Monkeys

1. 6-Month Oral Toxicity Study of Ursodiol in Monkeys (Fedorowski et al., Gastroenterology, 1978, 74: 75-81; reprint provided by sponsor).

Animals: Male and female (2.0 to 3.0 g; ages were not provided) rhesus monkeys.

Methods: Twenty-eight monkeys were randomly divided into 5 groups. Group I (3 males and 3 females) were controls and received orally administered placebo capsules. Group II (5 males and 4 females) received 40 mg/kg/day of orally administered chenodeoxycholic acid (CDCA) for 6 months; Group III (3 males and 2 females) received 100 mg/kg/day of CDCA; Group IV (2 males and 2 females) received 40 mg/kg/day of ursodeoxycholic acid (UDCA); Group V (2 males and 2 females) received 100 mg/kg/day of UDCA. Doses were approximated with combinations of 100, 75, 50 and 25 mg in gelatin capsules.

The following hematological and blood chemistry parameters were assessed at the initiation of studies, at monthly intervals thereafter and at the termination of the studies: total blood count, total and direct serum bilirubin, serum total protein and albumin, serum cholesterol, blood urea nitrogen, creatinine, electrolytes, blood glucose, serum glutamic oxaloacetic transaminase (SGPT), serum leucine amino peptidase (SLAP), and serum glutamic-pyruvic transaminase (SGPT).

Biliary bile acid concentration and composition was determined at sacrifice. Fecal bile acid concentration and composition was determined after 4 months of either CDCA or UDCA treatment. Hepatic microsomal HMG-CoA reductase activity was assessed at sacrifice.

Histological examination of liver tissue was done at sacrifice by both light and electron microscopy.

Results:

1. Observed Effects: The only clinical sign of toxicity that was reported was CDCA-induced liquid stools on several occasions throughout the study.

2. Mortality: There were no deaths.

3. Body Weight: Mean combined body weights of control males and females were approximately 2.5 and 2.7 kg at 0 and 6 months of treatment, respectively. There were no treatment-related effects on body weight.

4. Food Consumption: The sponsor reported that there were no treatment-related effects on food consumption. No data for food consumption were provided.

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

6. Blood Chemistry: As shown in the following table, CDCA produced dose-related increases in serum levels of serum leucine amino peptidase (SLAP), serum glutamine oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT). On the other hand, UDCA did not; in fact, there may have been a treatment-related decrease.

Effects of CDCA and UDCA on serum enzymes

Serum Enzyme	CDCA (mg/kg/day)		UDCA (mg/kg/day)	
	40	100	40	100
SLAP	100%	246%	-36%	-43%
SGOT	25%	239%	-17%	-22%
SGPT	188%	312%	-31%	-16%

7. Biliary and Fecal Bile Acid Compositions: As shown in the following table; both CDCA and UDCA produced decreases in the proportion of biliary cholic acid (CA) and deoxycholic acid (DCA). In the case of CDCA treatment, CDCA became the major biliary bile acid and biliary lithocholic acid (LCA) rose to over 10% of the total bile acids. In the case of UDCA treatment, UDCA became the major biliary bile acid at the 100 mg/kg/day dose of UDCA, but LCA only rose slightly with both doses of UDCA.

Biliary Bile Acid Composition (% of total bile acids)

Treatment	# Animals	LCA	DCA	CDCA	UDCA	CA
Control	6	3.2%	18.8%	15.2%	3.4%	64.4%
40 mg/kg CDCA	9	10.3%	2.8%	80.2%	3.4%	3.3%
100 mg/kg CDCA	5	13.3%	2.2%	78.4%	2.8%	3.3%
40 mg/kg UDCA	4	3.8%	8.5%	12.7%	32.5%	42.5%
100 mg/kg UDCA	4	3.4%	2.2%	15.3%	73.8%	5.4%

As shown in the following table, the proportion of LCA in feces was markedly increased in both CDCA-treated groups, did not differ from controls in both UDCA-treated groups.

Fecal Bile Acid Composition (% of total bile acids

Treatment	# Animals	LCA	DCA	CDCA	CA
Control	6	40.0%	32.0	8.0	20.0%
40 mg/kg CDCA	9	85.0%	9.0%	0	2.0%
100 mg/kg CDCA	5	93.0%	5.2%	0%	1.8%
40 mg/kg UDCA	4	53.0%	38.8%	0.5	10.0%
100 mg/kg UDCA	4	42.0%	42.5%	0%	15.0%

8. Hepatic Enzyme Activity: Hepatic HMG-CoA reductase was inhibited by -30% (% of difference from control) and -46% in the 40 and 100 mg/kg/day CDCA groups, respectively. Hepatic HMG-CoA reductase was inhibited by -21% and -38% in the 40 and 100 mg/kg/day UDCA groups, respectively. However, serum cholesterol levels were not affected by either CDCA or UDCA.

