as a dose-related increase in the mean revertants per plate of at least one tester strain with a minimum of two increasing concentrations of test article extract. For TA1535 and TA1537 the increase at the peak of the dose response must be equal to or greater than three (3) times the mean vehicle control value. For TA98, TA100 and WP2uvrA the increase at the peak of the dose response must be equal to or greater than two (2) times the mean vehicle control value.

Criteria for Valid Test:

Mean number of spontaneous revertants in the vehicle controls should be within the characteristic value range of : TA98, 10-50; TA100, 80-240; TA1535, 5-45; TA1537, 3-21; WP2uvrA, 10-60. Mean of each positive control should be at least three-fold the vehicle control value. A minimum of 3 non-toxic dose levels are required to evaluate assay data. A dose level is toxic if either there is a >50% reduction in the mean number of revertants as compared to control, or there is a reduction in the background lawn.

RESULTS:

Results of preliminary toxicity test showed toxicity at ≥ 3333 or at 5000 ug per plate. Precipitation was not observed. The maximum dose plated in the main assay was 5000 ug per plate.

Table 16 summarizes the results of the main assay. The data are from Experiment B1.

**APPEARS THIS WAY
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**Salmonella/E. coli Mutagenicity Assay
Summary of Results**

Table 16

Test Article Id : 6-decylaminoethyltrimethyl ammonium chloride
hydrochloride
Study Number : AA14N2.502.BTL Experiment No : B1

Average Revertants Per Plate \pm Standard Deviation

Liver Microsomes: None

Dose (μ g)	TA98		TA100		TA1535		TA1537		WP2 uvrA	
0.0	12 \pm 3	125 \pm 12	9 \pm 2	7 \pm 2	16 \pm 2					
25	12 \pm 0	145 \pm 11	12 \pm 4	4 \pm 1	16 \pm 2					
75	11 \pm 1	119 \pm 11	7 \pm 2	5 \pm 3	13 \pm 1					
200	13 \pm 1	132 \pm 8	7 \pm 2	5 \pm 2	16 \pm 2					
600	14 \pm 2	112 \pm 10	11 \pm 3	4 \pm 2	15 \pm 5					
1800	10 \pm 3	99 \pm 1	10 \pm 2	6 \pm 3	7 \pm 2					
5000	6 \pm 1	12 \pm 0	9 \pm 3	9 \pm 2	3 \pm 1					
Pos	301 \pm 16	663 \pm 26	324 \pm 60	1092 \pm 264	207 \pm 23					

Liver Microsomes: Rat liver S9

Dose (μ g)	TA98		TA100		TA1535		TA1537		WP2 uvrA	
0.0	16 \pm 3	137 \pm 19	11 \pm 4	8 \pm 1	13 \pm 3					
25	17 \pm 1	115 \pm 7	11 \pm 1	9 \pm 3	17 \pm 2					
75	16 \pm 1	136 \pm 11	6 \pm 3	5 \pm 1	14 \pm 2					
200	18 \pm 4	127 \pm 12	9 \pm 4	6 \pm 1	18 \pm 2					
600	12 \pm 2	119 \pm 10	9 \pm 3	5 \pm 2	14 \pm 2					
1800	12 \pm 2	96 \pm 33	10 \pm 4	8 \pm 5	10 \pm 3					
5000	7 \pm 5	27 \pm 2	8 \pm 3	4 \pm 2	7 \pm 6					
Pos	274 \pm 56	687 \pm 83	74 \pm 11	80 \pm 16	229 \pm 78					

0.0 = Vehicle plating aliquot of 50 μ L

Pos = Positive Control concentrations as specified in Materials and Methods section.

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Study Outcome:

7. According to the predefined criteria toxicity was generally observed at ≥ 1800 or at 5000 ug per plate.
8. No positive responses were observed with any of the tester strains in the absence or presence of S9 metabolic activation.

Study Validity:

The study was valid:

10. The mean number of spontaneous revertants in the vehicle controls were within the characteristic value ranges specified in the methods.
11. The mean number of revertants for each positive control was at least three-fold the vehicle control value.
12. A minimum of 3 non-toxic dose levels was available for all tester strains and conditions to evaluate the assay data.

SUMMARY:

Under the conditions of the assay, 6-decylaminohexyltrimethyl ammonium chloride hydrochloride was negative in the Ames bacterial reverse mutation assay with and without metabolic activation.

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**CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY (CHO) CELLS
CONDUCTED WITH A TEST ARTICLE EXTRACT**

Study title: Chromosome aberrations in chinese hamster ovary (CHO) cells
conducted with a test article (colesevelam HCl) extract
Study No: GT-02-TX-31
Study Type: Cytogenetics assay
Volume #, Page #: Vol. 1.17 (Appendix 5-21)
Conducting Laboratory: _____
Study Initiation Date: January 25, 1999
Study Completion Date: May 20, 1999
GLP Compliance: Yes
QA- Reports: Yes (x) No ()
Drug Lot Number: TMAC015-1868
Study Endpoint: *In vitro* clastogenesis

