NDA 20-675

FDA CDER CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC)
RODENT CARCINOGENICITY FACTSHEET

NDA: 20-675

DRUG CODE #: RU 22990

CAS #: 128-13-2

DATE:

DIVISION(s): Gastrointestinal and Coagulation Drug Products

DRUG NAME(s): Ursodiol (URSO™), ursodeoxycholic acid

SPONSOR: AXCAN PHARMA U.S. INC.
Plattsburgh, NY 12901

LABORATORY: [redacted]

P/T REVIEW DATE: January 9, 1997

CARCINOGENICITY STUDY REPORT DATE: March 28, 1985

THERAPEUTIC CATEGORY: Primary biliary cirrhosis

PHARMACOLOGICAL/CHEMICAL CLASSIFICATION: Bile acid

PRIOR FDA DOSE CONCURRENCE (Div./CAC)? (Y/N; Date): No

MUTAGENIC/GENOTOXIC (Y/N/equivocal/na; assay): No (Ames test, forward mutation assay in mouse lymphoma cells, sister chromatid exchange assay in human lymphocytes, chromosomal aberrations assay in mouse germ cells, micronucleus test in Chinese hamster bone marrow cells, and chromosomal aberrations assay in Chinese hamster bone marrow cells)

RAT CARCINOGENICITY STUDY (multiple studies? Std1; Std2 etc): Std1.

RAT STUDY DURATION (weeks): 104

STUDY STARTING DATE: February 2, 1980

STUDY ENDING DATE: March 30, 1983

RAT STRAIN: Fischer 344

ROUTE: Dietary

NDA 20-675

DOSING COMMENTS:

	Males	Females
No. Rats in Control (C1):	50	50
Low Dose (LD):	50	50
Middle Dose (MD):	50	50
High Dose (HD):		
No. Rats/sex/dose at each interim sacrifice:	10	10

RAT DOSE LEVELS (mg/kg/day)

Rat Low Dose: 500 ppm (22.5 and 28.5 mg/kg/day in M and F)
Rat Middle Dose: 1700 ppm (77.2 and 97.5 mg/kg/day in M and F)
Rat High Dose: 5000 ppm (239 and 300 mg/kg/day in M and F)

Basis for Doses Selected (MTD; AUC ratio; saturation; maximum feasible): The sponsor stated that dosage selection was based upon preliminary results that indicated appropriate growth rate suppression; details were not provided.

RAT CARCINOGENICITY (negative; positive; MF; M; F): Negative, MF

RAT TUMOR FINDINGS: There were no treatment-related incidences of neoplastic lesions.

RAT STUDY COMMENTS: The sponsor stated that dosage selection was based upon preliminary results that indicated appropriate growth rate suppression; details were not provided. In the present study, mean body weights of males and females in the 5,000 ppm group were reduced by approximately 8-10% during Weeks 65-104. Furthermore, the highest dose of UDCA in the diet was 5%. According to guidelines published in the Federal Register in 1995 [60 FR 11278], the maximum feasible dose by dietary administration is considered to be 5% of the diet.

1. 104-Week Dietary Carcinogenic Study of UDCA (Study No. 200; Report No. 537)

Testing Laboratory: [redacted]

Compliance with Good Laboratory Practices and Quality Assurance Requirements: Carcinogenicity study was performed according to the Investigation Method for Carcinogenesis in Animals (Odajima, 1978) and the guidelines proposed by the U.S. EPA (August 22, 1978).

Study Started: February 2, 1984

Study Completed: March 28, 1985

Animals: Male (85-104 g; 5 weeks of age) and female (72-88 g; 5 weeks of age) Fischer 344 rats.

Methods: The sponsor stated that dosage selection was based upon preliminary results that indicated appropriate growth rate suppression; details were not provided. Thus, four groups of 180 rats each (90 males and 90 females) were administered 0, 500, 1,700 and 5,000 ppm (0.5%, 1.7% and 5.0%) of ursodiol, respectively, admixed in the diet for 104 weeks. As shown in the following table (from Vol. 19/page 14 of the sponsor's submission), there were 2 groups per sex and dose at Week 104. One group consisted of 10 animals and the second group consisted of 50 animals. Data was provided from the groups of 10 for mortality, body weight and food consumption, but not for gross pathology and histopathology [redacted]

Total 720 animals were used in groups consisting of 90 males and 90 females each.

Sex	Group	Dose level (ppm)	Number of animals	I.D. No. of animal sacrificed at				
				week 26	week 52	week 78	week 104	week 104
Male	1	0	90	1081 ~ 1090	1071 ~ 1080	1061 ~ 1070	1051 ~ 1060	1001 ~ 1050
	2	500	90	1181 ~ 1190	1171 ~ 1180	1161 ~ 1170	1151 ~ 1160	1101 ~ 1150
	3	1,700	90	1281 ~ 1290	1271 ~ 1280	1261 ~ 1270	1251 ~ 1260	1201 ~ 1250
	4	5,000	90	1381 ~ 1390	1371 ~ 1380	1361 ~ 1370	1351 ~ 1360	1301 ~ 1350
Female	1	0	90	2081 ~ 2090	2071 ~ 2080	2061 ~ 2070	2051 ~ 2060	2001 ~ 2050
	2	500	90	2181 ~ 2190	2171 ~ 2180	2161 ~ 2170	2151 ~ 2160	2101 ~ 2150
	3	1,700	90	2281 ~ 2290	2271 ~ 2280	2261 ~ 2270	2251 ~ 2260	2201 ~ 2250
	4	5,000	90	2381 ~ 2390	2371 ~ 2380	2361 ~ 2370	2351 ~ 2360	2301 ~ 2350

* Analysis of chemical cumulation in the organs was performed in survival animals (Study No. 441,

Rats were observed twice daily (except holidays) for clinical signs of toxicity and morbidity. Animals were weighed once a week for the first 26 weeks of treatment, and once every 2 weeks thereafter. Food consumption was measured weekly.

Blood samples for hematological and blood chemistry examinations were obtained during interim sacrifice at Weeks 26, 52 and 78 of treatment and at terminal sacrifice (Week 104). Blood samples were obtained via the abdominal aorta in 8 to 10 anesthetized males and females per treatment group. Animals were then sacrificed by exsanguination and subjected to gross pathological examination. Organ weights were determined for brain, heart, lung, liver, kidneys, spleen, adrenals and testes/ovaries.

Twenty-four h urine samples for urinalysis were collected from 10 males and 10 females per treatment group at Weeks 26, 52, 78 and 104.

At interim sacrifices (9 to 10 males and females in each treatment group) and terminal sacrifice of all surviving animals, tissue specimens were obtained from brain, heart, lung, liver, kidneys, spleen, adrenals, testes/ovaries, lesions identified during gross pathological examination, tumors, pituitary, thyroid, thymus, gallbladder, bronchi, spinal cord, eye, salivary glands, trachea, esophagus, stomach, small intestine, large intestine, pancreas, urinary bladder, prostate, uterus, mesenteric lymph nodes, sciatic nerve, skin, mammary glands, bone and femoral muscle. Complete histopathological examinations were done on all animals subjected to interim sacrifice, all surviving animals in the control and 5,000 ppm groups, and all dead and moribund animals. Histological examination of liver tissue specimens and tumors was done in all animals.