9. Histopathology: Histopathological examinations were limited to the liver; incidences of hepatic lesions were not provided. It was reported that CDCA produced portal inflammation, hypertrophy of the smooth endoplasmic reticulum, and dilation of the bile cuniculi. On the other hand, UDCA only produced proliferation of the smooth endoplasmic reticulum.

Thus, orally administered CDCA (40 and 100 mg/kg/day) in monkeys produced dose-related increases in serum enzyme levels for SLAP, SGOT and SGPT; produced treatment-related increases in biliary and fecal levels of LCA; inhibited HMG-CoA reductase activity; and produced hepatic lesions (portal inflammation, hypertrophy of the smooth endoplasmic reticulum, and dilation of the bile canaliculi). On the other hand, orally administered UDCA (40 and 100 mg/kg/day) was well-tolerated. UDCA inhibited HMG-CoA reductase activity and produced proliferation of the smooth endoplasmic reticulum in the liver.

2. 1-Year Oral Toxicity Study of UDCA (Study No. was not provided).

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 rhesus monkeys (1.4 to 3.5 kg; ages were not provided).

Methods: Five groups of 8 monkeys each (4 males and 4 females) were orally administered 0, 50, 100, 300 and 900 mg/kg/day of UDCA via intragastric catheter for 1 year. Basis for dosage selection was not explicitly provided. Vehicle was 2% arabic gum solution; dosing volume was 5 ml/kg.

Animals were observed daily for clinical signs of toxicity. Body weights were measured on a weekly basis; food consumption on a daily basis.

Blood samples for hematology and blood chemistry evaluations were obtained via an unspecified vein of the forearm during the month before initiation of the study, at the beginning of the study, and during Months 3, 6, 9 and 12 of the study. Urinary examination was performed on fresh urine collected for 12 hours during the month before initiation of the study, at the beginning of the study, and during Months 3, 6, 9 and 12 of the study.

Electrocardiographic and blood pressure measurements were obtained one week before initiation of the study and during Months 3, 6, 9, and 12 of the study. Eye examinations were done before treatment and after completion of treatment.

Bile was collected by puncture of the gall bladder after termination. Bile acids were extracted from bile samples, and bile acids were separated and identified by gas-liquid chromatography.

Animals were anesthetized with Ketalar and sacrificed by exsanguination. Gross pathological examinations were performed on all animals. Organ weights were measured for brain, hypophysis, thyroid, thymus, heart, lung, liver, spleen, pancreas, kidneys, adrenals, testes/ovaries and prostate. Histopathological examinations were done on tissues from brain, pituitary gland, thyroid gland, thymus, gland, heart, lung, liver, spleen, pancreas, kidneys, suprarenal gland, urinary bladder, seminal vesicles, testicles, prostate, uterus, ovary, bone marrow, muscle, skin, stomach, small and large intestine, sciatic nerve, gall bladder, biliary duct, blood vessel and eye balls.

Data were subjected to analyses of variance.

Results:

1. Observed Effects: Some animals in the 900 mg/kg/day group vomited during drug administration during the first 2 weeks of treatment.

2. Mortality: One female in the 100 mg/kg/day group died during Week 24 of treatment. One female in the 300 mg/kg/day group died during Week 24; another female died during Week 36. One male in the 900 mg/kg/day group died during Week 18; one female died during Week 16; another female during Week 19. All animals were anorexic and were behaviorally inactive for 10-14 days before death. They were also dehydrated, anemic and had decreased body temperature.

3. Body Weight: Mean body weights of the control males and females were approximately 2.5 and 2.6 kg, respectively, at the start of the study (mean body weights were estimated from a figure provided by the sponsor). Mean body weight of the control males was approximately 3.5 kg during Week 50 of the study. Mean body weights of males during Week 50 of treatment were reduced by approximately -20% (% of difference from control) and -35% in the 300 and 900 mg/kg/day groups, respectively. Mean body weight of the control females was approximately 4.0 kg during Week 50 of the study. Mean body weights of females during Week 50 of treatment were reduced by approximately -35% (% of difference from control) and -40% in the 300 and 900 mg/kg/day groups, respectively.

4. Food Consumption: Mean food consumption of the control males and females was approximately 150 g/animal at the start of the study (mean food consumption were estimated from a figure provided by the sponsor). Mean food consumption of the control males was approximately 195 g/animal during Week 50 of the study. Mean food consumption of males during Week 50 of treatment was reduced by approximately -10% (% of difference from control) and -20% in the 300 and 900 mg/kg/day groups, respectively. Mean food consumption of the control females was approximately 200 g/animal during Week 50 of the study. Mean food consumption of females during Week 50 of treatment was reduced by approximately -25% (% of difference from control), -25% and -45% in the 100, 300 and 900 mg/kg/day groups, respectively.