METHODOLOGY

Cell line: Chinese Hamster Ovary cells (CHO cells)
Vehicle control (solvent): 0.1 N HCL (extraction blank control).
Negative Controls: 0.1 N HCl
Positive Controls: Mitomycin C (MMC) (non-activated assay); Cyclophosphamide
(activated assay)
Preparation of extract: Two (2) g of test article was extracted with 40 ml 0.1 N HCl for
three days, and extract was decanted and stored at room
temperature.
Dosing solutions used: Test article extract concentrations used in the aberration assays
were based on cell growth inhibition in concurrent toxicity tests.
Maximum concentrations used were not allowed to induce more
than ca. 70% of growth inhibition. In the definitive assay three
dose levels were selected from the results of the concurrent
toxicity tests. Test article extract concentrations in dosing
solutions were expressed as ul/ml, which means ul extract/ml
solvent.
Metabolic activation system: Aroclor 1254-induced rat liver S9
Aberration assay method: Duplicate cultures of CHO cells were exposed to test article
extract, positive or extraction blank controls by adding 500 ul of
dosing solution to 4.5 ml cell medium with/out S-9 mixture.
Cell exposure: Cells in non-activated assay were exposed for 4h or
continuously for 20h. Colcemid was added to duplicate flasks
(0.1 ug/ml) and flasks returned until cell collection.
Cells in S-9 activated study were exposed for 4h, washed,
returned to incubator and treated with Colcemid two hours before
collection.
Cell collection: Two h after colcemid addition, metaphase cells were harvested
by _____ Cells were collected approximately 20h after
treatment initiation, fixed, and mounted on slides.
ANALYSIS:
Scoring method: Mitotic index was determined for each group. A minimum of 200
metaphase spreads (100 per duplicate flask) were scored for
chromatid-type and chromosome-type aberrations. If a positive
result was obtained in the non-activated 4h exposure group, the
20h group was not evaluated for aberrations.
Cytotoxic endpoints: Cell growth inhibition

<u>Genetic toxicity endpoints:</u>	Number and types of aberrations/100 cells, % of structurally and numerically damaged cells, mean aberrations per cell. Gaps are not included in the % of cells with aberrations or in the frequency of structural aberrations/cell.
<u>Statistical methods:</u>	Fisher's exact test. Test was used to compare pairwise the % aberrant cells between treatment and control groups. When the Fisher test was positive at any dose level, the Cochran-Armitage test was used to measure dose-responsiveness.
<u>Criteria for Positive Results:</u>	A positive response is defined as a dose-responsive increase in the % of cells with aberrations, with one or more concentrations being statistically significant.
<u>Criteria for Valid Test:</u>	(1) Frequency of cells with structural chromosome aberrations in the extraction blank control in the range of the historical negative control. (2) Percentage of cells with aberrations in the positive control statistically increased ($p \leq 0.05$, Fisher's test) relative to extraction blank control.

RESULTS:

Precipitate/Osmolality

Visible precipitate was present in treatment medium at all dose levels tested. Osmolality of treatment medium of the highest concentrations tested (100 ug/ml) was 259 mmol/kg. Osmolality of extraction blank medium was 262 mmol/kg. The pH of the highest concentration treatment medium was 6.5.

Aberration test results

The following Tables (Table 2, Table 4, Table 6) show the results of the three aberration assays:
 Table 2: Without metabolic activation, 4h treatment, 16h recovery
 Table 4: With metabolic activation, 4h treatment, 16h recovery
 Table 6: Without metabolic activation, 20h treatment

Table 7 summarizes the results.

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TABLE 2
CYTOGENETIC ANALYSIS OF CHO CELLS TREATED WITH THE EXTRACT OF Colesevelam hydrochloride IN THE
ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

4 HOUR TREATMENT, 16 HOUR RECOVERY PERIOD

Treatment ^{1,2}	Flask	Mitotic Index ²	Cells Scored	Cells with Aberrations ³		Number of Structural Aberrations ⁴						Severely Damaged Cells ⁵	Average Structural Aberrations Per Cell ^{6,7}
				Numerical	Structural	Chromatid-type ⁴		Chromosome-type ⁴					
						Gaps	Breaks	Exch	Breaks	Dic	Ring		
0.1N HCl	A	2.4	100	2	2	0	2	0	0	0	0	0	0.020
	B	5.2	100	2	0	0	0	0	0	0	0	0	
Colesevelam hydrochloride extract													
13 µL/mL	A	5.0	100	1	5	0	1	0	2	2	0	0	0.050
	B	3.6	100	2	2	1	0	0	0	3	0	0	
25 µL/mL	A	3.8	100	1	1	1	0	0	0	1	0	0	0.010
	B	2.8	100	1	5	2	5	0	0	0	0	0	
50 µL/mL	A	3.4	100	3	5	1	3	0	1	1	1	0	0.060
	B	1.6	100	0	1	0	1	0	0	0	0	0	
MPC, 0.15 µg/mL	A	4.4	100	5	6	1	5	0	0	1	0	0	0.060
	B	3.6	100	3	7	0	6	1	0	0	0	0	

- ¹ CHO cells were treated for 4 hours at 37±1°C in the absence of an exogenous source of metabolic activation.
 - ² Mitotic index = number mitotic figures x 100/500 cells counted.
 - ³ Numerical: includes polyploid and endoreduplicated cells.; Structural: excludes cells with only gaps.
 - ⁴ Chromatid breaks include chromatid and isochromatid breaks and fragments; chromatid exchange figures (Exch) include quadriradials, triradials and complex rearrangements.
 - ⁵ Chromosome breaks include breaks and acentric fragments; dic, dicentric chromosome.
 - ⁶ Severely damaged cells includes cells with one or more pulverized chromosomes and cells with 10 or more aberrations.
 - ⁷ Severely damaged cells and pulverizations were counted as 10 aberrations.
- An additional dose level of 7 µL/mL was tested as a safeguard against excessive toxicity at higher dose levels but was not required for microscopic examination. Dose level 100 µL/mL was not analyzed due to excessive toxicity.