Data for body weight, food consumption, food efficiency, hematology, blood chemistry, organ weight, body and organ weight ratios were statistically analyzed with t-tests.

Results:

1. Achieved Doses: Mean achieved doses for males over 104 weeks were 22.5, 77.2 and 239 mg/kg/day for the 500, 1,700 and 5,000 ppm doses, respectively. Mean achieved doses for females over 104 weeks were 28.5, 97.5 and 300 mg/kg/day for the 500, 1,700 and 5,000 ppm doses, respectively.

2. Observed Effects: There were no treatment-related clinical signs of toxicity.

3. Mortality: As shown in the following table, there were no treatment-related mortalities. At termination, there were 47 (78.3%), 42 (70%), 42 (70%) and 42 (70%) surviving males in the 0, 500, 1,700 and 5,000 ppm groups, respectively, and 53 (8.8%), 51 (8.5%), 52 (8.7%), and 48 (80%) surviving females, respectively.

Cumulative mortality (# and %) during 104 weeks of treatment

Week No.	Males Ursodiol (ppm of diet)							
	0		500		1,700		5,000	
	#	%	#	%	#	%	#	%
26	0	0	0	0	0	0	0	0
52	0	0	0	0	1	1.7	0	0
78	1	1.4	2	3.3	2	3.3	3	5.0
104	13	21.7	18	30.0	18	30.0	18	30.0

Week No.	Females Ursodiol (ppm of diet)							
	0		500		1,700		5,000	
	#	%	#	%	#	%	#	%
26	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0
78	0	0	0	0	1	1.7	4	6.7
104	7	11.7	9	15.0	8	13.3	12	20.0

4. Body Weight: Mean body weights of control males and females were 94 and 80 g, respectively, during Week 0. Mean body weights of control males and females were 396 and 284 g, respectively, during Week 104. As shown in the following figures (from Vol. 19/ page 367 of sponsor's submission), mean body weights of males and females in the 5,000 ppm group were reduced by approximately 8-10% from Week 65 to the end of the experiment.

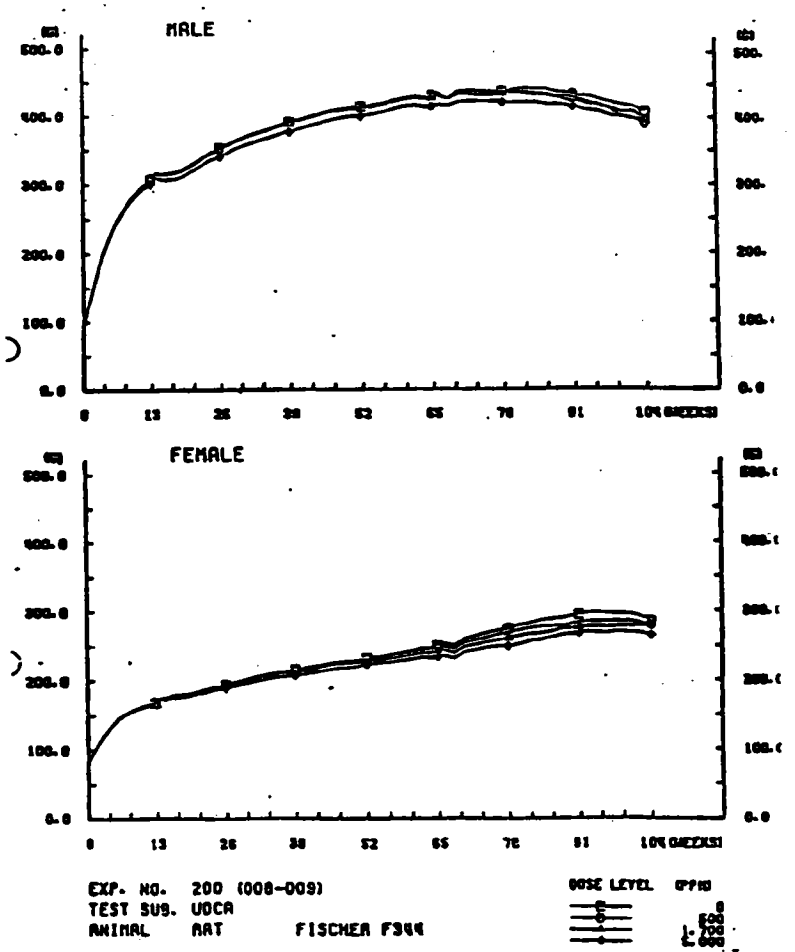

FIG.2 MEAN BODY WEIGHT

5. Food Consumption: Mean food consumption of control males and females was 12.1 and 10.3 g/day, respectively, during Week 1. Mean food consumption of control males and females was 15.7 and 12.9 g/day, respectively, during Week 104. There were no treatment-related effects on food consumption.

6. Hematology: There were no treatment-related effects.

7. Blood Chemistry: In males, glucose was increased by 29% (% of difference from control) and LAP was decreased by -17% at the 5,000 ppm dose during Week 104.

In females, glucose was increased by 15% at the 5,000 ppm dose and total cholesterol was reduced by -29% and -30% at the 1,700 and 5,000 ppm doses, respectively, during Week 104.

8. Urinalysis: There were no treatment-related effects.

9. Organ Weights: There were no treatment-related effects.

10. Gross Pathology: There were no treatment-related effects.

11. Histopathology:

Non-neoplastic

As shown in the following table, there were time-related and treatment-related increased incidences of bile duct fibrosis in males. The % incidence of bile duct fibrosis increased from Week 52 through 104 and as a function of UDCA dose. On the other hand, a high incidence of bile duct hyperplasia was already present in all interim sacrifice groups during Week 52.

Incidence of non-neoplastic lesions in males during 104 weeks of treatment

Group	Week	Lesion	Males Ursodiol (ppm of diet)			
			0	500	1700	5000
Interim Sacrifice	52	Bile duct fibrosis	0/10	0/10	1/10 (10%)	1/10 (10%)
	78		2/10 (20%)	3/10 (30%)	7/10 (70%)	8/10 (80%)
Main	104		36/50 (72%)	40/50 (80%)	48/50 (96%)	49/50 (98%)
Interim Sacrifice	52	Bile duct hyperplasia	7/10 (70%)	10/10 (100%)	10/10 (100%)	10/10 (100%)
	78		10/10 (100%)	10/10 (100%)	9/10 (90%)	10/10 (100%)
Main	Week		46/50 (92%)	50/50 (100%)	50/50 (100%)	47/50 (94%)

As shown in the following table, the % incidence of bile duct fibrosis increased from Week 78 to Week 104 and increased as a function of UDCA dose in females. The % incidence of bile duct hyperplasia was not time-related; incidences were relatively high during Week 52 in the UDCA groups, decreased during Week 78, and increased during Week 104. The % incidence of bile duct hyperplasia was dose-related during Week 104.