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

6. Blood Chemistry: Mean leucine aminophosphatase (LAP) levels in control males and females were 633 and 539 GR-units, respectively. Mean serum LAP level in males was increased by 164% (% of difference from control) in the 900 mg/kg/day group. Mean serum LAP levels in females were increased by 94%, 321% and 263% in the 100, 300 and 900 mg/kg/day groups, respectively.

Mean bilirubin levels in control males and females were 0.19 and 0.20 mg/dl, respectively. Mean bilirubin levels in males were increased by 221% (% of difference from control) and 89% in the 300 and 900 mg/kg/day groups, respectively. Mean bilirubin levels in females were increased by 450%, 280% and 415% in the 100, 300 and 900 mg/kg/day groups, respectively.

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

8. Biliary Bile Acids: Quantitative data were not provided. It was reported that for the 900 mg/kg/day group; proportion of cholic acid and deoxycholic acid increased in all animals, and UDCA became the predominant bile acid.

9. ECG, Blood Pressure, Ophthalmology: There were no treatment-related effects.

10. Organ Weights: Mean liver weights in males were increased by 12% (% of difference from control), 14%, 31% and 27% in the 50, 100, 300 and 900 mg/kg/day groups, respectively. Mean liver weights in females were increased by 5% (% of difference from control), 8%, 17% and 23% in the 50, 100, 300 and 900 mg/kg/day groups, respectively.

11. Gross Pathology: Incidences of macroscopic lesions were not provided. It was reported that cloudy swelling of livers was seen in males and females at the 300 and 900 mg/kg/day doses.

12. Histopathology: Incidences of histopathological lesions were not provided. It was reported that increased numbers of lysosomes and cell necrosis were observed in livers of males and females in the 900 mg/kg/day group. Furthermore, proliferation of epithelial cells in the bile duct were observed, along with inflammation and necrotic cells.

Thus, the no effect oral dose of UDCA was 50 mg/kg/day in the monkey in a 1-year toxicity study. Higher doses (100, 300 and 900 mg/kg/day) produced mortality, body weight loss, decreased food consumption, increased serum LAP and bilirubin levels, and increased liver weights. The 300 and 900 mg/kg/day doses produced gross pathological lesions and histopathological lesions in the liver; thus, the liver was a target organ for toxicity. Finally, UDCA was more toxic in the monkey after 1-year treatment than after 6-months treatment.

CARCINOGENICITY:

The sponsor provided 104-week dietary carcinogenic studies of ursodiol in CD-1 mice and $B6C3F_1$ mice, a 104-week dietary carcinogenic study of ursodiol in Fischer 344 rats, and a 126 to 138-week dietary study of ursodiol in Sprague-Dawley rats.

The sponsor also provided a copy of a published article describing oral carcinogenic studies of lithocholic acid in mice and rats [Bioassay of lithocholic acid for possible carcinogenicity, DHEW Publication No. (NIH) 79-1731]. Lithocholic acid is a metabolite of ursodiol in man, rhesus monkey and rodents. Lithocholic acid is mainly sulfated and excreted as the sulfate in man. In the rhesus monkey, lithocholic acid is absorbed, but not efficiently sulfated. In mice and rats, however, β-muricholic acid is a major metabolite of ursodiol, rather than lithocholic acid. The relatively small amount of lithocholic acid that is formed in rodents is not absorbed from the colon. The concern over lithocholic acid is illustrated by a published report in which either intrarectally administered lithocholic acid or taurodeoxycholate promoted N-methyl-N'-nitro-N-nitrosoguanidine-induced colonrectal neoplasms in rats (J. Natl. Cancer Inst. 1974, 53: 1093-1095). On the other hand, it is interesting to note that dietary ursodiol apparently protected rats against azoxymethane-induced colonic carcinogenesis (Cancer Res. 1994, 54: 5071-4).

All of the above mentioned submissions and published papers are reviewed in this CARCINOGENICITY: section.

[redacted]

Mice

COVERSHEET FOR CARCINOGENICITY STUDY IN MICE
([redacted] Report No. 406-006)

1. No. of Studies: 1

2. Name of Laboratory: [redacted]

3. Strain: CD-1

4. No./sex/group: 50

5. Doses (0, 0, L, M, H): 0, 0, 25, 150 and 1000 mg/kg/day

6. Basis for dose selection stated: Sponsor did not provide any explanation for dose selection.

7. Interim sacrifice: No.

8. Total duration (weeks): 104

9. Week/site for first tumor:

	Male	Female
0	71/hepatocellular carcinoma	104/hepatocellular carcinoma
0	75/hepatocellular carcinoma	104/hepatocellular carcinoma
L	97/hepatocellular carcinoma	104/hepatocellular carcinoma
M	94/hepatocellular carcinoma	104/hepatocellular carcinoma
H	104/hepatocellular carcinoma	93/hepatocellular adenoma

10. No. alive at termination:

	Male	% Survival	Female	% Survival
O	24	48	26	52
0	31	62	32	64
L	31	62	28	56
M	28	56	27	54
H	14	28	23	46

11. Statistical methods used: Incidence tables were provided for gross pathological lesions, and non-neoplastic and neoplastic microscopic lesions. These data were not statistically analyzed.