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TABLE 4

CYTOGENETIC ANALYSIS OF CHO CELLS TREATED WITH THE EXTRACT OF Colesevelam hydrochloride IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

4 HOUR TREATMENT, 16 HOUR RECOVERY PERIOD

Treatment ^{1,2}	Flask	Mitotic Index ²	Cells Scored	Cells with Aberrations ³		Number of Structural Aberrations						Severely Damaged Cells ⁴	Average Structural Aberrations Per Cell ^{5,7}
				Numerical	Structural	Chromatid-type Gaps	Chromatid-type Breaks	Chromosome-type Exch	Chromosome-type Breaks	Chromosome-type Dic	Chromosome-type Ring		
0.1N HCl	A	2.8	100	2	0	0	0	0	0	0	0	0	0.000
	B	3.4	100	3	0	0	0	0	0	0	0	0	0.000
Colesevelam hydrochloride extract													
25 μ L/mL	A	8.6	100	4	1	1	0	0	0	1	0	0	0.010
	B	7.8	100	3	1	0	0	0	0	0	1	0	0.010
50 μ L/mL	A	5.8	100	2	1	2	0	0	0	1	0	0	0.010
	B	7.2	100	3	1	3	0	1	0	0	0	0	0.010
100 μ L/mL	A	4.2	100	3	3	2	0	0	0	2	1	0	0.030
	B	4.2	100	2	6	3	2	2	1	1	0	0	0.060
EP, 10 μ B/mL	A	2.0	100	2	12	2	7	4	1	1	1	0	0.140
	B	2.2	100	3	13	1	11	6	2	1	1	0	0.210

¹ CHO cells were treated for 4 hours at 37 \pm 1°C in the presence of an exogenous source of metabolic activation.

² Mitotic index = number mitotic figures x 100/500 cells counted.

³ Numerical: includes polyploid and endoreduplicated cells.; Structural: excludes cells with only gaps.

⁴ Chromatid breaks include chromatid and isochromatid breaks and fragments; chromatid exchange figures (Exch) include quadriradials, triradials and complex rearrangements.

⁵ Chromosome breaks include breaks and acentric fragments; dic, dicentric chromosome.

⁶ Severely damaged cells includes cells with one or more pulverized chromosomes and cells with 10 or more aberrations.

⁷ Severely damaged cells and pulverizations were counted as 10 aberrations.

⁸ Additional dose levels of 7 and 15 μ L/mL were tested as a safeguard against excessive toxicity at higher dose levels but were not required for microscopic examination.

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TABLE 6
CYTOGENETIC ANALYSIS OF CHO CELLS TREATED WITH THE EXTRACT OF Colesevelam hydrochloride IN THE
ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

20 HOUR CONTINUOUS TREATMENT

Treatment ^{1,2}	Flask	Mitotic Index ²	Cells Scored	Cells with Aberrations ³		Number of Structural Aberrations, Chromatid-type						Severely Damaged Cells ⁴	Average Structural Aberrations Per Cell ^{5,7}
				Numerical	Structural	Gaps	Breaks	Exch	Breaks	Dic	Ring		
0.1N HCl	A	3.0	100	3	0	0	0	0	0	0	0	0	0.000
	B	3.6	100	4	1	0	0	0	0	0	1	0	0.010
Colesevelam hydrochloride extract													
7 µL/mL	A	5.8	100	3	1	2	0	0	0	1	0	0	0.010
	B	7.6	100	3	1	1	0	0	0	1	0	0	0.010
13 µL/mL	A	5.8	100	4	1	0	0	0	1	0	0	0	0.010
	B	4.6	100	4	0	0	0	0	0	0	0	0	0.000
25 µL/mL	A	4.0	100	2	0	2	0	0	0	0	0	0	0.000
	B	3.2	100	2	1	1	1	0	0	0	0	0	0.010
MMC, 0.08 µg/mL	A	2.6	100	4	11	0	4	4	1	2	0	0	0.110
	B	2.0	100	3	9	1	8	0	1	2	0	0	0.110

¹ CHO cells were treated for 20 hours at 37±1°C in the absence of an exogenous source of metabolic activation.

² Mitotic index = number mitotic figures x 100/500 cells counted.

³ Numerical: includes polyploid and endoreduplicated cells.; Structural: excludes cells with only gaps.

⁴ Chromatid breaks include chromatid and isochromatid breaks and fragments; chromatid exchange figures (Exch) include quadriradials, triradials and complex rearrangements.