Incidence of non-neoplastic lesions in females during 104 weeks of treatment

Group	Week	Lesion	Females Ursodiol (ppm of diet)			
			0	500	1700	5000
Interim Sacrifice	52	Bile duct fibrosis	0/10	0/10	0/10	0/10
	78		1/10 (10%)	2/10 (20%)	1/10 (10%)	5/10 (50%)
Main	104		7/50 (14%)	8/50 (16%)	21/50 (42%)	29/50 (58%)
Interim Sacrifice	52	Bile duct hyperplasia	1/10 (10%)	5/10 (50%)	8/10 (80%)	8/10 (80%)
	78		2/10 (20%)	0/10	3/10 (30%)	4/10 (40%)
Main	104		13/50 (26%)	23/50 (46%)	32/50 (64%)	38/50 (76%)

Neoplastic

As shown in the following table, there were no treatment-related neoplastic histopathological lesions. (These data were obtained from 50 main group animals/sex at the 0 and 5,000 ppm doses.) Historical control data from Charles River (Spontaneous neoplastic lesions in the CDF® [F-344]/CrlBR rat, February, 1990) describes a spontaneous incidence range of 0-15.7% for lymphoid cell leukemia in female rats. The spontaneous incidence range for adrenal pheochromocytoma has varied from 0 to 2.2% to 0-24.3% in male rats. Thus, the incidences of adrenal pheochromocytoma in the present study fall within the ranges of historical controls

Incidence of neoplastic histopathological lesions in main groups (0 and 5,000 ppm of UDCA) during 104 weeks of treatment

Lesion	Males Ursodiol (ppm of diet)				Females Ursodiol (ppm of diet)			
	0	500	1,700	5,000	0	500	1,700	5,000
Thyroid C-cell adenoma	4/50	---	---	5/50	6/50	---	---	4/50
Pituitary adenoma	25/50	---	---	25/50	22/50	---	---	9/50
Lymphoid cell leukemia	3/50	---	---	5/50	6/50	---	---	12/50
Mammary fibroadenoma	1/50	---	---	0/50	3/50	---	---	4/50
Adrenal pheochromo-cytoma	6/50	---	----	11/50	1/50	---	---	0/50
Testicular interstitial cell tumor	36/50	---	---	40/50	Not applicable			

In summary, there were no treatment-related incidences of neoplastic lesions in rats in the present 104-week carcinogenic study.

The sponsor stated that dosage selection was based upon preliminary results that indicated appropriate growth rate suppression; details were not provided. In the present study, mean body weights of males and females in the 5,000 ppm group were reduced by approximately 8-10% during Weeks 65-104. Furthermore, the highest dose of UDCA in the diet was 5%. According to guidelines published in the Federal Register in 1995 [60 FR 11278], the maximum feasible dose by dietary administration is considered to be 5% of the diet.

COVERSHEET FOR CARCINOGENICITY STUDY IN RATS

1. No. of Studies: 1
2. Name of Laboratory: [redacted]
3. Strain: Sprague-Dawley
4. No./sex/group: 0: 100; L,M,H: 50
5. Doses (O, L, M, H): 0, 33, 100 and 300 mg/kg/day
6. Basis for dose selection stated: None provided by sponsor.
7. Interim sacrifice: No
8. Total duration (weeks): 126-138
9. Week/site for first tumor:

	Male	Female
0	87/Adrenal pheochromocytoma	104/Adrenal pheochromocytoma
L	98/Adrenal pheochromocytoma	123/Adrenal pheochromocytoma
M	108/Adrenal pheochromocytoma	123/Adrenal pheochromocytoma
H	92/Adrenal pheochromocytoma	82/Adrenal pheochromocytoma

10. No. alive at termination (Week 126 in males; Week 138 in females):

	Male	% Survival	Female	% Survival
O	33	33	33	33
L	11	22	2	4
M	10	20	7	14
H	8	16	3	6

11. Statistical methods used: Data were analyzed student's t test, analyses of variance, and Fischer's exact test.

12. Appendix IV. Non-neoplastic and neoplastic histopathology data are provided on pages 248 - 265.

FDA CDER CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC)
RODENT CARCINOGENICITY FACTSHEET

NDA: 20-675 DRUG CODE #: RU 22990

CAS #: 128-13-2 DATE:

DIVISION(s): Gastrointestinal and Coagulation Drug Products

DRUG NAME(s): Ursodiol (URSOTM), ursodeoxycholic acid

SPONSOR: AXCAN PHARMA U.S. INC.
Plattsburgh, NY 12901

LABORATORY: [redacted]

P/T REVIEW DATE: January 9, 1997

CARCINOGENICITY STUDY REPORT DATE: April 2, 1981

THERAPEUTIC CATEGORY: Primary biliary cirrhosis

PHARMACOLOGICAL/CHEMICAL CLASSIFICATION: Bile acid

PRIOR FDA DOSE CONCURRENCE (Div./CAC)? (Y/N; Date): No

MUTAGENIC/GENOTOXIC (Y/N/equivocal/na; assay): No (Ames test, forward mutation assay in mouse lymphoma cells, sister chromatid exchange assay in human lymphocytes, chromosomal aberrations assay in mouse germ cells, micronucleus test in Chinese hamster bone marrow cells, and chromosomal aberrations assay in Chinese hamster bone marrow cells)

RAT CARCINOGENICITY STUDY (multiple studies? Std1; Std2 etc): Std2.

RAT STUDY DURATION (weeks): 126-138

STUDY STARTING DATE: March 13, 1978

STUDY ENDING DATE: April 2, 1981

RAT STRAIN: Sprague-Dawley

ROUTE: Dietary

DOSING COMMENTS:

No. Rats in Control1 (C1): 200 (100 males and 100 females)
Low Dose (LD): 100 (50 males and 50 females)
Middle Dose (MD): 100 (50 males and 50 females)
High Dose (HD): 100 (50 males and 50 females)

RAT DOSE LEVELS (mg/kg/day)

Rat Low Dose: 33 Rat Middle Dose: 100

Rat High Dose: 333

Basis for Doses Selected (MTD; AUC ratio; saturation; maximum feasible): None provided by sponsor.

RAT CARCINOGENICITY (negative; positive; MF; M; F): Positive; F

RAT TUMOR FINDINGS: There was a treatment-related increase in benign adrenal pheochromocytomas in females; 8/50 females in the 300 mg/kg/day dosage group had adrenal pheochromocytomas, compared to 2/50 females in the control group. There were no other treatment-related incidences of neoplastic lesions.

RAT STUDY COMMENTS: The sponsor stated that dosages of UDCA were selected with the purpose of reaching subtoxic levels for at least 2 of the 3 dosages; no further information was provided. A control group consisted of 100 males and 100 females. Dietary administration of UDCA was continued until a mortality rate of approximately 70% was reached in the control groups. Sponsor did not achieve the recommended maximum feasible dose (5%) by dietary administration. Furthermore, there is no evidence that a maximally tolerated dose was achieved. Any utilization of plasma AUC ratios for dosage selection of UDCA does not seem reasonable because of the extensive enterohepatic cycling of UDCA and its taurine and glycine conjugates

2. 126-138-Week Dietary Carcinogenic Study of UDCA (Report No. was not provided).