12. Appendix I: Non-neoplastic and neoplastic histopathology data are provided on pages 151 - 164.

APPEARS THIS WAY ON ORIGINAL

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 30, 1983

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).

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

MOUSE STUDY DURATION (weeks): 104

STUDY STARTING DATE: January 17, 1980

STUDY ENDING DATE: March 30, 1983

MOUSE STRAIN: CD-1

ROUTE: Dietary

DOSING COMMENTS:

	Males	Females
No. Mice in Control1 (C1):	50	50
Control2 (C2):	50	50
Low Dose (LD):	50	50
Middle Dose (MD):	50	50
High Dose (HD):	50	50

MOUSE DOSE LEVELS (mg/kg/day)

Mouse Low Dose: 25 Mouse Middle Dose: 150

Mouse High Dose: 1000

Basis for Doses Selected (MTD; AUC ratio; saturation; maximum feasible): Sponsor did not provide any explanation for dose selection.

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

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

MOUSE STUDY COMMENTS: The sponsor did not provide any explanation for the dosage selection. However, the recommended maximum feasible dose (5% of diet) was exceeded in males (7.19%) and females (6.44%) in the 1000 mg/kg/day dosage group.

APPEARS THIS WAY ON ORIGINAL

1. 104-Week Dietary Carcinogenic Study of UDCA (Study No. [redacted] & #406-006).

Testing Laboratory: [redacted]

Compliance with Good Laboratory Practices and Quality Assurance Requirements: Statements of compliance were provided.

Study Started: January 17, 1980

Study Completed: March 30, 1983

Animals: Male (mean body weight of 29 g; 6 weeks of age) and female (mean body weight of 23 g; 6 weeks of age) CD-1 mice.

Methods: Five groups of 100 mice each (50 males and 50 females) were administered 0 (Control I), 0 (Control II), 25, 150 and 1000 mg/kg/day of ursodiol, respectively, admixed in the diet for 104 weeks. The sponsor did not provide any explanation for the dosage selection. Although results of diet analysis were not provided, it is estimated that during Week 104 for intended dietary doses of 25, 150 and 1000 mg/kg/day in males, the diet contained 0.20%, 1.22% and 7.19% of ursodiol, respectively; in the case of females, 0.16%, 1.03% and 6.44%, respectively.

Mice were observed for clinical signs of toxicity and mortality three times daily from Monday through Friday and twice daily on weekends and holidays; detailed observations were recorded weekly. Body weights were recorded weekly for the first 14 weeks and once every 2 weeks thereafter. Food consumption was also recorded weekly for the first 14 weeks and once every 2 weeks thereafter.

Mice that died during treatment, those sacrificed *in extremis* and those sacrificed by carbon dioxide asphyxiation at the termination of the study underwent a thorough external examination. Contents of the abdominal, thoracic and cranial cavities were examined both *in situ* and after removal and dissection. Feces from colon and cecum of all surviving mice and animals found dead or sacrificed *in extremis* were also examined.

The control and high dosage groups were subjected to histopathological examination. Tissues from adrenals, brain, eye, ovary, testis with epididymis, heart, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, kidneys, urinary bladder, prostate/uterus, gallbladder, liver, lung and bronchi, lymph nodes, mammary gland, salivary gland, sciatic nerve, pancreas, pituitary, skeletal muscle, spinal cord, spleen, thymus, trachea, thyroid, parathyroid, sternum were studied. Liver tissues, but not other tissues, were examined in the 25 and 150 mg/kg/day dosage groups.

Body weight data were analyzed by Barlett's test for homogeneity of variances and analyses of variance. When appropriate, data were further analyzed with t-tests and Dunnett's multiple comparison test.

Incidence tables were provided for gross pathological lesions, and non-neoplastic and neoplastic microscopic lesions. These data were not statistically analyzed.

Results:

1. Achieved Doses: Mean achieved doses for males over 104 weeks were 25.3, 152 and 1014 mg/kg/day for the projected 25, 150 and 1000 mg/kg/day doses, respectively. Mean achieved doses for females over 104 weeks were 25.4, 152 and 1018 mg/kg/day for the projected 25, 150 and 1000 mg/kg/day doses, respectively. At the highest doses in males and females, the diet contained approximately 7.19% and 6.44% UDCA, respectively..

2. Observed Effects: Corneal opacities were seen in increased numbers in the 1000 mg/kg/day dosage group. During Weeks 80-89 of treatment; 9 male and 10 female mice at 1000 mg/kg/day had corneal opacities; none were seen in controls. During Weeks 90-104 of treatment, the number of males with corneal opacities decreased because of decreasing survival. On the other hand, nearly all surviving females at 1000 mg/kg/day had corneal opacities at study termination.