⁵ Chromosome breaks include breaks and acentric fragments; dic, dicentric chromosome.

⁶ Severely damaged cells includes cells with one or more pulverized chromosomes and cells with 10 or more aberrations.

⁷ Severely damaged cells and pulverizations were counted as 10 aberrations.

⁸ Dose levels 50 and 100 µL/mL were not analyzed due to excessive toxicity.

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TABLE 7

SUMMARY

Treatment	S9 Activation	Treatment ¹ Time (Hours)	Mitotic Index	Cells Scored	Aberrations Per Cell ² (Mean \pm SD)	Cells With Aberrations ³ (%)	
						Numerical	Structural
0.1N HCl	-	4	3.8	200	0.010 \pm 0.100	2.0	1.0
Colestesvelam hydrochloride extract							
13 μ L/mL	-	4	4.3	200	0.040 \pm 0.221	1.5	3.5
25 μ L/mL	-	4	3.3	200	0.030 \pm 0.171	1.0	3.0
50 μ L/mL	-	4	2.5	200	0.035 \pm 0.210	1.5	3.0
MHC, 0.15 μ g/mL	-	4	4.0	200	0.065 \pm 0.247	4.0	6.5**
0.1N HCl	+	4	3.1	200	0.000 \pm 0.000	2.5	0.0
Colestesvelam hydrochloride extract							
25 μ L/mL	+	4	6.2	200	0.010 \pm 0.100	3.5	1.0
50 μ L/mL	+	4	6.5	200	0.010 \pm 0.100	2.5	1.0
100 μ L/mL	+	4	4.2	200	0.045 \pm 0.208	2.5	4.5**
CP, 10 μ g/mL	+	4	2.1	200	0.175 \pm 0.544	2.5	12.5**
0.1N HCl	-	20	3.3	200	0.005 \pm 0.071	3.5	0.5
Colestesvelam hydrochloride extract							
7 μ L/mL	-	20	6.7	200	0.010 \pm 0.100	3.0	1.0
13 μ L/mL	-	20	5.2	200	0.005 \pm 0.071	4.0	0.5
25 μ L/mL	-	20	3.6	200	0.005 \pm 0.071	2.0	0.5
MHC, 0.08 μ g/mL	-	20	2.3	200	0.110 \pm 0.344	3.5	10.0**

¹ Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.

² Severely damaged cells were counted as 10 aberrations.

³ *, $p \leq 0.05$; **, $p \leq 0.01$; Fisher's exact test.

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Study Outcome:

1. Assay without metabolic activation, 4h treatment, 16h recovery: Growth inhibition was 69% at 50 ug/ml. Mitotic index at the highest dose level evaluated of 50 ug./ml was 34% reduced as compared to extraction blank control. There was no statistically significant elevation in the percentage of cells with structural or numerical chromosome aberration.
2. Assay with metabolic activation, 4h treatment, 16h recovery: Growth inhibition was 52% at 100 ug/ml. Mitotic index at the highest dose level of 100 ug/ml was not reduced as compared to extraction blank control, but was reduced by 48% as compared to the lowest extract dose level. There was a statistically significant elevation in the percentage of cells with structural chromosome aberrations at the dose level of 100 ug/ml ($p \leq 0.01$, Fishers's test). There was also a statistically significant positive dose-response for the % of cells with structural aberrations (Cochran-Armitage test, $p \leq 0.05$). There was no statistically significant elevation in the percentage of cells with numerical chromosome aberrations.

Reviewers Comment:

For the assay with metabolic activation (4h treatment, 16h recovery), the Sponsor noted that the % of structurally aberrant cells found at the 100 ug/ml dose level (4.5%) was within the % of aberrant cells observed in the historical negative control range (0-6.5%), see APPENDIX. Therefore, the Sponsor concluded that the statistically significant increase in the % of aberrant cells at this dose level was not biologically relevant. This however is not in agreement with the Sponsor's own criteria for a positive result, and this Reviewer does not agree with their conclusion.

3. Assay without metabolic activation, 20h treatment: Growth inhibition was 55% at 25 ug/ml. Mitotic index at the highest dose level of 25 ug./ml was not reduced as compared to extraction blank control. There was no statistically significant elevation in the percentage of cells with structural or numerical chromosome aberrations

Study Validity:

The study was valid:

- (1) The frequencies of cells with structural chromosome aberrations in the extraction blank controls were in the range of the historical negative controls.
- (2) All three positive controls (MMC in the two assays without metabolic activation; CP in the assay with metabolic activation) showed a significant elevation in the percentage of cells with structural chromosome aberrations.

Reviewers Comment: Guidelines recommend that relatively insoluble substances should be tested up to the limit of solubility. In this assay precipitate was observed at all dose levels (7-100 ug/ml). For this reason one might disqualify the findings of all three assays. However, this Reviewer feels the findings cannot be dismissed for this reason. At all dose levels precipitate was present, yet only at the high dose of 100 ug/ml in the test with metabolic activation a positive response was observed. Therefore it seemed that the presence of precipitate *per se* did not cause an artificial dose-dependent increase in chromosome aberrations.