Testing Laboratory: [redacted]

Compliance with Good Laboratory Practices and Quality Assurance Requirements: Sponsor provided a letter from [redacted] dated May 7, 1996, stating that studies performed at the above laboratory were conducted according to FDA requirements at that time.

Study Started: March 13, 1978

Study Completed: April 2, 1981

Animals: Male and female (approximate body weight of 70-75 g; 26 days of age) Sprague-Dawley rats.

Methods: According to the sponsor, dosages of UDCA were selected with the purpose of reaching subtoxic levels for at least 2 of the 3 dosages; no further information was provided. Thus, 3 groups of 100 rats each (50 males and 50 females) were administered 33, 100 and 300 mg/kg/kg of UDCA, respectively, admixed in the diet. Although results of diet analysis were not provided, it is estimated that during Week 126 for intended dietary doses of 33, 100 and 300 mg/kg/day in males, the diet contained 0.27%, 0.85% and 2.73% of ursodiol, respectively; in the case of females during Week 136; 0.26%, 0.78% and 2.40%, respectively. A control group consisted of 100 males and 100 females. Dietary administration of UDCA was continued until a mortality rate of approximately 70% was reached in the control groups.

Animals were observed for clinical signs of toxicity and mortality on a daily basis. Body weights were measured once a week through the first 13 weeks of treatment, and at 4 week intervals thereafter. Food consumption was also measured once a week through the first 13 weeks of treatment, and at 4 week intervals thereafter.

Blood samples were obtained under light ether anesthesia via the retrobulbar venous plexus at the end of the study in all surviving animals and, as far as possible, in moribund animals before sacrifice. Hematological assessments were done.

At the end of treatment, animals were subjected to ophthalmological examination, a simple noise test, and a dentation examination.

All surviving animals were sacrificed under ether anesthesia by cutting the aorta and subjected to gross pathological examination. Animals that died or were sacrificed in a moribund condition were subjected to similar examination. Organ weights were obtained for heart, kidneys, gonads, lungs, adrenals, thyroids, liver, thymus, brain, spleen and pituitary.

Histopathological examinations were performed on tissues from all animals in the 300 mg/kg/day dosage group and 50 males and 50 females in the control group. The method of selection for the control animals was not specified by the sponsor. Tissues from mammary gland, heart, esophagus, colon, kidneys, prostate, uterus, eye, bone marrow, pancreas, thymus, thyroids, stomach, liver, adrenals, testicles, brain, trachea, salivary glands, lungs and bronchi, duodenum, jejunum, ileum, rectum, spleen, urinary bladder, ovary, pituitary, bone, mesenteric lymph nodes, skeletal muscle, peripheral nerve and aorta were microscopically examined. Additionally, tissues from kidney, adrenals and urinary bladder were also examined in animals in the 33 and 100 mg/kg/day dosage groups.

Data were statistically analyzed with student's t tests, analyses of variance, and Fisher's exact tests.

Results:

1. Achieved Doses: Mean achieved doses for males over 126 weeks were 17.0, 50.9 and 163.7 mg/kg/day for the projected 33, 100 and 300 mg/kg/day doses, respectively. Mean achieved doses for females over 138 weeks were 21.5, 64.4 and 201.6 mg/kg/day for the projected 33, 100 and 300 mg/kg/day doses, respectively.

2. Observed Effects: There were no treatment-related clinical signs of toxicity.

3. Mortality: As shown in the following table, incidence of mortality was treatment-related at the 300 mg/kg/day dose in males and at the 33, 100 and 300 mg/kg/day doses in females. The percent incidence of mortality is also shown because there were 200 control animals (100 males and 100 females) and 100 animals per dosage group (50 males and 50 females). Dietary administration of UDCA was continued until a mortality rate of approximately 70% was reached in the control groups.

Cumulative mortality during 126 weeks of treatment in males and 138 weeks of treatment in females

Week No.	Males (mg/kg/day)				Females (mg/kg/day)			
	0	33	100	300	0	33	100	300
60	0	1 (2%)	0	2 (4%)	0	0	0	0
80	10 (10%)	3 (6%)	5 (10%	9 (18%)	4 (4%)	4 (8%)	4 (8%)	2 (4%)
100	27 (27%)	10 (20%)	13 (26%)	20 (40%)	16 (16%)	8 (16%)	11 (22%)	10 (20%)
126	67 (67%)	39 (78%)	40 (80%)	42 (84%)	49 (49%)	30 (60%)	30 (60%)	30 (60%)
138	---	---	---	---	67 (67%)	48 (96%)	43 (86%)	47 (94%)

4. Body Weight: Mean body weights of control males and females at the start of treatment were 126 and 115 g, respectively. Mean body weights of control males and females at the end of treatment were 448 and 302 g, respectively. There were no treatment-related effects.

5. Food Consumption: Mean food consumption of control males and females during Week 1 of treatment were 9.1 and 9.4 g/animal/day, respectively. Mean food consumption of control males and females at the end of treatment were 23.3 and 25.0 g/animal/day, respectively. There were no treatment-related effects.

5. Hematology: There were no treatment-related effects.

6. Ophthalmology/Auditory Test/Dental Examination: There were no treatment-related effects.

7. Organ Weights: There were no treatment-related effects.

8. Gross Pathology: There were no treatment-related effects.

9. Histopathology: Microscopic examination indicated that test compound had precipitated into the renal pelvis. Thus, tissues from kidneys, adrenals and urinary bladder were microscopically examined in all dosage groups. Other tissues were examined only in the 0 and 300 mg/kg/day groups.

Non-neoplastic

As shown in the following table, there were significant dose-related increases in the incidence of metaplasia of pelvic epithelium and pyleonephritis in kidneys and localized medullary

hyperplasia in adrenals of males. There was a treatment-related increase in the incidence of metaplasia of pelvic epithelium, chronic pyelitis and fatty degeneration of tubular epithelium in kidneys and localized medullary hyperplasia in adrenals and a dose-related increase in the incidence of calcareous deposits in pelvis of kidneys in females.