3. Mortality: As shown in the following table, incidence of mortality was treatment-related in males at the 1000 mg/kg/day dose. On the other hand, incidence of mortality was not treatment-related in females. At termination, there were 26, 31, 31, 28 and 14 surviving males in the 0 (Control 1), 0 (Control 2), 25, 150 and 1000 mg/kg/day groups, respectively, and 24, 32, 28, 27, and 23 surviving females, respectively.

Cumulative mortality during 104 weeks of treatment

Week No.	Males (mg/kg/day)					Females (mg/kg/day)				
	0 (C1)	0 (C2)	25	150	1000	0 (C1)	0 (C2)	25	150	1000
20	0	1	0	0	2	0	0	0	0	0
40	2	1	0	1	8	2	1	1	2	0
60	4	1	3	3	11	2	1	2	5	2
80	12	7	9	8	24	5	5	9	12	7
90	17	11	13	9	28	10	11	14	14	13
104	26	19	19	22	36	24	18	22	23	27

4. Body Weight: Mean body weights of control males and females were 28 and 22 g, respectively, during Week 0 in the Control 1 group and 29 and 22 g, respectively, in the Control 2 group. Mean body weights of control males and females were 39 and 35 g, respectively, during Week 104 in the Control 1 group and 37 and 34 g, respectively in the Control 2 group. There were no treatment-related effects on body weight.

5. Food Consumption: Mean food consumption of control males and females was 5.4 and 5.2 g/day, respectively, during Week 0 in the Control 1 group and 5.3 and 5.2 g/day, respectively, in the Control 2 group. Mean food consumption of control males and females was 5.0 and 5.3 g/day, respectively, during Week 104 in the Control 1 group and 4.7 and 5.1 g/day, respectively, in the Control 2 group. There were no treatment-related effects on food consumption.

6. Gross Pathology: As shown in the following table, there were increased incidences of cloudy/white eyes in males and females at the 1000 mg/kg/day dose. There were increased incidences of liver cysts in females at the 1000 mg/kg/day dose.

Total incidence of gross pathological lesions during 104 weeks of treatment

Lesion	Males Ursodiol (mg/kg/day)					Females Ursodiol (mg/kg/day)				
	0	0	25	150	1000	0	0	25	150	1000
Cloudy/white eyes	1/50	6/50	7/50	2/50	17/50	6/50	10/50	4/50	5/50	27/50
Liver cysts	0/50	1/50	1/50	5/50	6/50	0/50	1/50	1/50	3/50	9/50

7. Histopathology:

Non-neoplastic

As shown in the following table, there were treatment-related increased incidences of liver megalocytosis and liver karyomegaly in males. There were no treatment-related effects in females.

Total incidence of non-neoplastic histopathological lesions during 104 weeks of treatment

Lesion	Males Ursodiol (mg/kg/day)					Females Ursodiol (mg/kg/day)				
	0	0	25	150	1000	0	0	25	150	1000
Liver megalo-cytosis	4/50	6/50	9/50	11/50	24/50	3/50	1/50	0/50	0/50	5/50
Liver karyo-megaly	18/50	24/50	27/50	27/50	36/50	10/50	8/50	6/50	1/50	6/50

Neoplastic

As shown in the following table, there were no treatment-related neoplastic histopathological lesions in the liver. The incidence of kidney adenocarcinomas in males of the high-dose group is at the borderline of background incidence of kidney adenomas and adenocarcinomas (range of 0-6.0%) in Charles River CD-1 male mice. No kidney adenocarcinomas were seen in females of any dosage group. The range of background incidence of kidney adenomas in Charles River CD-1 female mice is 0-1.4%.

Total incidence of neoplastic histopathological lesions during 104 weeks of treatment

Lesion	Males Ursodiol (mg/kg/day)					Females Ursodiol (mg/kg/day)				
	0	0	25	150	1000	0	0	25	150	1000
Hepato-cellular carcinoma	7/50	5/50	10/50	5/50	1/50	0/50	1/50	3/49	1/50	0/50
Hepato-cellular adenoma	2/50	2/50	0/50	2/50	0/50	1/50	0/50	0/49	2/50	1/50
Hepato-cellular carcinoma and adenoma	9/50	7/50	10/50	7/50	1/50	1/50	1/50	3/50	3/50	1/50
Kidney:										
Adeno-carcinoma	0/50	1/50	*0/6	*1/9	3/50	0/50	0/50	*0/2	*0/5	0/50

* = Tissue samples, except those from liver, were microscopically examined in the low-dose and mid-dose groups only if related to a gross pathological lesion.