SUMMARY:

Under the conditions of the assay, colesevelam hydrochloride extract was negative in the chromosome aberration in Chinese Hamster Ovary (CHO) cells without metabolic activation. Under the conditions of the assay, colesevelam hydrochloride extract was positive in the chromosome aberration in Chinese Hamster Ovary (CHO) cells with metabolic activation.

APPENDIX

**IN VITRO MAMMALIAN CYTOGENETIC TEST USING
CHINESE HAMSTER OVARY (CHO) CELLS**

**HISTORICAL CONTROL VALUES
STRUCTURAL ABERRATIONS
1995-1997**

NON-ACTIVATED TEST SYSTEM

Historical Values	Aberrant Cells		
	Untreated Control -	Solvent Control ¹	Positive Control ²
Mean	1.1%	1.2%	27.5%
Standard Deviation	1.0%	1.3%	19.1%
Range	0.0% to 4.5%	0.0% to 6.0%	7.0% to 100.0%

S9-ACTIVATED TEST SYSTEM

Historical Values	Aberrant Cells		
	Untreated Control	Solvent Control ¹	Positive Control ³
Mean	1.3%	1.5%	39.0%
Standard Deviation	1.2%	1.4%	22.8%
Range	0.0% to 5.5%	0.0% to 6.5%	6.5% to 100.0%

¹Solvents include water, saline, dimethylsulfoxide, ethanol, acetone, non-standard solvents and Sponsor-supplied vehicles.

²Positive control for non-activated studies, triethylenemelamine (TEM, 0.25-0.5 µg/ml), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG, 0.75-2 µg/ml), and Mitomycin C (MMC, 0.08-0.15 µg/ml).

³Positive control for S9-activated studies, cyclophosphamide (CP, 10-50 µg/ml), and benzo(α)pyrene, (B[α]P, 30 µg/ml).

IN VITRO MAMMALIAN CHROMOSOME ABERRATION WITH DECYLAMINE HYDROCHLORIDE

Study Title: In Vitro Mammalian Chromosome Aberration Test
Study No: GT-0069-TX-2
Study Type: Cytogenetics assay
Volume #, Page #: Vol. 1.18 (Appendix 5-26)
Conducting Laboratory: _____
Study Initiation Date: February 18, 1999
Study Completion Date: May 28, 1999
GLP Compliance: Yes
QA- Reports: Yes (x) No ()
Drug Lot Number: P-1039-174A7
Study Endpoint: *In vitro* clastogenesis

METHODOLOGY

Cell line: Chinese Hamster Ovary cells (CHO cells)
Vehicle control (solvent): Distilled water
Negative Controls: Water
Positive Controls: Mitomycin C (MMC) (non-activated assay); Cyclophosphamide (activated assay)
Preparation of test article: Test article (decylamine HCL) was received from Sponsor and was dissolved in water. Dosing solutions were adjusted to compensate for the free-base portion of the test article (— g of free base/g test article)
Doses used in assays: Preliminary toxicity tests to determine cell growth inhibition were performed to select the dose levels to be tested in the aberration assays. Maximum dose in these tests was 5000 ug/ml. Concurrent toxicity tests with the selected dose levels were then performed to determine the dose levels to be tested in the definitive chromosome aberrations assays.
Metabolic activation system: Aroclor 1254-induced rat liver S9
Aberration assay method: Duplicate cultures of CHO cells were exposed to test article, positive control or solvent alone by adding 500 ul of dosing solution to 4.5 ml cell medium with/out S-9 mixture.
Cell exposure: Cells in non-activated assay were exposed to extract for 4h or continuously for 20h. Colcemid was added to duplicate flasks (0.2 ug/ml) and flasks returned until cell collection. Cells in S-9 activated study were exposed for 4h, washed, returned to incubator and treated with Colcemid two hours before collection.
Cell collection: Two h after colcemid addition, metaphase cells were harvested by _____. Cells were collected approximately 20h after treatment initiation, fixed, and mounted on slides.

ANALYSIS:

Scoring method: Mitotic index was determined for each group. A minimum of 200 metaphase spreads (100 per duplicate flask) were scored for chromatid-type and chromosome-type aberrations. If a positive result was obtained in the non-activated 4h exposure group, the 20h group was not evaluated for aberrations.
Cytotoxic endpoints: Cell growth inhibition
Genetic toxicity endpoints: Number and types of aberrations/100 cells, % of structurally and numerically damaged cells, mean aberrations per cell. Gaps are

not included in the % of cells with aberrations or in the frequency of structural aberrations/cell.

Statistical methods:

Fisher's exact test. Test was used to compare pairwise the % aberrant cells between treatment and control groups. When the Fisher test was positive at any dose level, the Cochran-Armitage test was used to measure dose-responsiveness.

Criteria for Positive Results:

A positive response is defined as a dose-responsive increase in the % of cells with aberrations, with one or more concentrations being statistically significant.

Criteria for Valid Test:

(1) Frequency of cells with structural chromosome aberrations in the extraction blank control in the range of the historical negative control. (2) Percentage of cells with aberrations in the positive control statistically increased ($p \leq 0.05$, Fisher's test) relative to extraction blank control.