Total incidence of non-neoplastic histopathological lesions during 126 to 138 weeks of treatment

Lesion	Males Ursodiol (mg/kg/day)				Females Ursodiol (mg/kg/day)			
	0	33	100	300	0	33	100	300
KIDNEYS								
Metaplasia of pelvic epithelium	1/50	2/50	*7/50	*12/50	0/50	3/50	4/50	*9/50
Calcareous deposits in pelvis	0/50	1/50	3/50	21/50	0/50	4/50	*12/50	*34/50
Chronic pyelitis	3/50	6/50	8/50	6/50	1/50	7*/50	5/50	*8/50
Fatty degeneration of tubular epithelium	19/50	13/50	27/50	28/50	14/50	13/50	18/50	*27/50
Pyelonephritis	1/50	1/50	*5/50	*6/50	2/50	1/50	1/50	1/50
Nephrosclerosis	5/50	6/50	7/50	6/50	5/50	11/50	4/50	12/50
Interstitial nephritis	9/50	8/50	5/50	5/50	3/50	5/50	4/50	6/50
Subcapsular scars	3/50	7/50	5/50	5/50	6/50	7/50	6/50	9/50
Congestion	34/50	36/50	25/50	27/50	20/50	18/50	18/50	15/50
ADRENALS								
Localized medullary hyperplasia	2/50	*9/50	*14/50	*10/50	0/50	1/50	2/50	*8/50
Thrombosed angiomatosis	0/50	0/50	3/50	1/50	9/50	8/50	11/50	7/50
Congestion	5/50	5/50	1/50	11/50	14/50	4/50	6/50	5/50
URINARY BLADDER								
Severe hyperplasia of transitional epithelium	0/50	0/50	2/50	6/50	0/50	0/50	3/50	1/50
Submucosal inflammation	4/50	5/50	2/50	2/50	1/50	0/50	5/50	4/50
Severe cystitis	0/50	3/50	1/50	1/50	0/50	0/50	1/50	3/50

Since carcinogenic studies in rats are usually terminated after 104 weeks of treatment, histopathological data for rats that died by Week 104 or earlier were examined separately. As shown in the following table, there was a treatment-related increase in incidence of localized medullary hyperplasia in adrenals in males. (There was missing individual data for males in the 300 mg/kg/day dosage group.)

In the case of females, there were treatment-related increases in incidence of calcareous deposits in pelvis and fatty degeneration of tubular epithelium in kidneys. Thus, in general, many non-neoplastic histopathological lesions in the kidneys, adrenals and urinary bladder emerged between 104 weeks of treatment and termination of the study (Week 126 in males and Week 138 in females).

Incidence of non-neoplastic histopathological lesions for up to 104 weeks of treatment

Lesion	Males Ursodiol (mg/kg/day)				Females Ursodiol (mg/kg/day)			
	0	33	100	300	0	33	100	300
KIDNEYS								
Metaplasia of pelvic epithelium	0/18	0/13	2/16	---*	0/12	0/10	1/11	3/14
Calcareous deposits in pelvis	0/50	0/13	0/16	---	0/12	2/10	2/11	9/14**
Chronic pyelitis	1/18	1/13	2/16	---	0/12	0/10	1/11	3/14
Fatty degeneration of tubular epithelium	7/18	5/13	7/16	---	5/12	3/10	6/11	9/14**
Nephrosclerosis	2/18	0/13	2/16	---	1/12	0/10	0/11	1/14
Interstitial nephritis	1/18	0/13	1/16	---	0/12	1/10	0/11	0/14
Subcapsular scars	1/18	2/13	0/16	---	0/12	0/10	1/11	1/14
Congestion	14/18	12/13	9/16	---	7/12	4/10	6/11	7/14
ADRENALS								
Localized medullary hyperplasia	0/18	0/13	5/16**	---	0/12	0/10	0/11	1/14
Thrombosed angiomatosis	0/50	0/13	1/16	---	2/12	0/10	0/11	2/14
Congestion	1/18	2/13	1/16	---	0/12	1/10	0/11	0/14
URINARY BLADDER								
Severe hyperplasia of transitional epithelium	0/18	0/13	0/16	---	0/12	0/10	0/11	0/14
Submucosal inflammation	1/18	1/13	0/16	---	0/12	0/10	1/11	1/14
Severe cystitis	0/18	0/13	0/16	---	0/12	0/10	0/11	1/14

*There was missing individual data.

**Different from control

Neoplastic

As shown in the following table, there was a treatment-related increase in benign adrenal pheochromocytomas in females; 8/50 females in the 300 mg/kg/day dosage group had adrenal pheochromocytomas, compared to 2/50 females in the control group.

Total incidence of neoplastic histopathological lesions during 126 to 138 weeks of treatment

Lesion	Males Ursodiol (mg/kg/day)				Females Ursodiol (mg/kg/day)			
	0	33	100	300	0	33	100	300
Thyroid C-cell carcinoma	2/50	---	---	2/50	2/50	---	---	2/50
Mammary fibroadenoma	0/50	---	---	1/50	15/50	---	---	14/50
Adrenal pheochromo-cytoma	6/50	7/50	5/50	8/50	1/50	3/50	2/50	8/50
Testicular interstitial cell tumor	13/50	---	---	8/50	Not applicable			

In summary, there was a treatment-related increase in benign adrenal pheochromocytomas in females; 8/50 females in the 300 mg/kg/day dosage group had adrenal pheochromocytomas, compared to 2/50 females in the control group. There were no other treatment-related incidences of neoplastic lesions.

The sponsor stated that dosages of UDCA were selected with the purpose of reaching subtoxic levels for at least 2 of the 3 dosages; no further information was provided. Sponsor did not achieve the recommended maximum feasible dose (5%) by dietary administration. Furthermore, there is no evidence that a maximally tolerated dose was achieved. The utilization of plasma AUC ratios for dosage selection of UDCA does not seem reasonable because of the extensive enterohepatic cycling of UDCA and its taurine and glycine conjugates.

3. 103-Week Oral Carcinogenic Study of Lithocholic Acid (Report No. [redacted]-175).

Testing Laboratory: [redacted]

Compliance with Good Laboratory Practices and Quality Assurance Requirements: Statements of compliance were not provided.

Study Started: Not provided.

Study Completed: Not provided.

Animals: Male and female Fischer 344 rats (approximately 6 weeks of age).

Methods: Since it was discovered that lithocholic acid was instable in diet, it was decided to administer lithocholic acid by gavage. In a 7-week oral dose-ranging study of lithocholic acid (464, 681, 1000, 1470 and 2150 mg/kg 3 times a week); there was a -6% to -20% reduction in body weight gain of males. There was no effect on body weight gain in females. There were no other clinical signs of toxicity. Thus, the high dose selected for the carcinogenic study was 500 mg/kg.

Therefore, 2 groups of 100 rats each (50 males and 50 females) were orally administered 250 and 500 mg/kg of lithocholic acid, respectively, 3 times a week for 103 weeks. Vehicle control groups consisted of 20 males and 20 females, respectively. Vehicle was shelf-grade A&P corn oil; dosing concentration of lithocholic acid was 5% to 10%. All animals were further observed for 1 week following cessation of lithocholic acid administration.

All animals were observed twice daily for clinical signs of toxicity and mortality. Body weights were recorded on a monthly basis throughout the study. Food consumption data were collected at monthly intervals from 20% of the animals in each group.

All surviving animals were sacrificed with carbon dioxide. Animals found dead, moribund animals that were sacrificed, and animals sacrificed at the end of the study were subjected to gross pathological examination whenever possible. Histopathological examinations were conducted on tissues from skin, subcutaneous tissue, lung and bronchi, trachea, bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, seminal vesicle, testis, prostate, brain, uterus, mammary gland, and ovary.