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

The sponsor did not provide any explanation for the dosage selection. However, the recommended maximum feasible dose (5% of diet) was exceeded in males (7.19%) and females (6.44%) in the 1000 mg/kg/day dosage group.

COVERSHEET FOR CARCINOGENICITY STUDY IN MICE ([redacted]
Report # 536)

1. No. of Studies: 1

2. Name of Laboratory: [redacted]

3. Strain: $B6C3F_1$

4. No./sex/group: 80

5. Doses (O, L, M, H): 0, 300, 900 and 2700 ppm; 0, 37.4, 116 and 362 mg/kg/day in males; 0, 49.4, 146 and 459 mg/kg/day in females.

6. Basis for dose selection stated: Growth rate suppression; details were not provided by sponsor.

7. Interim sacrifice: Weeks 26, 52 and 78 (N = 10/sex/group)

8. Total duration (weeks): 104

9. Week/site for first tumor:

	Male	Female
O	79/hepatocellular adenoma	104/hepatocellular adenoma
L	74/hepatocellular carcinoma	73/hepatocellular adenoma
M	91/hepatocellular adenoma	104/hepatocellular adenoma
H	88/hepatocellular adenoma	52/hepatocellular carcinoma

10. No. alive at termination:

	Male	% Survival	Female	% Survival
O	43	86	39	78
L	41	82	37	74
M	42	84	47	94
H	47	94	39	78

11. Statistical methods used: Non-neoplastic and neoplastic histopathology data were not subjected to statistical analyses.

12. Appendix II: Non-neoplastic and neoplastic histopathology data are provided on pages 165 - 208.

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)

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

MOUSE STUDY DURATION (weeks): 104

STUDY STARTING DATE: February 22, 1984

STUDY ENDING DATE: March 28, 1985

MOUSE STRAIN: $B6C3F_1$

ROUTE: Dietary

DOSING COMMENTS:

	Males	Females
No. Mice in Control1 (C1):	50	50
Low Dose (LD):	50	50
Middle Dose (MD):	50	50
High Dose (HD):	50	50
No. Mice/sex/dose at each interim sacrifice:	10	10

MOUSE DOSE LEVELS (mg/kg/day)

Mouse Low Dose: 300 ppm (37.4 and 49.4 mg/kg/day in M and F)
Mouse Middle Dose: 900 ppm (116 and 146 mg/kg/day in M and F)
Mouse High Dose: 2700 ppm (362 and 459 mg/kg/day in M and F)

Basis for Doses Selected (MTD; AUC ratio; saturation; maximum feasible): Growth rate suppression; details were not provided by sponsor.

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

MOUSE TUMOR FINDINGS: There were no treatment-related incidences of neoplastic tumors.

MOUSE STUDY COMMENTS: The sponsor stated that dosage selection was based upon preliminary results that indicated appropriate growth rate suppression; details were not provided. However, there were no treatment-related effects on body weight in the present study. On the other hand, there were treatment-related increased incidences of bile duct dilation and hyperplasia in males at the 900 and 2,700 ppm doses (achieved doses of 116 and 362 mg/kg/day) and in females at the 2,700 ppm dose (achieved dose of 459 mg/kg/day). These doses represent maximally tolerated doses

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2. 104-Week Dietary Carcinogenic Study of UDCA (Study No. 199/ Report No. 536).

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 22, 1984

Study Completed: March 28, 1985

Animals: Male (18.9-24.4 g; 6 weeks of age) and female (15.0-19.6 g; 6 weeks of age) $B6C3F_1$ mice.

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 160 mice each (80 males and 80 females) were administered 0, 300, 900 and 2,700 ppm (0.3%, 0.9% and 2.7%) of ursodiol, respectively, admixed in the diet for 104 weeks.

Mice 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 (N=9-10/sex/group) for hematological 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 9 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.

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 2,700 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, organ weight, and body and organ weight ratios were statistically analyzed with t-tests.

Results:

1. Achieved Doses: Mean achieved doses for males over 104 weeks were 37.4, 116 and 362 mg/kg/day for the 300, 900 and 2,700 ppm doses, respectively. Mean achieved doses for females over 104 weeks were 49.4, 146 and 459 mg/kg/day for the 300, 900 and 2,700 ppm doses, respectively.

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

3. Mortality: As shown in the following tables, there were no treatment-related mortalities. At termination, there were 43 (86%), 41 (82%), 42 (84%) and 47 (94%) surviving males in the 0, 300, 900 and 2,700 ppm groups, respectively, and 39 (78%), 37 (74%), 47 (94%), and 39 (78%) surviving females, respectively.