RESULTS:

Precipitate/Osmolality

Test article was soluble in treatment medium at all concentrations tested. Osmolality of treatment medium of the highest concentrations tested (30 ug/ml) was 291 mmol/kg. Osmolality of the solvent (water) in treatment medium was 293 mmol/kg. The pH of the highest concentration treatment medium was ca. 7.0.

Preliminary toxicity tests

Based upon the results of these toxicity tests the dose levels selected for testing in the aberration assay were as follows:

Treatment Condition	Treatment Time	Recovery Time	Dose Levels (ug/ml)
-S9	4h	16h	2, 4, 8, 13, 15, 23, 30
-S9	20h	0h	0.5, 1, 2, 4, 8, 13, 15
+S9	4h	16h	2, 4, 8, 13, 15, 23, 30

Aberration test results

The following Tables (Tables 5, 7, 9) show the results of the three definitive aberration assays:

Table 5: Without metabolic activation, 4h treatment, 16h recovery

Table 7: With metabolic activation, 4h treatment, 16h recovery

Table 9: Without metabolic activation, 20h treatment

Table 10 summarizes the results.

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TABLE 5
 CYTOGENETIC ANALYSIS OF CHO CELLS TREATED WITH Decylamine HCl IN THE
 ABSENCE OF EXOGENOUS METABOLIC ACTIVATION
 4 HOUR TREATMENT, 16 HOUR RECOVERY PERIOD

Treatment ^{1,2} Exch	Fask Breaks	Mitotic Index ²	Cells Scored Damaged	Cells with Aberrations ³ (%)		Number of Structural Aberrations ⁴						Severely Damaged Cells ⁵	Average Structural Per Cell ^{3,7}
				Numerical	Structural	Chromatid-type Gaps	Chromatid-type Breaks	Chromosome-type ⁶	Chromosome-type ⁶	Chromosome-type ⁶	Chromosome-type ⁶		
Water	A	7.6	100	3	0	0	0	0	0	0	0	0	0.000
	B	9.0	100	3	0	0	0	0	0	0	0	0	0.000
Decylamine HCl 8 µg/mL	A	5.0	100	2	3	1	3	0	0	0	0	0	0.030
	B	3.4	100	4	1	0	1	0	0	0	0	0	0.010
13 µg/mL	A	3.8	100	8	0	0	0	0	0	0	0	0	0.000
	B	3.0	100	9	1	1	1	0	0	0	0	0	0.010
15 µg/mL	A	2.4	100	7	5	0	4	1	0	0	0	0	0.050
	B	3.2	100	5	5	1	4	1	3	0	0	0	0.080
MNC, 0.08 µg/mL	A	6.0	100	4	8	0	6	3	1	0	0	0	0.100
	B	8.4	100	3	8	0	7	3	0	1	1	0	0.120

¹ CHO cells were treated for 4 hours at 37±1°C in the absence of an exogenous source of metabolic activation.
² Mitotic index = number mitotic figures x 100/500 cells counted.
³ Numerical: includes polyploid and endoreduplicated cells.; Structural: excludes cells with only gaps.
⁴ Chromatid breaks include chromatid and isochromatid breaks and fragments; chromatid exchange figures (Exch) include quadriradials, triradials and complex rearrangements.
⁵ Chromosome breaks include breaks and acentric fragments; dic, dicentric chromosomes.
⁶ Severely damaged cells includes cells with one or more pulverized chromosomes and cells with 10 or more aberrations.
⁷ Severely damaged cells and pulverizations were counted as 10 aberrations.
⁸ Additional dose levels of 2 and 4 µg/mL were tested as a safeguard against excessive toxicity at higher dose levels but were not required for microscopic examination. Dose levels 23 and 30 µg/mL were not analyzed due to excessive toxicity.

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TABLE 7
 CYTOGENETIC ANALYSIS OF CHO CELLS TREATED WITH Decylamine HCl IN THE
 PRESENCE OF EXOGENOUS METABOLIC ACTIVATION
 4 HOUR TREATMENT, 16 HOUR RECOVERY PERIOD

Treatment ^{1,2}	Flask	Mitotic Index ²	Cells Scored	Cells with Aberrations ³ (%)		Number of Structural Aberrations						Severely Damaged Cells ⁵	Average Structural Aberrations Per Cell ⁶
				Numerical	Structural	Chromatid-type Gaps	Chromatid-type Breaks	Chromosome-type Exch	Chromosome-type Breaks	Dic	Ring		
Water	A	6.8	100	5	0	0	0	0	0	0	0	0	0.000
	B	11.8	100	5	1	0	0	0	0	2	0	0	0.020
Decylamine HCl 4 µg/mL	A	4.8	100	4	2	0	1	1	0	0	0	0	0.020
	B	3.0	100	4	0	1	0	0	0	0	0	0	0.000
8 µg/mL	A	5.8	100	5	1	0	0	0	0	1	0	0	0.010
	B	5.4	100	4	1	0	0	0	0	1	0	0	0.010
13 µg/mL	A	2.4	100	4	3	4	4	0	0	0	0	0	0.040
	B	1.4	100	6	1	1	1	0	0	0	0	0	0.010
CP, 10 µg/mL	A	1.4	100	4	13	1	4	10	0	4	2	0	0.200
	B	2.2	100	3	15	2	9	2	2	4	0	0	0.170