Probabilities of survival were estimated by a product-limit procedure; dose-related effects on survival were evaluated by the method of Cox. Tumor data were statistically analyzed with Fischer's exact test, Cochran-Armitage test for linear trends, and time-adjusted analyses

Results:

1. Observed Effects: There were no treatment-related clinical signs of toxicity.

2. Mortality: Mortality data were provided in a figure; quantitative data were not provided. According to the authors of the report, neither the Tarone test nor the Cox tests indicated a significant positive association between dosage and mortality in either male or female rats. In the case of males, there were 16/20, 45/50 and 40/50 surviving rats in the 0, 250 and 500 mg/kg dosage groups, respectively, at the end of the study (Week 104). In the case of females, there were 16/20, 41/50 and 33/50 surviving rats in the 0, 250 and 500 mg/kg dosage groups, respectively, at the end of the study (Week 104).

3. Body Weight: Body weight data were provided in a figure; quantitative data were not provided. There was a moderate treatment-related reduction of body weight in males (up to approximately -10%). There was no treatment-related effect on mean body weight in females.

4. Food Consumption: Data were not provided.

5. Gross Pathology: Data were not provided.

6. Histopathology:

Non-neoplastic

As shown in the following table for males, there were apparent treatment-related, but low rate incidences of non-neoplastic lesions, that were not related to any neoplastic lesions.

Total incidence of non-neoplastic lesions in males

Lesion	Lithocholic acid (mg/kg, p.o., 3 times a week)		
	0	250	500
Lung hemorrhage	0/20	1/50	1/50
Heart thrombosis	0/20	1/50	1/50
Liver focal necrosis	0/19	0/49	1/50
Pancreatic islet hyperplasia	0/20	0/50	1/50

As shown in the following table for females, there were apparent treatment-related, but low rate incidences of non-neoplastic lesions, that were not related to any neoplastic lesions.

Total incidence of non-neoplastic lesions in females

Lesion	Lithocholic acid (mg/kg, p.o., 3 times a week)		
	0	250	500
Hepatic focal hyperplasia	0/20	4/49	7/49
Hepatic centrilobular necrosis	0/20	0/49	1/49
Hepatic periportal fibrosis	0/20	1/49	1/49
Urinary bladder papillary hyperplasia	0/17	0/40	1/40
Uteral polypoid hyperplasia	0/20	0/48	1/50

Neoplastic

As shown in the following table for males, there were no statistically significant treatment-related incidences of neoplastic lesions

[redacted]
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Total incidence of neoplastic lesions in males

Lesion	Lithocholic acid (mg/kg, p.o., 3 times a week)		
	0	250	500
Alveolar/ bronchiolar adenoma	1/20	4/50	1/50
Hepatocellular carcinoma or adenoma	0/19	1/49	3/50
Pituitary chromophobe adenoma	0/19	5/47	6/46
Adrenal pheochromo-cytoma	3/20	7/50	12/50
Follicular cell carcinoma or adenoma	0/9	3/39	1/27
Testicular interstitial cell tumor	19/20	45/49	47/50

As shown in the following table for females, there were no statistically significant treatment-related incidences of neoplastic lesions.

Total incidence of neoplastic lesions in females

Lesion	Lithocholic acid (mg/kg, p.o., 3 times a week)		
	0	250	500
Hematopoietic leukemia or malignant lymphoma	3/20	11/49	13/50
Pituitary chromophobe adenoma	10/20	18/46	21/48
Adrenal pheochromo-cytoma	0/20	3/47	1/50
Mammary gland fibroadenoma	1/20	6/49	6/50
Endometrial stromal polyp	1/20	7/48	7/50

In summary, orally administered lithocholic acid (250 and 500 mg/kg 3 times a week for 103 weeks) in rats was not carcinogenic. None of the statistical tests for any site in rats of either sex indicated a significant positive association between lithocholic acid administration and tumor incidence.

4. Narisawa, T. Magadia, N.E., Weisburger, J.H. and Wynder, E.L. Promoting effect of bile acids on colon carcinogenesis after intrarectal instillation of N-methyl-N'-nitro-N-nitrosoguanidine in rats. J. Natl. Cancer Inst. 1974, 53: 1093-1095.

Animals: Male and female Fischer 344 rats (6-7 weeks of age).

Methods: Five groups of 32 rats each (16 males and 16 females) were given lithocholic acid (LC), taurodeoxycholate (TDC), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) + LC, MNNG + TDC, and MNNG, respectively. In the LC group, each rat was intrarectally (ir) administered 1 mg/kg of LC 5 times weekly for 13 months; vehicle was 0.5 ml peanut oil. In the TDC group, each rat received TDC (1 mg/kg, ir) 5 times weekly for 13 months. In the MNNG + LC and MNNG + TDC groups, each rat received MNNG (4 mg/kg, ir); vehicle was 0.5 ml of 1% carboxymethyl cellulose in physiological saline. Seven days after the MNNG treatment, rats received either LC or TDC 5 times weekly until total doses of 240 mg/kg were achieved. In the MNNG group, rats received only a single dose of MNNG (4 mg/kg, ir) and were kept until the end of the experiment.

Rats in a moribund condition were sacrificed. All rats were terminated after 78 experimental weeks. The large intestine was opened and subjected to gross pathological examination. The liver, mesenteric lymph nodes, lungs and other (undefined) organs were assessed for metastases. All tumors and organs that were suspected of containing metastases were subjected to histo-pathological examinations.

Results: As shown in the following table, intrarectally administered LC alone and TDC alone did not produce any tumors in the distal colon and rectum. MNNG alone produced 10 neoplasms in 25% of the animals. MNNG + LC produced 30 neoplasms in 52% of the animals, while MNNG + TDC produced 28 neoplasms in 62% of the animals. Thus, LC alone and TDC alone were not carcinogenic, while both LC and TDC promoted MNNG-induced colonrectal neoplasms

Tumor incidence in distal colon and rectum after 40-78 weeks of treatment and incidence by histologic classification

Treatment	# of rats	# of rats with neoplasms (%)	Total # neoplasms	Incidence by histologic classification		
				Adeno-carcinoma	Polypoid adenoma	Sarcoma
LC*	32	0	-	-	-	-
TDC	32	0	-	-	-	-
MNNG + LC	29	15 (52%)	30	8	22	0
MNNG + TDC	29	18 (62%)	28	4	23	1
MNNG	32	8 (25%)	10	5	5	0

*LC = lithocholic acid, TDC = sodium taurodeoxycholate, MNNG = M-methyl-N'-nitro-N-nitrosoguanidine.

5. Earnest, D.L., Holubec, H., Wali, R.K., Jolley, C.S., Bissonette, M., Bhattacharyya, A.K., Roy, H., Khare, S. and Brasitus, T.A. Chemoprevention of azoxymethane-induced colonic carcinogenesis by supplemental dietary ursodeoxycholic acid. Cancer Res. 1994, 54: 5071-4.

Animals: Male Fischer 344 rats (90-130 g; ages were not provided).