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

Week No.	Males Ursodiol (ppm of diet)							
	0		300		900		2700	
	#	%	#	%	#	%	#	%
26	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0
78	1	0	0	0	0	0	0	0
104	7	14	8	16	8	16	3	6

Week No.	Females Ursodiol (ppm of diet)							
	0		300		900		2700	
	#	%	#	%	#	%	#	%
26	0	0	0	0	0	0	0	0
52	1	2	0	0	0	0	1	2
78	1	2	6	12	1	2	2	4
104	11	22	13	26	3	6	11	22

4. Body Weight: Mean body weights of control males and females were 21.8 and 16.9 g, respectively, during Week 1. Mean body weights of control males and females were 40.9 and 37.4 g, respectively, during Week 104. There were no treatment-related effects on body weight.

5. Food Consumption: Mean food consumption of control males and females was 4.3 and 4.2 g/day, respectively, during Week 1. Mean food consumption of control males and females was 5.1 and 5.6 g/day, respectively, during Week 104. There were no treatment-related effects on food consumption.

6. Hematology: There were dose-related decreases in white blood cell (WBC) counts in males. During Week 26, WBC counts were reduced by -39%, -50% and -64% at the 300, 900 and 2,700 ppm doses, respectively. During Week 52, WBC counts were reduced by -41%, -27% and -62% at the 300, 900 and 2,700 ppm doses, respectively. During Week 78, WBC counts were reduced by -18%, -24% and -52% at the 300, 9000 and 2,700 ppm doses, respectively. During Week 104, there was increased variability in WBC counts between animals in all treatment groups; reliable means could not be determined.

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

8. Gross Pathology: As shown in the following table for animals sacrificed at study termination (Week 104), there were increased incidences of liver cysts in males at the 900 and 2,700 ppm doses and in females at the 2,700 ppm dose. There were no treatment-related gross pathological lesions during interim sacrifice or in dead and moribund animals.

Incidence of gross pathological lesions for animals sacrificed at study termination (Week 104)

Lesion	Males Ursodiol (ppm of diet)				Females Ursodiol (ppm of diet)			
	0	300	900	2,700	0	300	900	2,700
Liver cyst	0/43	0/41	10/42	14/47	0/39	0/37	1/47	14/39

9. Histopathology:

Non-neoplastic

As shown in the following table, there was a time-related and dose-related increase in total incidence of bile duct dilation and hyperplasis in males. These bile duct lesions began to emerge at Week 78 in the 900 ppm dosage group and at Week 52 in the 2700 ppm dosage group. The % incidence increased as a function of dose for the 900 and 2700 ppm dosage groups.

Total incidence of bile duct dilation and hyperplasia in males during 104 weeks of treatment

Group	Week	Males Ursodiol (ppm of diet)			
		0	300	900	2,700
Interim Sacrifice	26	0/10	---	---	0/10
	52	0/10	0/10	0/10	2/10 (20%)
	78	0/10	0/10	3/10 (30%)	5/10 (50%)
Main	104	0/50	0/50	17/50 (34%)	23/50 (46%)

As shown in the following table, there was a time-related and dose-related increase in total incidence of bile duct dilation and hyperplasia in females. These bile duct lesions began to emerge at Week 78 in the 2700 ppm dosage group. The % incidence increased as a function of dose for the 2,700 ppm dosage group.

Total incidence of bile duct dilation and hyperplasia in females during 104 weeks of treatment

Group	Week	Females Ursodiol (ppm of diet)			
		0	300	900	2,700
Interim Sacrifice	26	0/10	---	---	0/10
	52	0/10	0/10	0/10	0/10
	78	0/10	0/10	0/10	2/10 (20%)
Main	104	0/50	2/50 (4%)	3/50 (6%)	25/50 (50%)

Neoplastic

As shown in the following table, there were no treatment-related neoplastic histopathological lesions in the main groups. In interim sacrifice groups during Week 78, 1 control male and 1 male in the 900 ppm dosage group each had a hepatocellular adenoma; 1 male in the 300 ppm dosage group had a hepatocellular carcinoma.

Total incidence of neoplastic histopathological lesions in the main groups during 104 weeks of treatment

Lesion	Males Ursodiol (ppm of diet)				Females Ursodiol (ppm of diet)			
	0	300	900	2,700	0	300	900	2,700
Hepato-cellular adenoma	14/50	15/50	9/50	4/50	3/50	2/50	4/50	3/50
Hepato-cellular carcinoma	5/50	2/50	2/50	1/50	0/50	1/50	0/50	3/80
Hepato-cellular adenoma and carcinoma	19/50	17/50	11/50	5/50	3/50	3/50	4/50	6/50

In summary, there were no treatment-related incidences of neoplastic lesions in mice 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. However, there were no treatment-related effects on body weight in the present study. On the other hand, there were time-related and treatment-related increases in incidence of bile duct dilation and hyperplasia. These doses represent maximally tolerated doses.

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

Testing Laboratory: [redacted]

[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 B6C3F$_1$ mice (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); 1/5 males died at the 464 mg/kg dose, 3/5 males and 1/5 females died at the 681 mg/kg dose, and all mice died at the 1000, 1470 and 2150 mg/kg doses. There were no other clinical signs of toxicity. Thus, the high dose selected for the carcinogenic study was 250 mg/kg.