¹ CHO cells were treated for 4 hours at 37±1°C in the presence of an exogenous source of metabolic activation.
² Mitotic index = number mitotic figures x 100/500 cells counted.
³ Numerical: includes polyploid and endoreduplicated cells.; Structural: excludes cells with only gaps.
⁴ Chromatid breaks include chromatid and isochromatid breaks and fragments; chromatid exchange figures (Exch) include quadriradials, triradials and complex rearrangements.
⁵ Chromosome breaks include breaks and acentric fragments; dic, dicentric chromosome.
⁶ Severely damaged cells includes cells with one or more pulverized chromosomes and cells with 10 or more aberrations.
⁷ Severely damaged cells and pulverizations were counted as 10 aberrations.
⁸ An additional dose level of 2 µg/mL was tested as a safeguard against excessive toxicity at higher dose levels but was not required for microscopic examination. Dose levels 15, 25, and 30 µg/mL were not analyzed due to excessive toxicity.

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TABLE 9
CYTOGENETIC ANALYSIS OF CHO CELLS TREATED WITH Decylamine HCl IN THE
ABSENCE OF ENDOGENOUS METABOLIC ACTIVATION

20 HOUR CONTINUOUS TREATMENT

Treatment ^{1,2}	Flask	Mitotic Index ²	Cells Scored	Cells with Aberrations ³		Number of Structural Aberrations					Severely Damaged Cells ⁴	Average Structural Aberrations Per Cell ^{5,7}	
				Numerical	Structural	Chromatid-type Gaps	Chromatid-type Breaks	Exchange	Chromosome-type Breaks	Dic			Ring
Water	A	5.8	100	2	0	0	0	0	0	0	0	0	0.000
	B	7.2	100	2	0	0	0	0	0	0	0	0	0.000
Decylamine HCl 4 µg/mL	A	6.0	100	5	1	0	1	0	0	0	1	0	0.020
	B	6.2	100	1	1	0	0	0	1	0	0	0	0.010
8 µg/mL	A	5.2	100	2	4	0	1	1	2	0	0	0	0.040
	B	5.8	100	1	2	1	2	0	1	2	0	0	0.050
13 µg/mL	A	3.6	100	3	0	0	0	0	0	0	0	0	0.000
	B	3.6	100	1	2	0	1	0	1	0	0	0	0.020
MPC 0.08 µg/mL	A	4.4	100	3	14	0	10	6	2	0	0	0	0.180
	B	3.6	100	0	12	0	11	2	0	0	0	0	0.130

¹ CHO cells were treated for 20 hours at 37±1°C in the absence of an exogenous source of metabolic activation.

² Mitotic index = number mitotic figures x 100/500 cells counted.

³ Numerical: includes polyploid and endoreduplicated cells.; Structural: excludes cells with only gaps.

⁴ Chromatid breaks include chromatid and isochromatid breaks and fragments; chromatid exchange figures (Exch) include quadriradiats, triradiats and complex rearrangements.

⁵ Chromosome breaks include breaks and acentric fragments; dic, dicentric chromosome.

⁶ Severely damaged cells includes cells with one or more pulverized chromosomes and cells with 10 or more aberrations.

⁷ Severely damaged cells and pulverizations were counted as 10 aberrations.

⁸ Additional dose levels of 0.5, 1, and 2 µg/mL were tested as a safeguard against excessive toxicity at higher dose levels but were not required for microscopic examination. Dose level 15 µg/mL was not analyzed due to excessive toxicity.

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TABLE 10

SUMMARY

Treatment	S9 Activation	Treatment ¹ Time (Hours)	Mitotic Index	Cells Scored	Aberrations Per Cell ² (Mean ± SD)	Cells With Aberrations ³ (%)	
						Numerical	Structural
Water	-	4	8.3	200	0.000 ± 0.000	3.0	0.0
Decylamine HCl							
8 µg/mL	-	4	4.2	200	0.020 ± 0.140	3.0	2.0
13 µg/mL	-	4	3.4	200	0.005 ± 0.071	8.5*	0.5
15 µg/mL	-	4	2.8	200	0.063 ± 0.318	6.0	5.0**
MNC, 0.08 µg/mL	-	4	7.2	200	0.110 ± 0.423	3.5	8.0**
Water	+	4	9.3	200	0.010 ± 0.141	5.0	0.5
Decylamine HCl							
4 µg/mL	+	4	3.9	200	0.010 ± 0.100	4.0	1.0
8 µg/mL	+	4	5.6	200	0.010 ± 0.100	4.5	1.0
13 µg/mL	+	4	1.9	200	0.025 ± 0.186	5.0	2.0
CP, 10 µg/mL	+	4	1.8	200	0.185 ± 0.522	3.5	14.0**
Water	-	20	6.5	200	0.000 ± 0.000	2.0	0.0
Decylamine HCl							
4 µg/mL	-	20	6.1	200	0.015 ± 0.158	3.0	1.0
8 µg/mL	-	20	5.5	200	0.045 ± 0.289	1.5	3.0*
13 µg/mL	-	20	3.6	200	0.010 ± 0.100	2.0	1.0
MNC 0.08 µg/mL	-	20	4.0	200	0.155 ± 0.438	1.5	13.0**

¹ Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.