Methods: Studies were conducted at the University of Chicago and the University of Arizona; data were pooled together. As shown in the following table, there were 7 treatment (diet) groups. All rats were fed their assigned diet for 2 weeks. All rats were then subcutaneously injected with azoxymethane (AOM; 15 mg/kg) once a week for 2 weeks; rats were concurrently continued on their assigned diet. All rats were maintained on their assigned diet for 28 more weeks. The authors did not provide achieved doses and did not provide amounts of diet consumed.

Treatments and numbers of animals/treatment group.

Treatment	Univ. of Chicago (N)	Univ. of Arizona (N)	Combined (N)
Group I Standard Diet (SD)	20	23	43
Group II SD + 0.2% (1.2 g/kg/day) Cholic Acid (CA)	23	24	47
Group III SD + 0.4% (2.4 g/kg/day) CA	23	24	47
Group IV SD + 0.2% (1.2 g/kg/day Ursodiol (URSO)	24	24	48
Group V SD + 0.4% (2.4 g/kg/day) URSO	23	22	45
Group VI SD + 0.2% (1.2 g/kg/day) CA + 0.2% (1.2 g/kg/day) URSO	24	23	47
Group VII SD + 75 ppm (4.5 mg/kg/day) Piroxicam	32	24	56

All rats were sacrificed, and their colons were removed and examined macroscopically and microscopically for the presence of tumors. Tumor size was measured with calipers at the University of Arizona and resected tumors were weighed at the University of Chicago. Macroscopic and microscopic neoplasms were classified as being either benign or malignant for each animals.

Data were statistically analyzed by either Fisher's exact test or unpaired t-tests; the a *priori* α level of acceptance was set at the 0.05 level.

Results:

1. Mortality: There were 2, 0, 2, 1, 1, 1 and 0 deaths in Groups I, II, III, IV, V, VI and VII, respectively. Cause of death was not provided in the article. Nevertheless, there was no statistically significant differences in incidence of deaths among treatment groups.

2. Body Weights: Body weights were not provided on a treatment group basis. Initial body weights of all animals ranged from 125 g to 137 g at the University of Chicago and from 92 g to 102 g at the University of Arizona. Final body weights of all animals ranged from 410 g to 448 g at the University of Chicago and from 414 g to 480 g at the University of Arizona.

3. Colon Tumors: As shown in the following table, the # of tumor-bearing animals dose-dependently increased as a function of cholic acid dose (Groups II and III), compared to the group receiving standard diet (Group I). The # of tumor-bearing animals decreased in the group fed SD + 0.4% URSO (Group V), compared to the group fed standard diet (Group I). Groups IV (SD + 0.2% URSO), VI (SD + 0.2% CA + 0.2% URSO) and VII (SD + 75 ppm piroxicam) did not differ from Group 1.

The mean number of tumors/tumor-bearing animal dose-dependently increased as a function of cholic acid dose (Groups II and III), compared to the group receiving standard diet (Group I). The other groups (IV, V, VI and VII) did not differ from Group I.

The ratio of benign/malignant tumors increased dramatically in Group V (SD + 0.4% URSO), compared to the group receiving standard diet (Group I). On the other hand, differences between Group I and Groups II, III, IV, VI and VII were probably not biologically significant

Effects of cholic acid, ursodiol and prioxicam on azoxymethane-induced colonic tumors in rats

Treatment	Combined (N)	# Tumor-bearing animals	Mean # tumors/tumor-bearing animal	Tumors (Benign/ Malignant)
Group I Standard Diet (SD)	43	20 (46.5%)	1.10	14/6
Group II SD + 0.2% Cholic Acid (CA)	47	30 (63.8%)	1.80	18/12
Group III SD + 0.4% CA	47	34 (72.3%)	2.26	20/14
Group IV SD + 0.2% Ursodiol (URSO)	48	23 (47.9%)	1.26	14/9
Group V SD + 0.4% URSO	45	10 (22.2%)	1.30	10/0
Group VI SD + 0.2% CA + 0.2% URSO	47	20 (42.5%)	1.55	8/12
Group VII SD + 75 ppm Piroxicam	56	19 (33.9%)	1.53	16/3

In summary, cholic acid dose-dependently promoted the incidence of AOM-induced colonic tumors. On the other hand, 0.4% URSO decreased the incidence of AOM-induced colonic tumors. 0.4% URSO also dramatically increased the ratio of benign/malignant tumors; that is, completely abolished malignant tumors

MUTAGENICITY:

1. Ames test in Salmonella typhimurium (Report No. was not provided).

Testing Laboratory: [redacted]

Compliance with Good Laboratory Practice and Quality Assurance Requirements: Sponsor provided a letter from [redacted] dated May 7, 1996, stating that studies performed at the above laboratory were conducted according to FDA requirements at that time.

Date Study Started: Not provided.

Date Study Completed: January 17, 1979

The sponsor did not originally provide data for this study. A short summary was submitted on May 7, 1996. Thus, the review is abbreviated.

Salmonella typhimurium strains: TA 1535, TA 1537, TA 1538, TA 98 and TA 100.

Methods: Ursodeoxycholic acid (UDCA) concentrations of 5.25 and 125 x 10^{-3}, 0.5 and 2.5 mg/plate were used. S-9 fraction from rats pretreated with Arochlor 1354 was used for metabolic activation experiments. Positive controls were aflatoxin B_1 (1 μg/plate) and 2-aminofluorine (10 μg/plate).

Results: UDCA did not increase mutation rates either with or without metabolic activation. The solvent DMSO had no effects. UDCA did not produce any cytotoxic effects. (No quantitative data were provided.)

Sponsor mentioned that concentrations of DMSO were not optimal, especially during metabolic activation experiments. Thus, according to the sponsor; UDCA was not mutagenic without metabolic activation, but no conclusions can be made about the experiments with metabolic activation.

2. Forward mutation assay in mouse lymphoma cells (Report No. was not provided).

Testing Laboratory: [redacted]

Compliance with Good Laboratory Practice and Quality Assurance Requirements: Sponsor provided a letter from Dr. J. Leuschner, dated May 7, 1996, stating that studies performed at the above laboratory were conducted according to FDA requirements at that time.

Date Study Started: April 26, 1982

Date Study Completed: July 30, 1982

Cells: Mouse lymphoma cells from Fischer L5178Y line.

Methods: In a preliminary study, ursodeoxycholic acid (UDCA) appeared soluble in the assay medium up to 125 μg/ml, but a white precipitate and a yellow color was noted at concentrations from 250 to 1000 μg/ml. Nonetheless, 10 concentrations of UDCA in the range of 50 to 1000 μg/ml were assessed for mutagenic activity; data were only provided for UDCA concentrations of 50, 125 and 250 μg/ml.

Thus, 3 x 10^6 cells were seeded in soft agar plates with Fischer's mouse leukemia medium, supplemented with L-glutamine, sodium pyruvate and horse serum, for 10 days. Colony growth was assessed in the presence of 5-bromo-2'-deoxyuridine (BRDU). Effects of UDCA were studied in presence and absence of S-9 fraction from rat liver homogenate; studies were done in duplicate.