Therefore, 2 groups of 100 mice each (50 males and 50 females) were orally administered 125 and 250 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 2.5% to 5%. All animals were further observed for 2 weeks following cessation of lithocholic acid administration.

All animals were observed twice daily for clinical signs of toxicity and mortality. Body weights were recorded once a week for the first 6 weeks, every 2 weeks for the next 4 weeks, and at monthly intervals thereafter. 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, gallbladder, 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 mice. In the case of males, there were 15/20, 37/50 and 32/50 surviving mice in the 0, 125 and 250 mg/kg dosage groups, respectively, at the end of the study (Week 105). In the case of females, there were 13/20, 34/50 and 41/50 surviving mice in the 0, 125 and 250 mg/kg dosage groups, respectively, at the end of the study (Week 105).

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

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	125	250
Mesenteric lymph node hyperplasia	0/20	0/46	2/42
Bile duct cysts	0/20	0/47	2/47
Pituitary hyperplasia	0/10	0/22	1/28
Mesentery fat necrosis	0/20	1/48	3/47

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	125	250
Spleen hyperplasia	0/16	0/41	1/49
Follicular cyst	0/16	0/35	1/45

Neoplastic

As shown in the following table for males, there were apparent treatment-related increases in incidence of alveolar/bronchiolar carcinoma or adenoma, hepatocellullar carcinoma, and hepatocellular carcinoma or adenoma in males. However, the background incidence of these neoplastic lesions in male $B6C3F_1$ mice is 20.81% ± 6.34 (mean ± standard deviation), 19.00% ± 7.51, and 48.57% ± 15.43, respectively. Thus, when compared to background incidence, these neoplastic lesions are not treatment-related.

Total incidence of neoplastic lesions in males

Lesion	Lithocholic acid (mg/kg, p.o., 3 times a week)		
	0	125	250
Alveolar/ bronchiolar carcinoma or adenoma	1/20 (5%)	7/48 (14.6%)	5/46 (10.9%)
Hepatocellular carcinoma	5/20 (25%)	14/47 (29.8%)	6/47 (12.8%)
Hepatocellular carcinoma or adenoma	6/20 (30%)	17/47 (36.2%)	9/47 (19.1%)

As shown in the following table for females, there were apparent treatment-related increases in incidence of alveolar/bronchiolar adenoma, hematopoietic leukemia or malignant lymphoma, and hepatocellular carcinoma in females. However, the background incidence of these neoplastic lesions in female $B6C3F_1$ mice is 5.00% ± 2.66, 19.16% ± 6.51, and 6.35% ± 6.05, respectively. Thus, when compared to background incidence, these neoplastic lesions are not treatment-related.

Total incidence of neoplastic lesions in females

Lesion	Lithocholic acid (mg/kg, p.o., 3 times a week)		
	0	125	250
Alveolar/ bronchiolar adenoma	0/19 (0%)	1/44 (2.3%)	3/50 (6%)
Hematopoietic leukemia or malignant lymphoma	5/19 (26.3%)	17/45 (37.8%)	12/50 (24%)
Hepatocellular carcinoma	0/18 (0%)	1/45 (2.2%)	3/50 (6%)

In summary, orally administered lithocholic acid (125 and 250 mg/kg 3 times a week for 103 weeks) in mice was not carcinogenic.

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Rats

COVERSHEET FOR CARCINOGENICITY STUDY IN RATS [redacted]
Report # 537)

1. No. of Studies: 1

2. Name of Laboratory: [redacted]

3. Strain: Fischer 344

4. No./sex/group: 90

5. Doses (O, L, M, H): 0, 500, 1700 and 5000 ppm; 0, 22.5, 77.2 and 239 mg/kg/day in males; 0, 28.5, 97.5 and 300 mg/kg/day in females.

6. Basis for dose selection stated: Growth rate suppression; details were not provided by the sponsor.

7. Interim sacrifice: Weeks 26, 52 and 78 (N = 10/sex/group/interval)

8. Total duration (weeks): 104

9. Week/site for first tumor:

	Male	Female
O	79-104/Testiticular interstitial cell	53-78/Pituitary adenoma
L	Not analyzed	Not analyzed
M	Not analyzed	Not analyzed
H	79-104/Testiticular interstitial cell	79-104/Pituitary adenoma

10. No. alive at termination:

	Male	% Survival	Female	% Survival
O	47	78.3	53	88.3
L	42	70	51	85
M	42	70	52	86.7
H	42	70	48	80

11. Incidence data for non-neoplastic and neoplastic microscopic lesions were not statistically analyzed.

12. Appendix III: Non-neoplastic and neoplastic histopathology data are provided on pages 209 - 247.

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