² Severely damaged cells were counted as 10 aberrations.

³ *, p<0.05; **, p<0.01; Fisher's exact test.

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Study Outcome:

- (1) Assay without metabolic activation, 4h treatment, 16h recovery: Growth inhibition was 52% at 15 ug/ml. Mitotic index at the highest dose level evaluated of 50 ug./ml was 66% reduced as compared to vehicle control. There was a statistically significant elevation in the percentage of cells with structural chromosome aberrations at 15 ug/ml ($p \leq 0.01$). The dose-response test for the % of cells with structural aberrations was also statistically significant ($p \leq 0.05$). There was also a statistically significant elevation in the percentage of cells with numerical chromosome aberrations at 13 ug/ml. The dose-response test for the % of cells with numerical aberrations was also positive ($p \leq 0.05$).

Reviewers Comment:

The Sponsor concluded that, since the % of structurally aberrant cells found at the 15 ug/ml dose level (5.0%) in this study was within the range of structurally aberrant cells observed with the historical solvent control (0-6%, see APPENDIX), the statistically significant increase in the % of aberrant cells at this dose level was not considered biologically relevant. This is not in agreement with the Sponsor's own criteria for a positive result, and this Reviewer does not agree with their conclusion.

The Sponsor also concluded that, since the % of numerically aberrant cells found at the 13 ug/ml dose level (8.5%) was only 2% outside the range of the % of aberrant cells observed with the historical solvent control (0-6.5%), the statistically significant increase in the % of aberrant cells at this dose level was not considered biologically relevant. In the opinion of this Reviewer, the significance of the increase in numerical aberrations in this test is unclear.

- (2) Assay with metabolic activation, 4h treatment, 16h recovery: Growth inhibition was 63% at 13ug/ml. Mitotic index at the highest dose level of 13ug./ml was 80% reduced as compared to the vehicle control. There was no statistically significant elevation in the percentage of cells with structural or numerical chromosome aberrations.
- (3) Assay without metabolic activation, 20h treatment: Growth inhibition was 58% at 13 ug/ml. Mitotic index at the highest dose level of 13 ug./ml was 45% reduced as compared to vehicle control. There was a statistically significant elevation in the percentage of cells with structural chromosome aberrations at the mid dose of 8 ug/ml ($p \leq 0.01$). However, the dose-response test for the % of structurally aberrant cells was not statistically significant. There was no statistically significant elevation in the percentage of cells with numerical chromosome aberrations.

Reviewers Comment:

The Sponsor concluded that, since the % of structurally aberrant cells found at the 8 ug/ml dose level (3.0%) in Study (3) was within the % of aberrant cells observed in the historical negative control range (0-6.5%, see APPENDIX), the statistically significant increase in the % of aberrant cells at this dose level was not considered biologically relevant. In the opinion of the Reviewer, however, it can be concluded that the test results was negative on the basis of the other criterion, i.e., the dose-response test being negative.

Study Validity:

The study was valid:

- (3) The frequencies of cells with structural chromosome aberrations in the vehicle controls were in the range of the historical negative controls.
- (4) All three positive controls (MMC in the two assays without metabolic activation; CP in the assay with metabolic activation) showed a significant elevation in the percentage of cells with structural chromosome aberrations.
- (5) For evaluation of numerical aberrations, this study appears to be inadequate. The positive controls did not induce significant increases in the % of cells with these types of aberrations.

SUMMARY:

Under the conditions of the assay (4h treatment, 16h recovery), the chromosome aberration test with decylamine HCl in CHO cells without metabolic activation was positive.

Under the conditions of the assay (20h treatment), the chromosome aberration test with decylamine HCl in CHO cells without metabolic activation was negative.

Under the conditions of the assay, the chromosome aberration test with decylamine HCl in CHO cells with metabolic activation was negative.

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APPENDIX

**IN VITRO MAMMALIAN CYTOGENETIC TEST USING
CHINESE HAMSTER OVARY (CHO) CELLS**

**HISTORICAL CONTROL VALUES
STRUCTURAL ABERRATIONS
1995-1997**

NON-ACTIVATED TEST SYSTEM

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Range	0.0% to 4.5%	0.0% to 6.0%	7.0% to 100.0%

S9-ACTIVATED TEST SYSTEM

Historical Values	Aberrant Cells		
	Untreated Control	Solvent Control ¹	Positive Control ³
Mean	1.3%	1.5%	39.0%
Standard Deviation	1.2%	1.4%	22.8%
Range	0.0% to 5.5%	0.0% to 6.5%	6.5% to 100.0%

¹Solvents include water, saline, dimethylsulfoxide, ethanol, acetone, non-standard solvents and Sponsor-supplied vehicles.

²Positive control for non-activated studies, triethylenemelamine (TEM, 0.25-0.5 µg/ml), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG, 0.75-2 µg/ml), and Mitomycin C (MMC, 0.08-0.15 µg/ml).

³Positive control for S9-activated studies, cyclophosphamide (CP, 10-50 µg/ml), and benzo(α)pyrene, (B[α]P, 30 µg/ml).

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