Negative controls were untreated cells and cells exposed to the solvent. Positive control with metabolic activation was ethylmethane sulfonate (EMS, 0.5 μl/ml). Positive control without metabolic activation was dimethylnitrosamine (DMN, 0.3 μl/ml).

Parameters that were studied and assessed included suspension growth, total mutant colonies, total viable colonies, cloning efficiency, relative growth and mutant frequency. In the case of mutant frequency, a range up to approximately 30 x 10^6 was considered normal.

Results: In the presence of metabolic activation, UDCA produced cell toxicity and death; relative cell growth [(relative suspension growth x relative cloning efficiency)/100] ranged from 15.7 to 18.1% for the 250 μg/ml concentration. In the absence of metabolic activation, UDCA produced cell toxicity and death; relative cell growth ranged from 8.0 to 17.9% for the 250 μg/ml concentration. Thus, mutagenic effects of UDCA could only be assessed at concentrations of 50 and 125 μg/ml; at these concentrations UDCA was not mutagenic in either the presence or absence of metabolic activation. Positive controls produced significant increases in total mutant colonies. Thus, UDCA was not mutagenic in this assay, but UDCA-induced cell toxicity and death limited the assessment.

3. Sister chromatid exchange assay in human lymphocytes (Report No. was not provided).

Testing Laboratory: [redacted]

Compliance with Good Laboratory Practice and Quality Assurance Requirements: Sponsor provided a letter from [redacted] dated May 7, 1996, stating that studies performed at the above laboratory were conducted according to FDA requirements at that time.

Date Study Started: June 25, 1982

Date Study Completed: August 18, 1982

Methods: Human lymphocytes were separated from venous blood by centrifugation, washed and condensed, and 2 x 10^5 cells/ml were placed in culture medium. Cells were either incubated at 37° C for about 7 days and test compound added on the 7th day or test compound was added on the 1st day and cells were incubated at 37° C for about 7 days. In a preliminary study, 1 mg/ml of ursodeoxycholic acid (UDCA) in the culture medium formed a white precipitate. Some precipitation also occurred at UDCA concentrations of 500 and 750 µg/ml, but the precipitate rapidly redissolved after gentle mixing at 37° C. Thus, UDCA was studied at concentrations of 1, 10, 50, 100, 500 and 750 µg/ml. Studies were done in duplicate.

In both cases, cell division was stimulated on the 8th day by addition of 1% phytohemagglutinin (PHA) and 25 µM bromodeoxyuridine (BRDU). Stimulated cells were cultured for 3 days; cell division was arrested by addition of colcemid (0.1 µg/ml). A negative control consisted of non-stimulated cells and a solvent control was treated with 1% DMSO. A positive control was treated with ethylmethane sulfonate (EMS; 0.10 or 0.125 µl/ml) at about 24 h after initiation of cell division by PHA and BRDU. Cell suspensions were centrifuged, washed and stained.

M_2 cells were scored for frequency of sister chromatid exchange (SCE) per cell and per chromosome. An increase in SCE was considered significant if there was a two-fold increase over the negative and solvent controls for a minimum of 3 concentrations of test compound or a statistically significant increase (student's t test) for a minimum of 3 concentrations of test compound and evidence for a positive dose-response effect.

Results: UDCA concentrations of 50 µg/ml and higher produced cytotoxicity. Thus, SCE could only be studied at UDCA concentrations of 1 and 10 µg/ml; UDCA did not produce significant increases in either SCEs/cell or SCEs/chromosome.

The positive control did produce significant increases in SCEs/cell and SECs/chromosome. Thus, UDCA was not mutagenic in the sister chromatid exchange assay, but UDCA-induced cell toxicity limited the assessment.

4. Chromosomal aberrations in mouse germ cells (Report No. was not provided).

Testing Laboratory: [redacted]

Compliance with Good Laboratory Practice and Quality Assurance Requirements: Sponsor provided a letter from [redacted], dated May 7, 1996, stating that studies performed at the above laboratory were conducted according to FDA requirements at that time.

Date Study Started: July, 1982

Date Study Completed: September 3, 1982

Animals: Male (17-23 g; ages were not provided) NMRI mice.

Methods: Groups of 6 mice each were orally administered 0, 200, 400 and 800 mg/kg ursodeoxycholic acid (UDCA), respectively, twice within 24 h. Vehicle was 0.8% aqueous hydroxypropyl-methylcellulose; dosing volume was 20 ml/kg. Animals were intraperitoneally administered 4 mg/kg desacetylmethylcolchicine at 7 h prior to preparation of testes. Animals were sacrificed via cervical dislocation and slides of testicular spermatogonia were prepared. A positive control group received a single dose of 0.25 mg/kg Mitomycin C, i.p., 24 h before desacetylmethyl-colchicine treatment.

Fifty spermatogonia in metaphase per animal were microscopically examined for normal metaphases, gaps, chromatid exchange and breaks. Data were statistically analyzed with analyses of variance and student's t-tests.

Results: There were no differences between groups in number of normal metaphases, gaps and breaks. The positive control group had significant increases in incidence of chromatid exchange; UDCA did not produce any chromatid exchange. Thus, UDCA was not mutagenic in the chromosomal aberrations assay in testicular spermatogonia in mice.

5. Micronucleus test in Chinese hamster bone marrow cells (Report No. was not provided).

Testing Laboratory: [redacted]

Compliance with Good Laboratory Practice and Quality Assurance Requirements: Sponsor provided a letter from [redacted] dated May 7, 1996, stating that studies performed at the above laboratory were conducted according to FDA requirements at that time.

Date Study Started: February, 1982

Date Study Completed: March 6, 1981

Animals: Male (38-44 g; ages were not provided) Chinese hamsters.

Methods: Four groups of 6 hamsters each were orally administered 0, 200, 400 and 800 mg/kg ursodeoxycholic acid (UDCA) twice within 24 h. (A dose-ranging study was mentioned, but details of the study were not provided). Vehicle was 0.8% aqueous hydroxypropylmethylcellulose; dosing volume was 10 mg/kg. A positive group were orally administered methylmethanesulfonate (100 mg/kg). At 6 h after the last treatment, animals were sacrificed and slides of bone marrow smears were prepared.

A total of 1000 polychromatic erythrocytes were microscopically examined for presence of micronuclei for each animal. Data were analyzed with student's t tests.

Results: Although the sponsor reported that there were no UDCA-induced increases in incidence of micronuclei, quantitative data were not provided. The positive control did produce a significant increase in incidence of micronuclei (quantitative data were provided). Thus, UDCA was not mutagenic in Chinese hamster bone marrow cells.

6. Chromosomal aberrations in Chinese hamster bone marrow cells (Report No. was not provided).

Testing Laboratory: [redacted]

Compliance with Good Laboratory Practice and Quality Assurance Requirements: Sponsor provided a letter from [redacted] dated May 7, 1996, stating that studies performed at the above laboratory were conducted according to FDA requirements at that time.